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Microbial and hydrolase activity after release of low molecular weight organic compounds by a model root surface in a clayey and a sandy soil

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ABSTRACT

The stimulatory effects of low molecular weight organic compounds (LMWOC) such as glucose, citrate, oxalate, glutamate, on microbial and hydrolase activity, in the rhizosphere and bulk soil layers of a clayey and a sandy soil were studied. Incubation units reproducing the rhizosphere environment were used, allowing additions and precise sampling of soil. All LMWOC stimulated respiration in both soils although the degree of LMWOC mineralization was different in the two soils. The double-stranded DNA (dsDNA) content increased significantly in the rhizosphere layer of the clayey soil treated with glucose or glutamate, but not with citrate or oxalate. In the sandy soil, a significant increase in the dsDNA content was only observed in the rhizosphere layer upon release of oxalate, whereas differences of the other treatments over the control were not significant. Hydrolase activity in the rhizosphere soil layer was generally stimulated. The alkaline phosphatase activity was significantly stimulated by glucose, glutamate and citrate in both the clayey and sandy soils, whereas the acid phosphatase activity was significantly stimulated by citrate in the clayey soil and by glutamate, citrate and oxalate in the sandy soil. Phosphodiesterase activity was significantly stimulated by glutamate and citrate in the clayey soil and by citrate only in the sandy soil. Urease activity was significantly stimulated by glucose and citrate in the clayey soil, whereas, the stimulation of urease activity in the sandy soil upon release of glucose, citrate and oxalate was not significant. No response to LMWOC release was observed for proatease activity in the rizosphere or bulk soil layer of both soils. It was concluded that in the rhizosphere, different LMWOC commonly released in the root exudates stimulate soil microbial activity and hydrolase activity differently depending on the compound released and soil type.

1. Introduction

Enzyme activity is generally higher in rhizosphere than in bulk soil as a result of greater microbial activity, sustained by root exudates or due to the release of enzymes from roots (Tarafdar and Jungk, 1987; Grierson and Adams, 2000; Marschner et al., 2005; George et al., 2005). It has been frequently postulated that hydrolase activity in the rhizosphere is of crucial importance for plant nutrition in both agricultural and forest soils, as it is responsible for N and P supply to plants (Nannipieri et al., 2003). However, knowledge on the specific capacity of low molecular weight organic compounds released in root exudates to stimulate hydrolase activity in the rhizosphere indifferent soils is still poor. Recent contributions on this topic

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have elucidated the effects of specific root exudates on the composition of the microbial community and on the biochemical properties of the rhizosphere (Baudoin et al., 2003; Falchini et al., 2003; Landi et al., 2006; Renella et al., 2006), but always using one soil type. The aim of the present work was to study the stimulatory effects of some low molecular weight organic compounds (LMWOC), such as glucose, citrate, oxalate or glutamate released by an artificial root surface, on microbial activity and hydrolase activity in the rhizosphere of two soils. Glucose and citrate were chosen as they are the most common compounds released in root exudates (Uren, 2001), oxalate as it is mineralized by specilized microrganisms (Falchini et al., 2003) and glutamate, as it is representative of N-containing compounds in root exudates.

2. Materials and methods

2.1. Soil characterisitics and treatments

An agricultural soil left under set aside management for 6 years located at the Couhins Experimental Farm, Bordeaux (France), classified as sandy clay Arenic Udifluvent (USDA, 1998) and a forest soil located at Vallombrosa Forest, Tuscany (Central Italy), classified as sandy loam Fragic Disdrudept (USDA, 1998) were sampled. Samples were taken from the A_P (0–30 cm) and A_0 (0–10 cm) horizons, respectively. Five soil samples were collected from each soil, mixed and bulked into pooled samples from where three subsamples were taken for analysis. The main chemical and physical parameters of both soils are reported in Table 1. Soils were sieved (<2 mm) at field moisture, moistened to 40% WHC and preincubated at a constant 25 °C in the dark for 7 days. After preincubation, soil samples of 70 g (dry weight equivalent) were placed in incubation units reproducing the rhizosphere environment, allowing the release of LMWOC from a cellulose round filter paper acting as a root surface, and sampling of soil layers at different distance from the surface (Falchini et al., 2003). The LMWOC solutions contained glucose, citrate, oxalate or glutamate, added to give an amount of 300 mg of LMWOC-C kg^{-1} soil. Soils treated with deionized H_2O served as controls. All treatments and determinations were carried out on three separate incubation units for each treatment. The units were incubated for 7 days at 25 °C in 1 l air tight jars. The incubation time was chosen because 7 days is an average estimate for the turnover time of microbial assimilation of root-derived C inputs to soils (Treonis et al., 2004). At the end of the incubation period, soils were sampled from 0 to 2 mm (rhizoshere soil) and >4 mm (bulk soil) distance from the surface, and analyzed for their microbial respiration and biomass content and different hydrolase activities.

2.2. Biochemical measurements

Soil respiration was determined by measuring the CO_2–C evolution from the whole soil by back titration of alkali after precipitation of carbonates by 0.75N $BaCl_2$ (Stotzky, 1965). Microbial biomass in rhizosphere and bulk soil was estimated by the total soil DNA content, extracted by a bead beating method (FastDNA SPIN Kit for soil, Bio 101 Inc., USA). The extracted dsDNA was quantified by fluorometry (Hoefer DyNA Quante 200) using bisbenzimide-dye (Hoechst H 33258). Acid and alkaline phosphatase activities were assayed according to Tabatabai and Bremner (1969) and phosphodiesterase activity as reported by Browman and Tabatabai (1978). Urease activity was measured as reported by Nannipieri et al. (1974). Protease activity was determined by the hydrolysis of *N*-benzoylargininamide (*N*-BAA) according to Ladd and Butler (1972). All enzyme activities were assayed at 37 °C for 1 h, with centrifugation of soil slurries at 6000 × *g* at 4 °C. Concentration of *p*-nitrophenol (*p*-NP) produced in the assays of acid and alkaline phosphatase and phosphodiesterase activities was calculated from a calibration curve after subtracting the absorbance of the control at 400 nm wavelength using a UV–Vis spectrophotometer Lambda 2 (Perkin Elmer). The NH_4^+-N produced by urease and *N*-BAA-hydrolysing activities was extracted by 2 M KCl and quantified by a flow injection analyzer (FIAStar, Tecator, Sweden). To account for the NH_4^+-N fixation by soils, NH_4^+-N solutions with concentrations in the range of those released by urease and protease activities were incubated with these soils. Recovery of NH_4^+-N were 95% and 98% for the clayey and the sandy soil, respectively. Statistical analysis to evaluate the effects of the treatments on the measured biochemical parameters of the two soils was conducted by two-way ANOVA using the Tukey HSD pairwise multiple comparison test at *P* level of 5% using the Systat 11 software (SYSTAT software, 2004).

3. Results

All added LMWOCs stimulated respiration in both soils (Fig. 1). In the clayey soil, by subtracting the basal respiration value, it was calculated that glucose, citrate and glutamate acid were mineralized whereas oxalate was apparently not completely mineralized in the clayey soil after 7 days (Fig. 1). In the sandy soil, a priming effect was observed in soils treated with glutamate, citrate and oxalate (Fig. 1).

The dsDNA content increased in the rhizosphere layer of both soils, whereas no significant increase was observed in the bulk soil layer (Fig. 2). Significant differences were found in relation to soils, treatments and interactions between soils and treatments (Table 2). The increase over respective controls was significant upon release of glucose or glutamate in the clayey soil and upon release of oxalate in the sandy soil (Fig. 2).

Table 1 – Main physico-chemical characteristics of the two studied soils

Soil type	pH (H_2O)	Sand (%)	Silt (%)	Clay (%)	C org (%)	N tot (%)	CEC (cmol kg^{-1})
Arenic udifluvent (clayey)	6.9	67.5	15.0	17.5	0.7	0.10	14.5
Fragic disrudept (sandy)	5.1	77.8	20.0	2.2	3.7	0.21	10.9

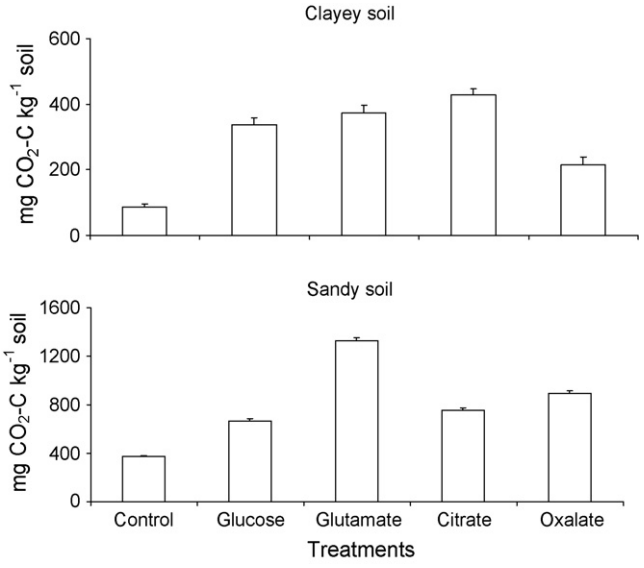

Fig. 1 – Cumulative respiration of the clayey and sandy soils treated with H_2O (control) or LMWOC, after 7 days of incubation. The error bars represent the standard deviation of the means ($n = 3$).

Significant stimulation of phosphatase and urease activites was observed in the rhizosphere layer of both the clayey and sandy soils, whereas, no response to LMWOC release was observed for protease activity (Figs. 3–4). Stimulation in the bulk layer was not significant in any case.

Alkaline phosphatase activity was significantly stimulated by glucose, glutamate and citrate in both the clayey and sandy soils. Significant differences were found in relation to soils, treatments and interactions between soils and treatments (Table 2). Stimulation (+48%) upon release of oxalate which was observed in the sandy soil was not significant (Fig. 3). Acid phosphatase activity was significantly higher in the sandy than in the clayey soil (Table 2). In the sandy soil acid

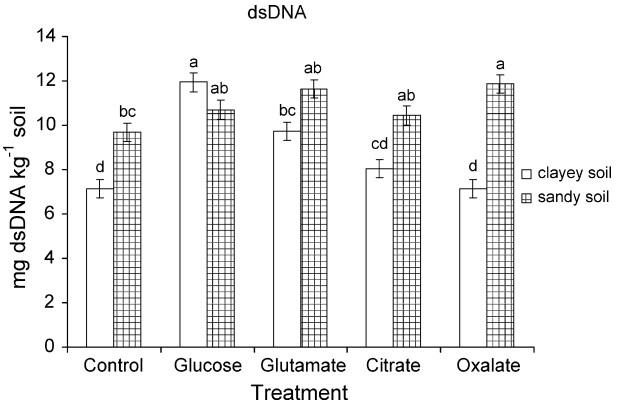

Fig. 2 – The dsDNA content of the rhizosphere soil layer (0–2 mm) of the clayey and sandy soils treated with H_2O (control) or LMWOC, after 7 days of incubation. Different letters indicate significant differences at $P < 0.05$ between soils and treatments. The error bar is the standard error.

Table 2 – Results of the two-way ANOVA the (*F* and *P* values) for the main factors and interactions

Source	F-ratio	P
dsDNA		
Soil	62.443	<0.001
Treatment	15.93	<0.001
Treatment × soil	13.419	<0.001
Alkaline phosphatase		
Soil	28.35	<0.001
Treatment	30.202	<0.001
Treatment × soil	5.54	0.004
Acid phosphatase		
Soil	477.091	<0.001
Treatment	9.479	<0.001
Treatment × soil	4.382	0.010
Phosphodiesterase		
Soil	165.731	<0.001
Treatment	87.432	<0.001
Treatment × soil	52.714	<0.001
Urease		
Soil	39.796	<0.001
Treatment	12.634	<0.001
Treatment × soil	17.682	<0.001
Protease		
Soil	9.183	0.007
Treatment	0.885	0.490
Treatment × soil	0.749	0.570

phosphatase activity was significantly stimulated by glutamate, citrate and oxalate, whereas significant stimulation of acid phosphatase in the clayey soil was observed only upon release of citrate (Fig. 3).

Phosphodiesterase activity was significantly stimulated by glutamate and citrate in the clayey soil and by citrate only in the sandy soil (Fig. 3). Significant differences were found in relation to soils, treatments and interactions between soils and treatments (Table 2). Urease activity was significantly stimulated by glucose and citrate in the clayey soil (Fig. 4). Significant differences were found in relation to soils, treatments and interactions between soils and treatments (Table 2). Stimulation of urease activity in the sandy soil upon release of glucose, glutamate, citrate and oxalate (27, 39, 23 and 49%, respectively) were not statistically significant (Fig. 4).

4. Discussion

Release of LMWOC stimulated respiration in the two soils differently depending on the compound released. The reduced mineralization of oxalate observed in the clayey soil was in agreement with the data reported by Hamer and Marschner (2005), and confirms that the utilization of LMWOC in root exudates depends on soil type (Bremer and van Kessel, 1990; Nguyen and Guckert, 2001). Lower mineralization of oxalate in the clayey soil could be due to its lower availability due to a greater adsorption or precipitation by soil minerals as compared with glucose and glutamate (Darrah, 1991a,b; van Hees et al., 2003). Greater diffusion of glucose and glutamic acid as compared to oxalic acid in an alkaline soil under similar incubation conditions was reported by Falchini et al.

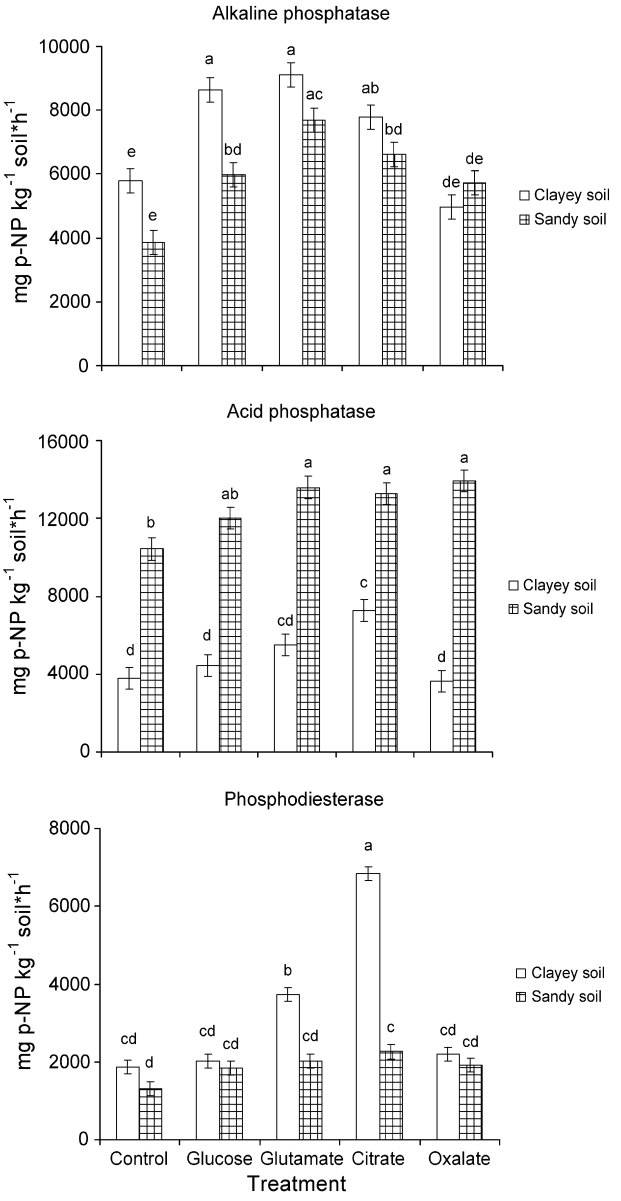

Fig. 3 – Phosphatase activity of the rhizosphere soil layer (0–2 mm) of the clayey and sandy soils treated with H_2O (control) or LMWOC, after 7 days of incubation. Different letters indicate significant differences at $P < 0.05$ between soils and treatments. The error bar is the standard error.

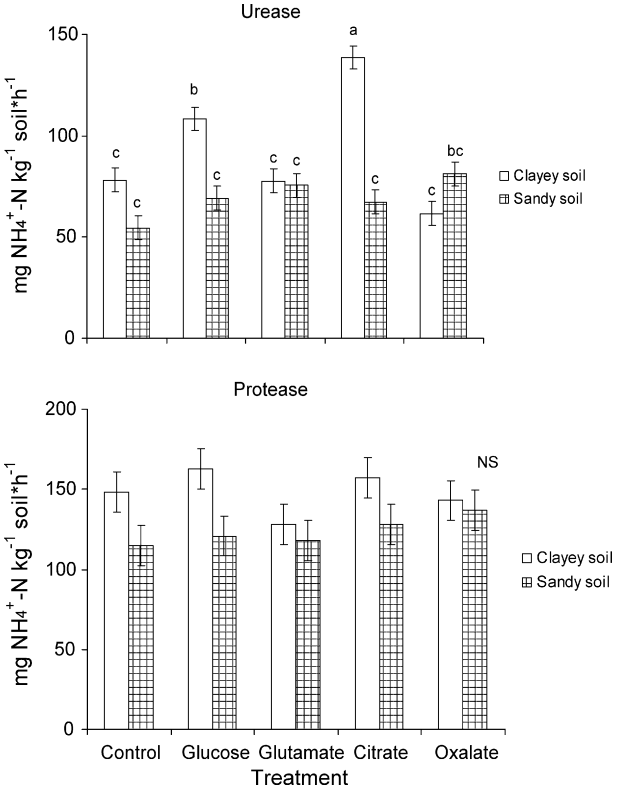

Fig. 4 – Urease and protease activities of the rhizosphere soil layer (0–2 mm) of the clayey and sandy soils treated with H_2O (control) or LMWOC, after 7 days of incubation. 'NS' for the protease activity indicate that differences between the means were not significant. The error bar is the standard error.

(2003). The positive priming effect observed in the sandy soil treated with citrate, oxalate and glutamate, was likely the result of enhanced microbial decomposition of organic matter (Kuzyakov et al., 2000). In particular, the large priming effect induced by glutamate could be due to the fact that in this soil mineralization of organic matter is N-limited (Landi et al., 2006), and probably the addition of glutamic acid-N was sufficient to overcome such limitation. However, without the use of istotope-labelled substrates the origin of the priming effect is difficult to explain.

The increase in dsDNA content in the rhizosphere layer of both the sandy and clayey soils, although significant only for the latter soil, demonstrated that the released LMWOCs were able to induce microbial growth. Marstorp and Witter (1999) demonstrated that quantification of the dsDNA content can be used to estimate microbial growth in soil in the presence of readily mineralizable substrates. The fact that the LMWOC-induced microbial growth in the sandy soil was significant only upon release of oxalate even though decomposition of all the LMWOC occurred (Fig. 1), was probably due to the soil acidic pH value. Reduced growth of soil microrganisms in acidic soils has been reported (Bååth, 1998). Quantitative results of glucose- and glutamate-induced microbial growth in the clayey soil were comparable with those reported by Marstorp (1996), whereas, to our knowledge no similar data have been published for LMWOC in acidic forest soils. Different effects of LMWOC in the two soils can be ascribed to several factors. The fast mineralization and stimulatory effects of oxalate on both microbial growth and enzyme production in the sandy soil could be explained by the presence of a selected microflora due to the fact that oxalic acid is present at relatively high concentrations in the A horizon of forest soil (Certini et al., 2000). Selection of bacteria upon release of oxalic acid was demostrated by Falchini et al. (2003). Simple sugars and organic acids are primarily used for microbial biosynthesis and growth; however, the substrate-use efficiency of C rich compounds of root exudates depends on the availability of N and other nutrients in the rhizosphere.

Nutrient limitation to respiration and growth of soil microrganisms has been reported (Aldén et al., 2001). Landi et al. (2006) reported that the release of glucose or oxalic acid decreased the mineral N content of the rhizosphere soil due to N immobilization, by using the same model system as in our work. It should be noted that in our model rhizosphere system plant uptake was not present. Therefore, we have determined the potential stimulatory effects of the LMWOC since the presence of plants can reduce N and nutrient availability.

Increase in hydrolase activity during the mineralization of the LMWOC was likely due to the enhancement of microbial activity and microbial growth, particularly in the rhizosphere layer (Fig. 2). Although with the methods used it was not possible to distinguish between the activity of intracellular and stabilized extracellular enzymes (Nannipieri et al., 2003), both intracellular and pericellular enzymes associated with actively growing cells may have contributed to the measured enzyme activity. Schmidt et al. (1987) demonstrated that both intracellular and pericellular phosphohydrolases were increased in *Pseudomonas* sp. by the addition of glucose. The lack of response of protease activity within the experimental period might be due to the fact that protease activity is characterized by a late response to soil organic amendments, as compared with other hydrolases (Nannipieri et al., 1979, 1983). Increase in hydrolase activity during microbial growth induced by LMWOC addition to soil is well established (Zantua and Bremner, 1976; Nannipieri et al., 1978; Dilly and Nannipieri, 2001; Allison and Vitousek, 2005), but the occurrence of this phenomenon in the rhizosphere is not well documented, even if it might be relevant for nutrient cycling and crop production. For example, the hydrolysis of organic phosphate monoesters by phosphatases may account for 30–80% of P taken up by plants in agricultural soils (Gilbert et al., 1999).

In conclusion, our work demonstrated that different LMWOC present in root exudates stimulate soil microbial activity and hydrolase activity in the rhizosphere differently depending on the compound released and soil type. Further studies are needed to better understand the mechanisms responsible for the differential stimulatory effects of root exudates in different soil types and the influence of root exudates on nutrient turnover in the rhizosphere.

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