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Microbial and hydrolase activity after release of indoleacetic acid and ethylene–polyamine precursors by a model root surface

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ABSTRACT

Effects of indoleacetic acid (IAA) and ethylene (E) precursors on microbial biomass, respiration, and various hydrolase activities of the rhizosphere and bulk soil, were studied using a model system simulating this environment. The effects of IAA and E precursors were compared to those of glucose-C, N and S (GNS) applied at the same rate to soils. None of the treatments significantly affected respiration rates and ATP contents of soils. The IAA precursor significantly increased phosphatase, β-glycosidase, urease and protease activities of the rhizosphere layer of both soils; the E precursor significantly increased phosphodiesterase, urease and protease activities of both soils. The GNS treatment did not significantly increase any hydrolase activity. The IAA precursor also stimulated the phosphatase activity of the bulk layer of the sandy soil after 7 d of incubation, possibly due to its diffusion from the rhizosphere to the bulk soil, whereas no stimulation in the bulk soil layer was observed in either E or GNS treatments. The increased hydrolase activities in the rhizosphere upon addition of both IAA and E precursors may be due to the role of these precursors as microbial metabolic activators, and may be involved in stimulation of plant growth through other processes involving IAA and E producing root associated microorganisms.

1. Introduction

Phytohormones such as indole-3-acetic acid (IAA) and ethylene (E) are naturally present in soil at concentrations in the order of 1–5 mg kg^{-1} (Lebuhn and Hartmann, 1993; Martens and Frankenberger, 1993; Smaill et al., 2010). Despite their low concentrations the production of phytohormones by rhizobacteria or pathovars can affect root development, plant nutrient uptake, crop yield, and development of plant disease (Glick, 2005). Phytohormones such as IAA and E are produced by precursor molecules such as tryptophan or indolic derivatives and methionine analogs, respectively, which can be released during the decomposition of biological debris or secreted into root exudates, present in humic substances and dissolved organic matter (Sorge et al., 1993; Knicker et al., 2002); they can be taken up by microorganisms through high affinity transport systems (Marlowe and Kosuge, l972). Application of IAA precursors promote plant growth due to their rapid (2 hto 1 d) microbial conversion into IAA (García Mina et al., 2004), whereas ethylene plant growth promotion is due to its microbial synthesis from precursors such as 2-hydroxy-4-methyl thiobutanoic acid (HMTB) and its derivatives, through the methionine pathways (Billington et al., 1979). The role of phytohormones within microbial cells is still not completely clear, but *in vitro* studies have shown that indole and methionine analogs control several bacterial processes such as gene expression, sporulation, plasmid stability, biofilm formation (Wang et al., 2001), and production of signal molecules (Spaepen et al., 2007). While it is known that many soil microorganisms are able to synthesize active phytohormones by various precursors through different biosynthetic pathways (Leinhos, l994; Bonkowski and Brandt, 2002), and also depending on soil and plantation management (Smaill et al., 2010), the effects of IAA and E precursors on microbial activity in soil are unknown. Despite their trace concentrations in soil, metabolism of indolic and methionine precursors may play a significant role in soil microbial ecology and plant–bacteria relationships.

In this research we hypothesised that IAA and E precursors added at concentrations in the order of those found naturally in soils may stimulate soil respiration and the activity of hydrolases involved in C, P, and N cycling in soil. The effects of IAA and E precursors on microbial biochemical activity were studied using an artificial system reproducing the rhizosphere environment, as the study of real rhizospheres would be complicated by the fact that plant roots would respond to the phytohormones and eventually modify root exudate profiles which in turn affect microbial activities in soil.

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Table 1
Cumulative respiration of the Vallombrosa and Romola soils. Values are in mg CO_2-C kg^{-1} soil. Values in brackets are the standard deviation of the mean values ($n = 3$).

Treatment	Incubation time (d)				
	2	4	7	10	14
Vallombrosa					
Control	73.3 (4.6)	146.0 (7.8)	250.8 (12.4)	345.3 (10.2)	474.9 (15.7)
IAA	73.6 (6.2)	149.6 (9.4)	259.9 (9.5)	357.2 (12.7)	487.1 (14.4)
E	74.2 (4.4)	151.2 (8.6)	266.1 (11.3)	361.9 (15.3)	485.2 (12.6)
GNS	76.2 (6.1)	150.6 (9.0)	261.9 (10.2)	361.2 (12.9)	388.6 (18.7)
Romola					
Control	12.4 (1.3)	23.4 (1.7)	42.6 (2.5)	59.7 (3.2)	79.8 (6.6)
IAA	14.8 (2.5)	29.9 (2.4)	50.2 (2.3)	68.1 (5.3)	85.9 (4.5)
E	14.6 (1.5)	26.5 (2.3)	51.0 (4.7)	66.2 (5.5)	83.8 (3.0)
GNS	18.7 (2.4)	29.5 (2.5)	56.3 (4.3)	74.7 (4.4)	95.5 (5.8)

2. Materials and methods

Effects of indoleacetic acid (IAA) and ethylene (E) precursors were studied in two soils: a sandy clay Eutric Cambisol (WRB) located at Romola (Tuscany, Central Italy), with 11% clay content, a $pH_{(H_2O)}$ value of 7.0, 0.7% total organic C and 0.07% total N, and a sandy loam Fragic Disdrudept (USDA, 1998), located at Vallombrosa (Tuscany, Central Italy) with 2% clay content, a $pH_{(H_2O)}$ value of 5.2, 3.5% total organic C and 0.22% total N. Samples were taken from the A horizons in three replicates of 1 kg each, kept separate, sieved (<2 mm) at field moisture, moistened to 40% WHC and preincubated at 25 °C in the dark for 7 d. After preincubation, soil samples of 70 g (dry weight equivalent) were placed in incubation units reproducing the rhizosphere environment, with the release of organic compounds through a cellulose filter acting as an artificial root surface, and sampling of soil layers at different distances from the cellulose filter surface (Renella et al., 2007). Treatment solutions contained distilled H_2O (control), IAA and E precursors or a mixture of glucose-C + $(NH_4)_2SO_4$ (GNS), to account for C, N and S added with the IAA and E treatments. The IAA precursor contained a mixture of free L-tryptophane and esters at concentration of 0.053 M (García Mina et al., 2004), in which L-tryptophane is converted into IAA in 1:1 ratio. The E precursor solution contained 0.346 M of 2-hydroxy-4-methyl thiobutanoic acid (HMTB), patented as metabolic activator and precursor of the synthesis of ethylene and polyamines in plants (Garcia Mina et al., 2007a,b). The GNS solution contained 0.4 M glucose-C + 0.5 M $(NH_4)_2SO_4$. All solutions were added to soils to provide the equivalents of 10 mg of C kg^{-1} soil. All soils and treatments were incubated at 25 °C in 1 l air tight jars and sampled after 0, 2, 4, 7 and 14 d. After each incubation period the cumulative soil respiration was measured by gas chromatography (Blackmer and Bremner, 1977); afterward the soils were sampled from 0 to 2 mm (rhizosphere) and 4 to 6 mm (bulk soil) distance from the surface for determining the soil ATP content according to Ciardi and Nannipieri (1990), the acid and alkaline phosphomonoesterase activities according to Tabatabai and Bremner (1969), the phosphodiesterase activity according to Browman and Tabatabai (1978), the β-glucosidase and β-galactosidase activities according to Tabatabai (1982), the urease activity as reported by Nannipieri et al. (1974) and the protease activity by the hydrolysis of N-BAA (Ladd and Butler, 1972). All treatments were replicated three times with three independent incubation units, and the significance of differences between mean values was assessed by one-way ANOVA followed by the Tukey–Kramer HSD test.

3. Results

The Vallombrosa and Romola soils differed largely in respiration rates, ATP content and hydrolase activities. In both soils, the indole-3-acetic acid (IAA) and ethylene (E) precursors, and the GNS treatment slightly increased the cumulative soil respiration but differences between daily respiration rates of control and treated soils were not significant for any incubation time (Table 1). The ATP content of soil was not significantly changed either in the artificial rhizosphere or in the bulk soil, regardless of the treatment and incubation time. Mean values of ATP concentration (mM) for the artificial rhizosphere and bulk soil were 2.26 (±0.18) and 2.21 (±0.22) for the Vallombrosa soil, and 1.33 (±0.10) and 1.31 (0.09) for the Romola soil.

Hydrolase activities of the artificial rhizosphere layer, were increased significantly by the IAA and E treatments in both soils, whereas the hydrolase activities of the control and GNS amended soils did not change during the incubation period. In the Vallombrosa soil, the IAA treatment significantly increased all the measured hydrolase activities by 22 and 165% over control, and among them, the acid phosphomonoesterase activity showed a permanent and significant increase until the end of the incubation period (Fig. 1). The E treatment significantly increased the phosphodiesterase (+67%), urease (+25%) and protease (+22%) activities (Fig. 1). In the Romola soil the IAA treatment significantly increased all the measured hydrolase activities by 26 and 67% over control, whereas the E treatment significantly increased the acid phosphomonoesterase (+25%), urease (+24%) and protease (+19%) activities (Fig. 2). Smaller but significant increases of the acid phosphomonoesterase (+28%), phosphodiesterase (+48%) and β-glucosidase (+22%) activities in the bulk layer of the Vallombrosa soil treated with the IAA precursor were detected after 7 d of incubation (data not shown), whereas no significant changes were detected in the bulk layer of the Romola soil regardless of the treatments and incubation time.

4. Discussion

The lack of significant changes in the soil cumulative respiration and ATP content were likely due to the low amount of C, N and S supplied to soils, not sufficient to induce microbial growth even in the artificial rhizosphere layer. Microbial growth in soil is generally stimulated by glucose-C and N additions in the order of mg kg^{-1} soil (e.g. Aldén et al., 2001).

Marked effects of indole-3-acetic acid (IAA) and ethylene (E) availability on the measured hydrolase activities, particularly in the Vallombrosa soil, were likely due to the metabolic activation of its large microbial biomass. Stimulation of phosphatase activities in plants by IAA of microbial origin has been previously reported (Palmer, 1970; Grappelli and Rossi, 1981). *In vitro* studies have shown that microbial synthesis of IAA and other phytohormones from tryptophan and other precursors is associated with the synthesis of enzymes such as 1 aminocyclopropane-1-carboxylic acid (ACC) synthase (Patten and Glick, 1996; Patten and Glick,

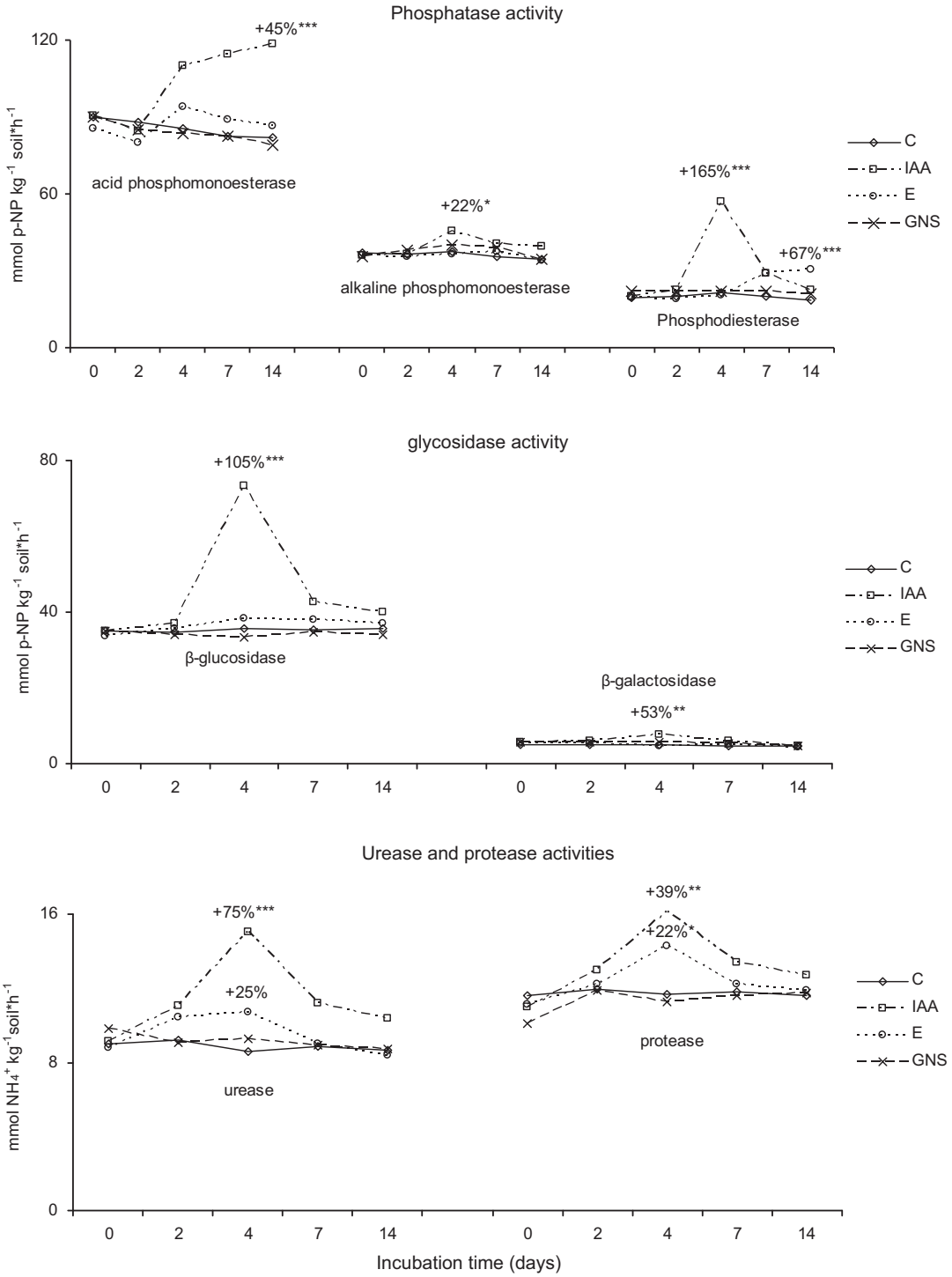

Fig. 1. Hydrolase activity in the artificial rhizosphere soil layer (0–2 mm) of the Vallombrosa soil treated with H_2O (C), glucose, N and S (GNS), IAA precursor or E precursor. Symbols *, ** and *** indicate significant differences between control and IAA, E and GNS treatments.

2002), and synthesis of glycolytic enzymes that can hydrolyze inactive IAA-glucosides into the active hormone (Gaudin et al., 1994). Stimulation of dehydrogenase, glycosidase and phosphatase activities associated with IAA production by rhizobacteria has also been reported (Leinhos and Vacek, 1994; Vivas et al., 2005). The observed increase of soil phosphatase activity in the rhizosphere layer of the IAA treatment could also be due to the increased microbial P requirement because of the formation of phosphorylated intermediates involved in the microbial indole metabolism (Smith and Yanofsky, 1960). The increase in urease and protease activities in the IAA treatment may indicate a more rapid protein turnover within microbial cells, related to an increase in microbial protein synthesis. In fact, methionine, the metabolic intermediate of the ethylene synthesis, is also the aminoacid initiator of the protein synthesis (Loenen, 2006). *In vitro* studies have shown that the supply of exogenous HMTB to microorganisms can decrease the proportion of microbial N synthesised from inorganic sources, confirming that HMTB may be not only a methionine precursor, but also a precursor of S-adenosylmethionine, which is also involved in the synthesis of polyamines (Noftsger et al., 2003).

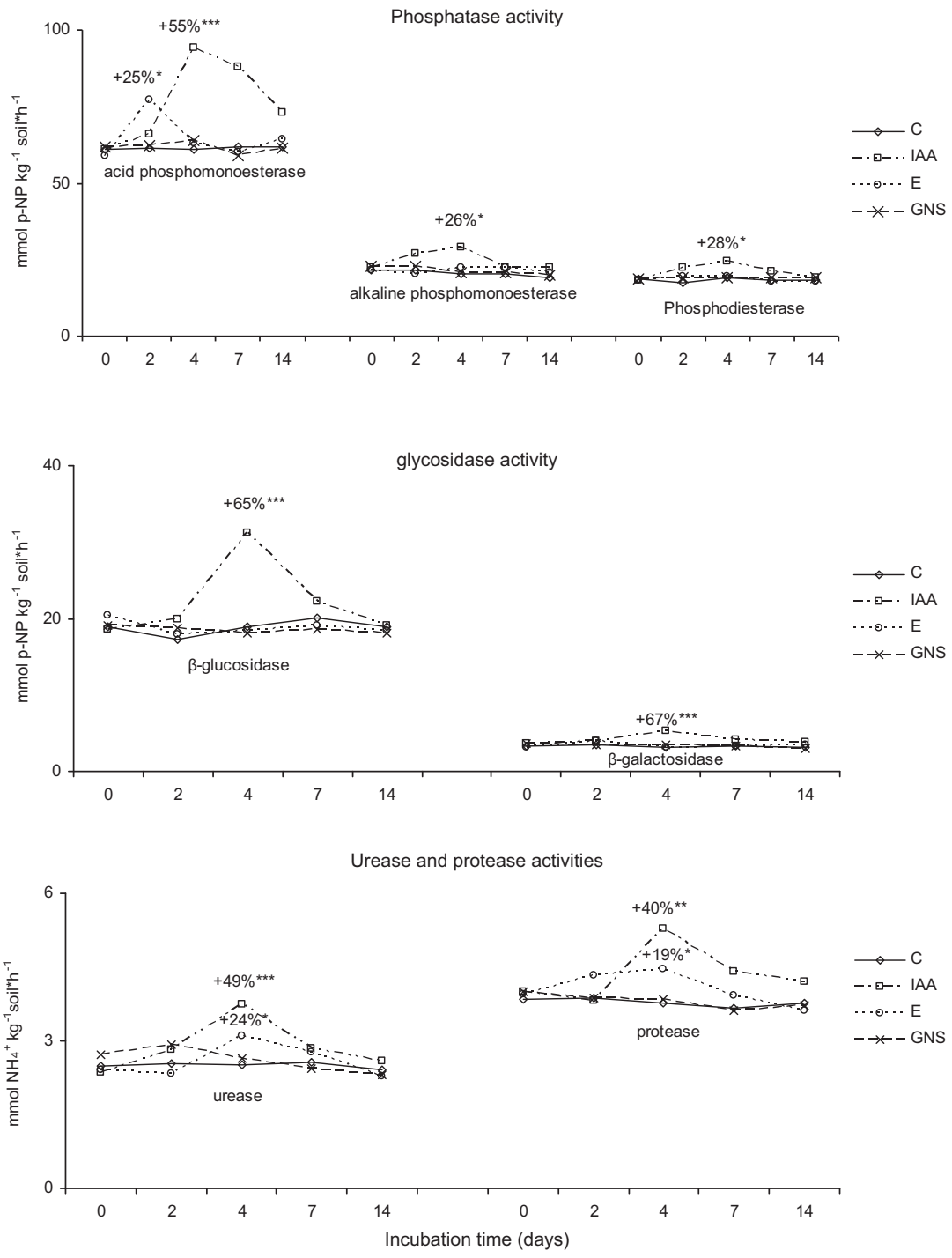

Fig. 2. Hydrolase activity in the artificial rhizosphere soil layer (0–2 mm) of the Romola soil treated with H_2O (C), glucose, N and S (GNS), IAA precursor or E precursor. Symbols *, ** and *** indicate significant differences between control and IAA, E and GNS treatments.

Microbial indole and its derivatives can act as extracellular signals for gene activation (Wang et al., 2001); indeed, IAA production is involved in the colonization strategy of rhizosphere bacteria, which is based on the exchange of signal molecules among microorganisms to coordinate the species behavior in complex microbial communities (Lee and Lee, 2009). Because IAA precursors have been also detected in organic wastes (Arkhipchenko et al., 2006), the increase in microbial activity and hydrolase activities frequently observed after soil amendment may depend not only on the microbial utilization of the added phytohormone precursors, but also by their stimulative effects on microbial enzyme activities.

In conclusion, the data presented demonstrate that phytohormone precursors can stimulate soil microbial activity, and suggest that the availability of IAA and E precursors in the rhizosphere may be responsible for the greater microbial and hydrolase activity of the rhizosphere than the bulk soil. The fact that glucose-C + N and S added at the same rate as in the phytohormone precursors did not have the same effects, suggests that the observed effects were not due to the microbial use of such precursors as nutrient sources.

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