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CHANGES IN INORGANIC N AND CO<sub>2</sub> EVOLUTION IN  
SOIL INDUCED BY L-METHIONINE-SULPHOXIMINEL. LANDI,<sup>1</sup>\* L. BADALUCCO<sup>2</sup> and P. NANNIPIERI<sup>1</sup><sup>1</sup>Dipartimento di Scienza del Suolo e Nutrizione della Pianta, P. le delle Cascine 15, 50144 Firenze, Italy and <sup>2</sup>Dipartimento di Agrobiologia ed Agrochimica, Via S. C. De Lellis, 01100 Viterbo, Italy

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**Summary**—Short-term (up to 48 h) incubation assays were conducted in the presence of L-methionine-DL-sulphoximine (MSX), an inhibitor of glutamine synthetase, to assess its effect on exchangeable NH<sub>4</sub><sup>+</sup>-N, NO<sub>3</sub><sup>-</sup>-N production, and CO<sub>2</sub> evolution. A sandy-clay-loam (Pistoia) and a sandy (Romola) soil were moistened and either amended with glucose (200 μmol glucose-C g<sup>-1</sup> soil), glucose + (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> [50 μmol N g<sup>-1</sup> soil as (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>] or left unamended. In the two unamended soils, NH<sub>4</sub><sup>+</sup>-N concentration was increased by the highest MSX level (1 μmol g<sup>-1</sup> soil), while the lowest inhibitor concentration (0.5 μmol g<sup>-1</sup> soil) had less influence. NH<sub>4</sub><sup>+</sup>-N concentrations were higher with 1 μmol MSX than without the inhibitor in the glucose-only-treated Pistoia soil in the 0–12 h period; thereafter the opposite situation was observed. Probably the CO<sub>2</sub> evolution increased as a result of inhibitor mineralization after 12 h. In the glucose-treated Romola soil both MSX concentrations were generally effective in increasing NH<sub>4</sub><sup>+</sup>-N concentrations with respect to the same amendment without the inhibitor. These increases were probably due to glutamine synthetase inhibition by MSX and not to the presence of mineralization of the inhibitor because CO<sub>2</sub> evolution was only slightly increased at 48 h by MSX. Probably, as a result of this inhibition, NO<sub>3</sub><sup>-</sup>-N was used as an alternative N source in the glucose-amended Romola soil. The inhibitor had no significant effect on NH<sub>4</sub><sup>+</sup>-N concentration when both soils were amended with glucose + (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> probably because, in the presence of high NH<sub>4</sub><sup>+</sup>-N concentrations, NH<sub>3</sub> assimilation occurred more through glutamate dehydrogenase than through glutamine synthetase–glutamate synthase enzymes. NO<sub>3</sub><sup>-</sup>-N concentrations were decreased by MSX in the glucose-amended Romola but not in the glucose-amended Pistoia soil.

## INTRODUCTION

Ammonium (NH<sub>4</sub><sup>+</sup>), the end product of N mineralization process, is often re-used and incorporated into organic molecules by soil microorganisms. Thus, N mineralization and immobilization occur simultaneously and for this reason it is very difficult to determine the gross rates of the two processes (Jansson, 1958; Nannipieri *et al.*, 1983; Stevenson, 1986). NH<sub>4</sub><sup>+</sup> reacts with glutamate to give glutamine through the reaction catalysed by the glutamine synthetase (GS); then glutamine reacts with α-ketoglutarate producing glutamate through glutamate synthase, known as glutamine oxoglutarate amino transferase (GOGAT). However, when the NH<sub>4</sub><sup>+</sup> concentration is high (>1 mM), the NH<sub>4</sub><sup>+</sup> is incorporated as glutamate via glutamate dehydrogenase (GDH). In some microorganisms, NH<sub>4</sub><sup>+</sup> is also incorporated by the reaction producing asparagine from aspartate and catalysed by asparagine synthetase (Reitzer and Magasanik, 1987). Moreno-Vivian *et al.* (1983) showed that when GS is inactive, reductive amination of pyruvate is the obligatory way for NH<sub>4</sub><sup>+</sup> incorporation in phototrophic bacteria. The K<sub>m</sub> for GS is less than those of both GDH and asparagine

synthetase, indicating a relatively higher affinity of GS for NH<sub>4</sub><sup>+</sup>. In fact, when NH<sub>4</sub><sup>+</sup>-N concentrations are <0.1 mM, NH<sub>4</sub><sup>+</sup>-N is only incorporated through the reaction catalysed by GS (Magasanik and Neidhardt, 1987). Since NH<sub>4</sub><sup>+</sup> concentrations in the soil solution are usually very low, microbial N immobilization in soil probably occurs through the GS/GOGAT pathway.

L-Methionine-DL-sulphoximine (MSX) is an effective GS inhibitor and is commonly used in pure culture experiments to study the pathways of NH<sub>4</sub><sup>+</sup>-N assimilation and to measure nitrate reductase activity (Rowe and Meister, 1970; Rigano *et al.*, 1982; Arp and Zumft, 1983). It is more efficient than the corresponding D-isomer and other putative GS inhibitors (Brenchley, 1973). However, MSX could also be utilized as a N and C source by some microorganisms, as found for some antibiotics (e.g. streptomycin and cycloheximide) applied to soil (Badalucco *et al.*, 1994). The effect of MSX on N metabolism can therefore be rather complex; for example, the addition of MSX to a NO<sub>3</sub><sup>-</sup>-assimilating phototrophic bacteria prevented both growth and NO<sub>3</sub><sup>-</sup> uptake with simultaneous excretion of NH<sub>3</sub> to the medium (Moreno-Vivian *et al.*, 1983). The GS activity was inactivated by MSX while NO<sub>3</sub><sup>-</sup> reductase was unaffected.

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In soil this inhibitor has been used to study the effect of various N compounds on the microbial synthesis of urease (McCarty *et al.*, 1992), the regulation of assimilatory  $\text{NO}_3^-$  reductase activity (McCarty and Bremner, 1992a,b) and the microbial uptake of  $\text{NH}_4^+$  (Schimel and Firestone, 1989a). MSX inhibited  $\text{NH}_4^+$  incorporation by 24 and 13% in submerged  $\text{O}_2$  and  $\text{O}_3$  subhorizons of a forest soil, respectively (Schimel and Firestone, 1989a). Soil slurries permit less variability than aerated soils, but waterlogged conditions are markedly different with respect to those occurring *in situ*. Particularly, the submersion of soil in water severely limits  $\text{O}_2$  diffusion to the microflora with the consequent shift from aerobic to anaerobic metabolism.

Our work was to follow changes in exchangeable  $\text{NH}_4^+$ -N,  $\text{NO}_3^-$ -N production and  $\text{CO}_2$  evolution in soils treated with MSX to test the possibility of developing a method for determining gross rates of N mineralization by blocking the N immobilization process. Soils were also treated with glucose so as to favour N immobilization.

#### MATERIALS AND METHODS

A sandy-clay-loam (Pistoia) and a sandy (Romola) textured soil were sampled (0–20 cm), air-dried, sieved (2 mm) and stored (Table 1). Particle size was determined by using the pipette method (Day, 1965); pH by a glass electrode with a 1:2 soil-to-water ratio; organic C by the Walkley–Black procedure (Nelson and Sommers, 1982) and total N by Kjeldahl digestion (Bremner and Mulvaney, 1982).

In a short-term incubation experiment, each soil was treated with distilled water (unamended), dissolved glucose (glucose-amended) (200  $\mu\text{mol}$  glucose-C  $\text{g}^{-1}$  d.w. soil) or glucose +  $(\text{NH}_4)_2\text{SO}_4$  [50  $\mu\text{mol}$  N  $\text{g}^{-1}$  d.w. soil as  $(\text{NH}_4)_2\text{SO}_4$ ] in the presence of 0, 0.5 or 1.0  $\mu\text{mol}$  MSX  $\text{g}^{-1}$  d.w. soil. In each treatment after all the additions and prior to incubation, the soil moisture content corresponded to 50% of the water holding capacity (WHC). Each treatment was replicated 3 times. MSX was purchased from Sigma Chemical Co. Glass beakers with soil samples (20 g oven d.w.), were placed in airtight jars (1l) containing a vial with 4 ml 1 N NaOH to adsorb  $\text{CO}_2$ , and a vial with 5 ml distilled water to maintain a moist atmosphere. Soils were kept at 25°C for 48 h. After 6, 12, 24, 36 and 48 h of incubation, the evolved  $\text{CO}_2$  was determined and 10 g of soil were transferred to 100 ml centrifuge tubes and extracted with 40 ml 2 M KCl solution. Tubes were shaken for 1 h, centrifugated for 10 min at 4000g, filtered through Whatman No. 40 paper and the extracts

stored at 4°C. The  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N contents were determined by steam distillation of the soil extracts (30 ml) with MgO and Devarda's alloy (Keeney and Nelson, 1982).

$\text{CO}_2$  evolved during the incubation was determined using a Radiometer autotitrator after precipitating the carbonate with 8 ml of 0.75 N  $\text{BaCl}_2$ ; the excess of base was titrated with 0.1 M HCl; the end-titration point was pH 8.8. A control without soil was used for each incubation time to correct for atmospheric  $\text{CO}_2$  and to standardize the NaOH (Tinsley *et al.*, 1951).

#### RESULTS

##### $\text{NH}_4^+$ -N concentration

Without glucose, the  $\text{NH}_4^+$ -N concentration was immediately greater in the presence of 1  $\mu\text{mol}$  MSX in Pistoia soil and after 12 h in Romola soil (Figure 1). The effect of the lowest MSX concentration in increasing  $\text{NH}_4^+$ -N concentration was not always significant.

The addition of glucose only caused a significant decrease in  $\text{NH}_4^+$ -N concentration in both soils (Figure 1); this decrease was more marked in Romola than in Pistoia soil. In the glucose-treated Pistoia soil the addition of the inhibitor caused a greater decrease further with the exception of the 0–12 h incubation period at the highest MSX concentration (Figure 1). However, the 0.5  $\mu\text{mol}$  treatment only slightly affected the  $\text{NH}_4^+$ -N concentration with respect to control values. By contrast, in the glucose-treated Romola soil  $\text{NH}_4^+$ -N concentration was generally higher with than without MSX. The greatest difference in  $\text{NH}_4^+$ -N concentration was observed at 12 h, then the difference decreased and at the end of the incubation all the three treatments were the same (Figure 1).

Higher concentrations of  $\text{NH}_4^+$ -N were generally observed with than without the inhibitor in the glucose +  $(\text{NH}_4)_2\text{SO}_4$ -treated Pistoia soil (Figure 1). The differences in  $\text{NH}_4^+$ -N concentration between the two MSX concentrations were only significant at 48 h. The addition of both inhibitor concentrations to the glucose +  $(\text{NH}_4)_2\text{SO}_4$ -treated Romola soil significantly increased the  $\text{NH}_4^+$ -N concentration in comparison to the respective control values only after 24 h.

##### $\text{NO}_3^-$ -N concentration

The content of  $\text{NO}_3^-$ -N in the unamended Pistoia soil without the inhibitor decreased rapidly during the first 12 h, increased until 24 h and then remained almost constant (Figure 2). Greater  $\text{NO}_3^-$ -N concentrations were observed in the Pistoia soil amended with

Table 1. Properties of soils

Soil	Sand (%)	Silt (%)	Clay (%)	Organic-C (%)	Total-N (%)	pH		CEC (mequiv 100 $\text{g}^{-1}$ )
						$\text{H}_2\text{O}$	KCl	
Pistoia	63.2	17.6	19.2	3.64	0.26	6.7	6.1	23.5
Romola	90.05	3.5	5.6	0.86	0.08	7.2	5.9	ND

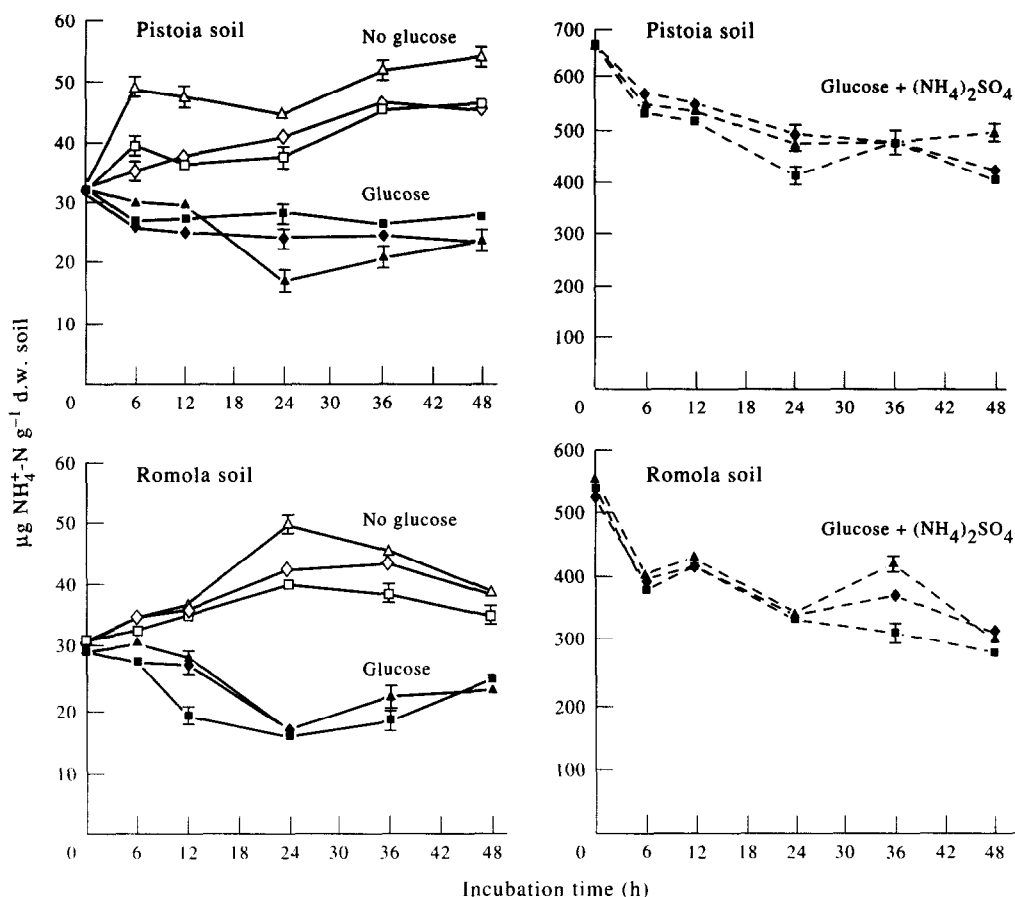


Fig. 1. Changes in  $\text{NH}_4^+$ -N during the incubation:  $\square$ ,  $\blacksquare$  no MSX;  $\diamond$ ,  $\blacklozenge$  +0.5  $\mu\text{mol}$  MSX;  $\triangle$ ,  $\blacktriangle$  +1  $\mu\text{mol}$  MSX. Bars represent SDs; SDs not reported are smaller than symbol size.

both MSX concentrations with respect to the control from 6 to 12 h, no significant differences were observed between the two MSX treatments. After 24 h the two inhibitor treatments presented different patterns; the greatest MSX concentration showed greater  $\text{NO}_3^-$ -N values with respect to the 0.5  $\mu\text{mol}$  MSX treatment and to the respective control. By contrast, in the unamended Romola soil the inhibitor decreased the  $\text{NO}_3^-$ -N content with respect to the control and the major decline was observed with the highest MSX concentration after 24 h (Figure 2). However, due to the initial lower  $\text{NO}_3^-$ -N concentration,  $\text{NO}_3^-$ -N fluctuations were much smaller in the unamended and glucose-amended Romola soil than in the respective Pistoia treatments.

The  $\text{NO}_3^-$ -N content of the glucose-treated Pistoia soil markedly decreased during the incubation. By 24 h it had fallen to about 7% of its initial value, then it remained constant until the end of experiment (Figure 2). The only significant inhibitor effect on the  $\text{NO}_3^-$ -N concentration was observed at 6 h with the highest MSX concentration (Figure 2). In Romola soil, the glucose addition also decreased  $\text{NO}_3^-$ -N concentration; the presence of both MSX concentrations lowered  $\text{NO}_3^-$ -N concentrations with respect to control values after 12 h.

$\text{NO}_3^-$ -N concentrations also decreased in the glucose +  $(\text{NH}_4)_2\text{SO}_4$ -treated Pistoia soil during the incubation (Figure 2). The addition of 1  $\mu\text{mol}$  MSX gave the highest  $\text{NO}_3^-$ -N concentrations during the 0–12 h. Then a marked decrease was observed and values were lower than those without the inhibitor. The 0.5  $\mu\text{mol}$  MSX in glucose +  $(\text{NH}_4)_2\text{SO}_4$ -treated Pistoia soil showed lower  $\text{NO}_3^-$ -N contents than the respective control but significant differences were only observed after 6 h. In the glucose +  $(\text{NH}_4)_2\text{SO}_4$ -treated Romola soil, the  $\text{NO}_3^-$ -N concentration decreased at 6 h and then increased up to 12 h (Figure 2). After 12 h, the  $\text{NO}_3^-$ -N concentration fluctuated but almost reached the initial value at the end of the incubation. The addition of the inhibitor decreased the  $\text{NO}_3^-$ -N concentration with respect to control values and the most marked effect was generally observed with the highest MSX concentration.

#### *CO<sub>2</sub> evolution*

The  $\text{CO}_2$  evolution plots of both unamended soils are shown together with those of glucose-amended soils so as to maintain the same presentation of  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N concentration patterns. Due to the scale used, differences in  $\text{CO}_2$  evolution among unamended soils are often not clear (Figure 3). The

CO<sub>2</sub> evolution of the unamended Romola soil was not affected by both of MSX concentrations, with the exception of respiration values at 12 h (both an 18% increase) and 24 h (22 and 37% increase for the lowest and the highest MSX concentrations, respectively). On the contrary, the inhibitor affected respiration immediately in the unamended Pistoia soil. However, the highest MSX concentration showed the most marked effect throughout (41, 100, 77, 74 and 53% increases over the control at 6, 12, 24, 36 and 48 h, respectively).

The addition of 0.5  $\mu\text{mol}$  MSX to the glucose or glucose + (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>-treated Pistoia soil did not alter CO<sub>2</sub> evolution in comparison with the respective control up to 24 h (Figure 3). Thereafter this MSX concentration inhibited CO<sub>2</sub> evolution with the exception of the glucose + (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> treatment at 48 h. The presence of 1  $\mu\text{mol}$  MSX rapidly and markedly stimulated CO<sub>2</sub> evolution after 6 h in both glucose and glucose + (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>-amended Pistoia soil (about 34 and 45% increase over the control, respectively). By contrast, in the glucose-treated Romola soil, the inhibitor did not influence the CO<sub>2</sub> evolution rate with the exception of 1  $\mu\text{mol}$  MSX at

12 and 48 h and of 0.5  $\mu\text{mol}$  MSX at 12 h (Figure 3). Both concentrations of MSX depressed the respiration in the glucose + (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> amended Romola soil after 12 h (Figure 3). No significant differences were observed between the two MSX treatments except at the end of incubation.

## DISCUSSION

Although organic C and total N contents were greater in the sandy-clay-loam Pistoia than in the sandy Romola soil (Table 1), higher mineralization rates of soil organic matter, as determined by CO<sub>2</sub> evolution, were generally found in the sandy than in the sandy-clay-loam soil when both soils were incubated without glucose from 0 to 48 h. The lower mineralization rate in sandy-clay-loam soil may be due to the higher physical protection (e.g. by clays) of organic material or the lower protozoan predator activity in Pistoia than in Romola soil (Heynen *et al.*, 1988). In fact, the degradation rate of organic matter in soil is strongly influenced by the physico-chemical properties of soil (Catroux *et al.*, 1987; Hassink, 1992; Kretschmar and Ladd, 1993). With glucose the

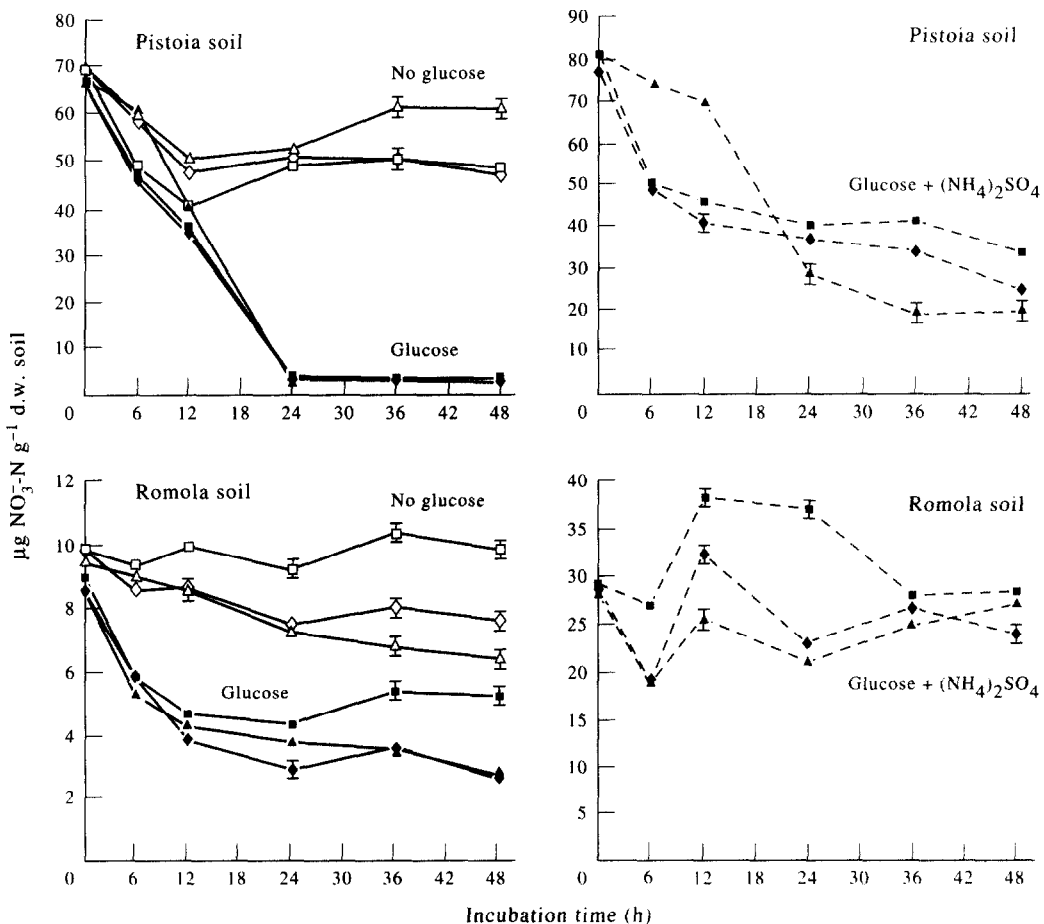


Fig. 2. Changes in NO<sub>3</sub><sup>-</sup>-N during the incubation: □, ■ no MSX; ◇, ◆ +0.5  $\mu\text{mol}$  MSX; △, ▲ +1  $\mu\text{mol}$  MSX. Bars represent SDs; SDs not reported are smaller than symbol size.

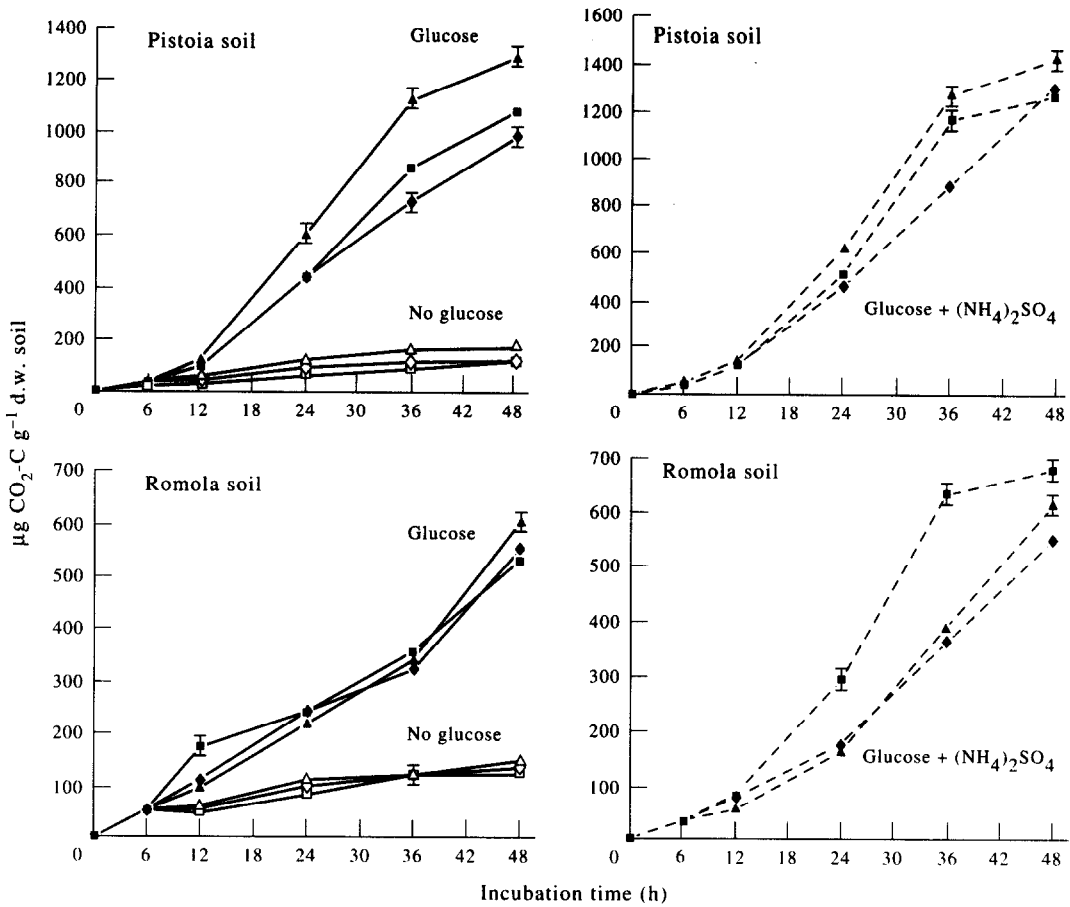


Fig. 3. Changes in cumulative CO<sub>2</sub> evolved during the incubation: □, ■ no MSX; ◇, ◆ +0.5 µmol MSX; △, ▲ +1 µmol MSX. Bars represent SDs; SDs not reported are smaller than symbol size.

cumulative CO<sub>2</sub> evolved at 48 h was greater from the Pistoia than from the Romola soil. This behaviour probably did not depend on the presence of more available N in the Pistoia than in the Romola soil. Despite the fact that the addition of a N source as (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> with glucose increased the cumulative CO<sub>2</sub> evolved from Romola at 48 h with respect to the glucose-amended soil, the total CO<sub>2</sub> evolved was still lower than that of the glucose + (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>-amended Pistoia soil. The difference in CO<sub>2</sub> evolution between the two glucose-treated soils may depend on the different size of the microbial biomass or the different composition of soil microflora rather than on the amount of available N. Opportunistic microorganisms (zymogenous according to Winogradsky) exhibit high rates of activity and rapid growth on easily utilizable substrates (Atlas and Bartha, 1981). The number of these microorganisms might be greater in the Pistoia than in the Romola soil.

Increases in the NH<sub>4</sub><sup>+</sup>-N concentration in the presence of the inhibitor can be due to an effective inhibition of N immobilization or MSX-N mineralization. Inhibition of NH<sub>3</sub> incorporation by microorganisms can also stimulate intracellular N mineralization. Indeed, effective inhibition of NH<sub>4</sub><sup>+</sup>-N

incorporation in amino acids was found to increase the N mineralization of cytoplasmic protein-N (Reitzer and Magasanik, 1987). Both net N mineralization and CO<sub>2</sub> evolution were increased from an organic sandy soil amended with MSX, or L-methionine or methionine sulphoxide another methionine analogue (Hopkins *et al.*, 1995). These increases were attributed to the mineralization of the added compounds by soil microflora. Thus, the increases in NH<sub>4</sub><sup>+</sup>-N concentration and CO<sub>2</sub> evolution may both be indicative of inhibitor mineralization and not GS inhibition in soil. This behaviour was evident in the unamended Pistoia soil with the highest MSX concentration but not in the unamended Romola soil, where both MSX concentrations positively affected the NH<sub>4</sub><sup>+</sup>-N concentration and CO<sub>2</sub> evolution only at 12 and 24 h (Figure 3). The increase in the NH<sub>4</sub><sup>+</sup>-N concentration in the unamended Romola soil might be due to the release of only NH<sub>4</sub><sup>+</sup>-N by the deamination of the inhibitor. When glucose was applied to the Romola soil, NH<sub>4</sub><sup>+</sup>-N concentrations were higher with both MSX concentrations than without the inhibitor in the 0–24 h period (Figure 1). No differences in CO<sub>2</sub> evolution were observed in the glucose treatment except a decrease at 12 h for both MSX concentrations

and an increase at 48 h for the highest MSX concentration. The observed patterns in  $\text{NH}_4^+$ -N concentrations and  $\text{CO}_2$  evolution seem to indicate that microbial  $\text{NH}_4^+$  incorporation had been inhibited by MSX in the glucose-treated Romola soil before 24 h. The deamination of MSX was probably not responsible for the differences in  $\text{NH}_4^+$ -N concentrations observed in the glucose-amended Romola soil between the MSX treatments and the respective control because any available inorganic N source would have been immediately immobilized by microorganisms during the rapid oxidation of glucose. The addition of 1  $\mu\text{mol}$  MSX to the glucose-treated Pistoia soil gave slightly higher  $\text{NH}_4^+$ -N concentrations in comparison to the respective control in the 0–12 h period. Probably, the highest MSX concentration slightly inhibited GS enzyme during this period (Figure 1); then, the inhibitor was used as an N and C source by soil microorganisms as it seems to be proved by the higher  $\text{CO}_2$  evolution over the control after 12 h. The mineralization of the inhibitor was not reflected in higher  $\text{NH}_4^+$ -N concentrations probably because  $\text{NH}_4^+$  was incorporated by microorganisms in the presence of glucose, that is under conditions favouring N immobilization. Probably, microbial  $\text{NH}_4^+$  was incorporated in the glucose-amended Pistoia soil with 0.5  $\mu\text{mol}$  MSX; however, it is difficult to explain the inhibition of  $\text{CO}_2$  evolution observed after 24 h in this treatment.

The addition of MSX to both soils treated with glucose +  $(\text{NH}_4)_2\text{SO}_4$  did not influence significantly  $\text{NH}_4^+$ -N concentrations. Under non-limiting  $\text{NH}_4^+$ -N concentrations,  $\text{NH}_4^+$  is probably incorporated through reactions catalysed not only by both GS/GOGAT enzymes but also by the GDH enzyme, which is unaffected by the MSX (Magasanik and Neidhardt, 1987).

The concentration of  $\text{NO}_3^-$  in soil depends on various processes:  $\text{NO}_3^-$  is formed by nitrification while it is consumed by denitrification and microbial and plant uptake (Stevenson, 1986). Higher  $\text{NO}_3^-$ -N concentrations with respect to the control were observed with the highest MSX concentration in unamended Pistoia soil (Figure 2). The addition of 1  $\mu\text{mol}$  MSX to glucose +  $(\text{NH}_4)_2\text{SO}_4$ -amended Pistoia soils increased the  $\text{NO}_3^-$ -N concentration only in the 0–12 h period (Figure 2). This increase might be due to the inhibition of  $\text{NO}_3^-$  uptake by microorganisms as it has been observed for  $\text{NO}_3^-$ -assimilating phototrophic bacteria treated with MSX (Moreno-Vivian *et al.*, 1983) and not as the result of higher nitrification rates due to the slightly higher  $\text{NH}_4^+$ -N concentrations with respect to control values (Figure 1). If the increased nitrification rate had been caused by the slight increase in  $\text{NH}_4^+$ -N concentration, higher  $\text{NO}_3^-$ -N contents would have been also observed in the glucose +  $(\text{NH}_4)_2\text{SO}_4$ -amended Pistoia soil with 0.5  $\mu\text{mol}$  MSX. In fact, this treatment also showed slightly higher  $\text{NH}_4^+$ -N concentrations than the respective

control (Figure 1). Probably, MSX at the lowest concentration was ineffective in inhibiting  $\text{NO}_3^-$  uptake by microorganisms because at this level most of the inhibitor was adsorbed by soil particles. Both MSX applications decreased  $\text{NO}_3^-$ -N concentrations in the unamended and glucose-amended Romola soil in comparison with the respective control, while this did not occur in the respective Pistoia treatments.  $\text{NO}_3^-$  might have been used as an alternative N source in the unamended and glucose-amended Romola soil because GS was inhibited by the MSX, as has been hypothesized by Magasanik and Neidhardt (1987). However, as discussed before, GS was probably inhibited in the glucose-amended but not in the unamended Romola soil. In addition, with the present data we cannot exclude that differences in the composition as well in the physiological state of the nitrifiers may be the cause of the different  $\text{NO}_3^-$  patterns between the two soils. No published data are available on the effect of MSX on the nitrifying activity of soil.

The addition of glucose in Pistoia soil caused a marked decrease of  $\text{NO}_3^-$ -N as a result of N immobilization (Nannipieri *et al.*, 1983) or denitrification (Lalisse-Grundmann *et al.*, 1988). If  $\text{NO}_3^-$  immobilization is the process responsible for the decrease of  $\text{NO}_3^-$ -N it remains to provide as explanation of why  $\text{NH}_4^+$ -N was not completely utilized by microorganisms. It has been demonstrated that  $\text{NO}_3^-$  is utilized by microorganisms when the  $\text{NH}_3$  concentration is lower than 0.1  $\mu\text{g N g}^{-1}$  soil (Rice and Tiedje, 1989). Probably, in the glucose-amended Pistoia soil anaerobic conditions prevailed and  $\text{NO}_3^-$  was used as an electron acceptor, while in the glucose-amended Romola soil  $\text{NO}_3^-$  was used partly as an alternative N source in the presence of MSX.  $\text{O}_2$  shortage due to intense microbial activity promoted by glucose may be more severe in fine than in coarse textured soil (Atlas and Bartha, 1981).

In conclusion, MSX was probably effective in inhibiting GS enzymes in glucose-amended Romola soil up to 24 h. It might have been also effective in the glucose-amended Pistoia soil at the highest concentration only in the 0–12 h period. Probably, in the unamended and glucose-amended Pistoia soil (after 12 h for the highest MSX concentration) MSX was mineralized to  $\text{CO}_2$  and  $\text{NH}_4^+$ -N, while in the unamended Romola soil it was deaminated to  $\text{NH}_4^+$ -N. However, more studies are needed to ascertain the complex effect of this inhibitor on N metabolism and to monitor the fate of MSX in soil. By using the  $^{15}\text{NH}_4$  dilution technique (Nishio *et al.*, 1989; Barraclough, 1991; Geens *et al.*, 1991) it may be possible to determine the gross rates of the N mineralization and immobilization processes and how these processes are affected by MSX. In addition, more direct evidence of the MSX effect on the GS activity of soil is required through an enzyme assay to determine the GS activity in soil.

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