Scientific registration n° : 406
Symposium n° : 41
Presentation : poster

Sites of microbial decomposition of soil organic matter. Impact of substrate-soil matrix relationships. Sites microbiens de décomposition de la matière organique. Importance des relations sol-substrat.

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Soils are composed of complex assemblages of solid particles and voids thus presenting variable habitats for the soil biota. As part of research programmes at CSIRO and ORSTOM dealing with the influence of soil structure and substrate concentration on the activities and distribution within soil matrix of decomposer organisms, we have determined the sites of microbial assimilation and turnover of 14C-labelled soluble and particulate substrates incorporated in a highly aggregated Vertisol.
Soil subsamples were conditioned in the presence of 14C-labelled soluble glucose and unground but cut segments (<500 µm) of legume leaves for up to 66d. At 3, 38 and 66d, soils were partially dispersed by the Brückert (1979) procedure, which disrupted macroaggregates (>250 µm), but which allowed recoveries of undecomposed plant residues and undisrupted microaggregates (<50 µm).
After 3 d, total biomass C in the glucose-amended soil was the same as that in the unamented soil, 58% of total biomass C of the amended soil being derived from the glucose. By contrast, total biomass C was highest in soil amended with legume residues, of which only 27% was derived from the legume leaves. In the glucose-amended soil, 67% of biomass 14C was associated with the microaggregates 2-50 µm, whereas in the legume treatment biomass 14C was bimodally distributed in the light fraction >250 µm, comprised of organic residues, and in the microaggregates 2-50 µm. During the 3-66 days period, biomass 14C decreased in both treatments, the extent of the decrease varying according to the particle size fractions. The entire biomass 14C sheltered in the light fraction <250 µm at 3d in the legume-amended soil disappeared. By contrast, microaggregates 2-50 µm are more efficient at protecting microorganisms, since respectively 46% and 73% of the biomass present at 3d were still sheltered in this fraction in the glucose- and legume-amended soils at 66d.
Particulate organic residues and microaggregates 2-50 µm arethe two main sites of soil organic matter decomposition, the relative responses of their biomass varying according to the nature of the substrate. Soluble substrate stimulated the growth of microorganisms located in microaggregates, whereas particulate plant residues presented new sites for colonization. Increasing incubation time revealed the greater capacity of microaggregates, compared with plant residues, to protect their associated biota.

Key words: Microbial sites, Soluble substrate, Particulate substrate, Soil matrix.
Mots clés: Habitats microbiens, substrat soluble, substrat particulaire, matrice minérale.

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INTRODUCTION

The effects of soil structure on the distribution and activities of soil biota are well documented (Elliott, 1986; Gupta and Germida, 1988; Hattori, 1988; Kabir et al., 1994; Killham et al., 1993). Amato and Ladd (1992) obtained a positive correlation between glucose-derived or plant residues-derived biomass C and the clay content of 23 Australian soils (pH > 7.0). Amato and Ladd (1992) and Ladd et al. (1992) indicated that soil microporosity per se did not explain differences in biomass ^{14}C turnover between soils. Biomass ^{14}C stability was greater in a Vertisol than in an Alfisol, although the volume of pores of neck dia > 6 µm, and in which most of the biomass ^{14}C was expected to reside, was similar in both soils. They suggested that volume of water-filled pore space could be of higher importance in biological activities (Linn and Doran, 1984). However, very little of the extensive literature on soil structure and soil biota relationships deal with soil biomass turnover associated with soil fractions.
This work is part of a broader study aimed at investigating the sites of microbial assimilation and turnover of ^{14}C-labelled soluble and particulate plant residues incorporated in a highly aggregated Vertisol (Chotte et al., in press). It focusses on the impact of substrate-soil matrix relationships on the location and the turn-over of substrate derived biomass ^{14}C and soil organic matter derived biomass ^{12}C. We hypothesized that clay surfaces would affect more extensively the behaviour of soluble substrate C than of particulate plant residue C and that this would modify the sites of microbial trun-over.

MATERIALS AND METHODS

Soil, substrates and incubation conditions

Soil used was sampled from the 0-10 cm layer of a Vertisol (Black Earth). Pending further use, the soil was stored at 4°C at its field moisture content, which was equivalent to 20% of its water holding capacity (WHC). It was thoroughly mixed and sieved (8 mm mesh) and its water content adjusted to 40% WHC, and pre incubated for 2 weeks at 28°C. Subsamples of soil (equivalent to 50 g oven dried-soil) were incubated in triplicate

with substrates, added to a final concentration of 1 mg C g^{-1} soil. Labelled-glucose (2.769 MBq g^{-1} C) was added as solution to the soil, whereas legume leaves (2.769 MBq g^{-1} C) were added as oven-dried (60°C, overnight) segments unground but cut (< 500 µm). An unamended soil was included as a control. Vessels containing the amended-soils were placed in 1L glass jar sealed with a screw cap. Carbon dioxide mineralized during the incubation was trapped by a NaOH solution (0.1 N) (results not shown). The incubation lasted for up to 66 d at 25°C.

Soil fractionation

Soils incubated for 3d and 66d were partially dispersed by the Bruckert (1979) procedure. A soil subsample (equivalent to 10 g oven dried soil), was taken from each of the triplicate incubation vessels. The subsamples were pooled and rolled for 16 h in water (200 ml of cold, <10°C, distilled water) with 5 agate marbles. Then, the subsamples were sieved to collecte the coarse (> 250 µm) and fine sand-size fractions (50-250 µm). Light fractions (Lf) were separated from the these fractions by flotation in water. The heavy sand-size fractions (>250 µm and 50-250 µm) were mixed to give a single sand-size fraction (Hf > 50 µm). The suspension < 50 mm was then centrifuged (90 g for 6 min) to separate the fractions 2-50 µm and <2 µm.

Biomass ^{12}C and biomass ^{14}C

Soil organic matter (Som)-derived biomass ^{12}C and substrate-derived biomass ^{14}C were determined in triplicate in moist unfractionated soils sampled after 3,and 66 d of incubation, and in duplicate in fractions prepared from them. Free water was removed from slurries of fractions by centrifugation, prior to biomass assay. Biomass C and ^{14}C of moist unfractionated soil and moist particle fractions were estimated by fumigation-extraction methods (Amato and Ladd, 1988; Ladd and Amato, 1988). Total biomass C of unfractionated soil and soil fractions was estimated from the gain in ninhydrin-reactive N after fumigation, multiplied by 21 (Amato and Ladd, 1988). Biomass ^{14}C of unfractionated soil and soil fractions was determined from the gain of extractable ^{14}C after fumigation, multiplied by 3.05 for unfractionated soil and 2.65 for soil fractions to take into account the effect of the soil moisture content on assay performance (Chotte et al., in press).

RESULTS

Weight distribution of the particle size fractions

Weight recoveries after soil fractionation approximated to 100%. Any difference between treatments and sampling dates have been recorded. The silt-size fraction (Hf 2-50 µm) was the predominant fraction, representing 50.8 % of total soil weight. The sand-size fraction (Hf > 50 µm) accounted for 21% of the soil weight, being equivalent to the amount of the mineral particles in the sand size range (> 50 µm) determined by conventional particle size analysis after the destruction of organic matter and the entire dispersion of the soil. Clay mineral particles recovered by this method amounted to 50% of the soil weight, thus being more adundant than clay-size fraction dispersed by the

Bruckert method (17.8% of the total soil weight). Thus, 65% of clay mineral particles remained as clay-aggregates in the fraction of silt-size range.

Biomass C (Total, Substrate-derived and Som-derived) of unfractionated soil

After 3d, total biomass C in the glucose-amended soil was similar to that in the unamended soil (1020 µg C g^{-1} soil and 1050 µg C g^{-1} soil, respectively) (Figure 1). Unlike for the soluble amendment, legume amendment yielded highest total biomass C (1300 µg C g^{-1} soil). At that time, substrate-derived biomass C in the glucose- and legume-amended soils amounted respectively to 550 and 350 µg C g^{-1} soil, thus representing 54% and 27% of total biomass C. Som-derived biomass C, calculated by the difference between total and substrate-derived biomass C, was higher in the legume-amended soil (950 µg C g^{-1} soil) than in the glucose- amended soil (470 µg C g^{-1} soil). For the legume-amended soil this amount equalled to that of the unamended soil. By contrast, Som-derived biomass C in the glucose-amended soil was lower than in the unamended-soil.
By the end of the incubation, total biomass C was of similar concentration in amended and unamended soils (about 940 µg C g^{-1} soil). Substrate-derived biomass C represented lower proportions of total biomass C than those at 3d (respectively 27% and 14% in the soil amended with glucose and legume leaves).

Substrate-derived biomass ^{14}C in particle size fractions

The distribution of substrate-derived biomass ^{14}C in soil fractions differed according to treatments (Figure 2).

Particle size fraction Lf > 250 µm. After 3 d, biomass ^{14}C associated with the Lf > 250 µm amounted only to 30 µg ^{14}C g^{-1} soil in the glucose-amended soil, these quantity being far less than that in the soil amended with legume leaves (150 µg ^{14}C g^{-1} soil). Substrate-derived biomass located in this fraction represented about 5% and 40% of the biomass ^{14}C recovered from the unfractionated soil amended with glucose and legume, respectively. On further incubation, biomass ^{14}C disappeared entirely in this fraction from both treatments.

Particle size fraction Lf >50 µm. Substrate-derived biomass ^{14}C in the fraction Lf 50-250 µm accounted for 70 µg C g^{-1} soil in the glucose-amended soil and for 40 µg C g^{-1} soil in the legume-amended soil, these amounts being of similar proportion (about 14%) of recovered biomass ^{14}C in each of the treatments. By the end of incubation, substrate-derived biomass ^{14}C associated with this fraction declined in both treatments.

Particle size fraction Hf > 50 µm. Whatever the treatment, this fraction sheltered any susbtrate-derived biomass ^{14}C.

Particle size fraction Hf 2 -50 µm. At 3d, the substrate-derived biomass ^{14}C located in the particles of the silt size range of the glucose-amended soil was highest than that sheltered in the others fractions (370 µg ^{14}C g^{-1} soil), thus accounting for 67% of the recovered biomass ^{14}C. By contrast, biomass ^{14}C associated with the silt size fraction in

the legume-amended soil amounted only to 40% of the recovered biomass ^{14}C. Thereafter, substrate-derived biomass ^{14}C decreased in both treatments. However, the decrease was deeper in the glucose-amended soil, since biomass ^{14}C sheltered in the Hf 2-50 µm at the end of incubation corresponded only to 45% of that isolated at 3d. For the legume-amended soil, it amounted to 72% of the biomass ^{14}C associated to this fraction at 3 days incubation.

Particle size fraction Hf 0-2 µm. Substrate-derived biomass ^{14}C associated with the clay-size fraction, Hf 0-2 µm, represented broadly similar proportions of recovered biomass ^{14}C in amended soils at 3d (14% and 20% in the glucose-amended and legume-amended soils, respectively). On continued incubation, these proportions declined in each treatment.

DISCUSSION

Microbial sites

The incorporation into a highly aggregated Vertisol of a soluble or a particulate substrate resulted in the turnover of microbial biomass located in different sites of the soil matrix. Site differences were concluded from the differences in the the turnover of biomass ^{14}C associated with fractions of the soil during the two main important phases of the incubation, viz. the 0-3 d period when no ^{14}C turnover occured and the 3-66 d period when ^{14}C turnover was evident (Chotte et al., in press).
By definition, a site is favourable for microbial growth if it shelters more than 50% of soil biomass at 3 days incubation. A site is referred to as protective if its biomass decline, during the 3-66 days period, represents less than 50% of its biomass at 3 days of incubation. The combination of these two parameters allowed the comparison of the microbial sites to be made (Figure 3). The incorporation of fresh particulate organic residues temporarily increases soil biomass by offering new sites for microorganisms. However these residues are unprotecting sites since their associated biomass disappeared entirely thereafter. By contrast, soluble compounds promote soil biomass located in microaggregates. However, these sites are unprotecting as well.

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Figure 1 : Biomass C (substrate-derived and Som derived) in unfractionated soils.

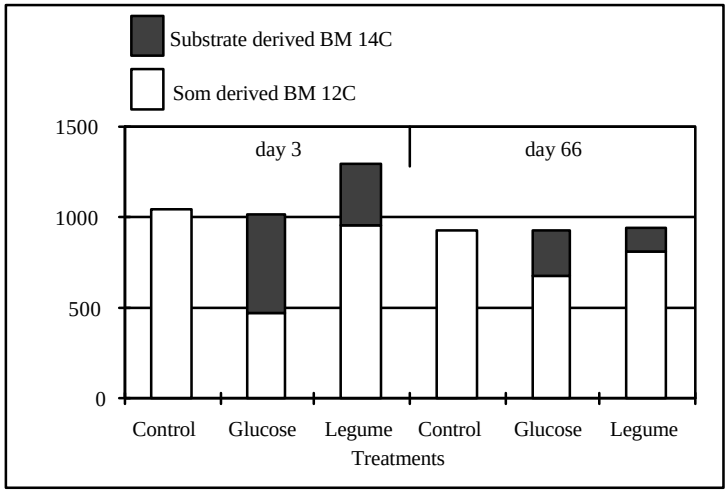

Figure 2 : Substrate- derived biomass ^{14}C in the particle size fractions in the glucose (A) and legume-amended (B) soils at 3d and 66d of incubation.
F1 : Lf > 250 µm ; F2 : Lf 50-250 µm ; F3 : Hf > 50 µm ; F4 : Hf 2-50 µm ; F5 : Hf 0-2 µm.

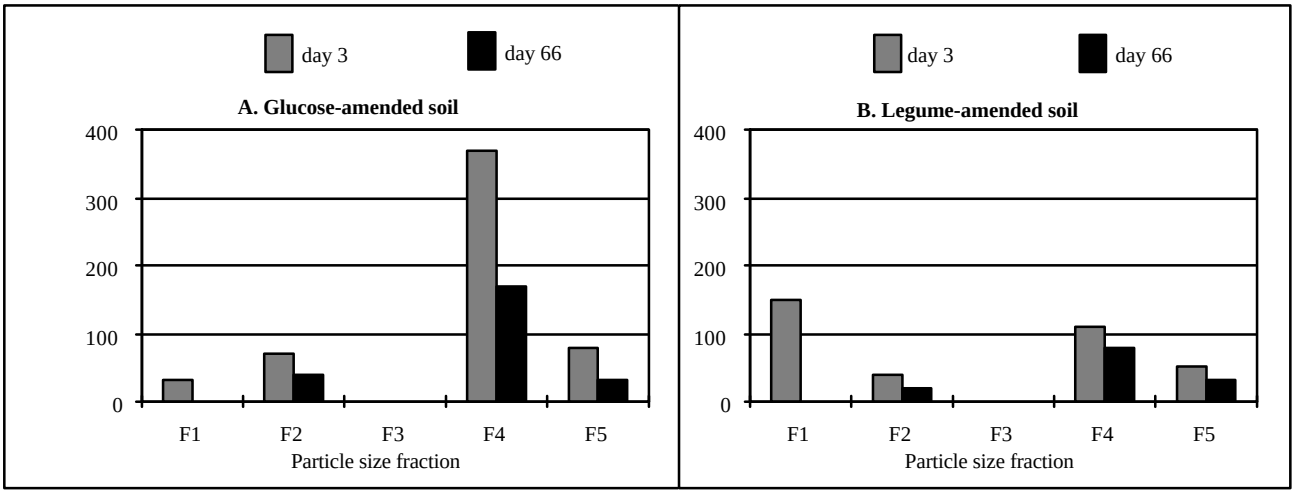

Figure 3 : Schematic classification of microbial sites.