Beyond the Biomass
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CHAPTER 18

Priming effects of macroorganisms on microflora: A key process of soil function?

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Bingeman et al. (1953) described the priming effect as the stimulation of soil organic matter decomposition by the addition of fresh organic material. Jenkinson (1966) described it in more general terms as any positive or negative change in the decomposition rate of soil organic matter caused by the addition of fresh organic matter. He illustrated this using a simple experiment in which ryegrass foliage uniformly labelled with ^{14}C was mixed with soil, and carbon dioxide evolution was monitored during an incubation period of 78 days. The production of unlabelled carbon dioxide resulting from soil organic carbon mineralisation was greater in the mixture than in the control soil. The difference was the result of a positive priming action.

Most examples of priming effects described in the literature are positive (reviewed by Jenkinson et al., 1985). They indicate that the addition of fresh organic matter as green manure to soils or of mineral nitrogen as fertiliser often stimulates mineralisation of soil organic matter. However, some authors have observed negative effects. For example, Bingeman et al. (1953) reported such effects in the first few days following the addition of glucose to an organic soil. The observed priming effects may be apparent or real. Apart from experimental factors (such as the exchange of labelled for unlabelled carbon in calcareous soils, or errors resulting from heterogeneous labelling of introduced material), some observed priming effects may be attributable to changes in pH or oxygen supply following the introduction of organic material (Parr and Reuszer, 1959; Barrow, 1960). In experiments where labelled material is added, apparent priming effects may also result from the release of unlabelled material turned over in the microbial biomass (Dalenberg and Jager, 1981).

Real priming effects may stem from three factors:

- the germination of spores, which increases overall microbial activity
- interactions between compounds derived from the added and 'native' organic matter, which render the latter more labile (Mandl and Neuberg 1956)
- an increase in the concentration of extra-cellular enzymes produced by microorganisms, resulting in accelerated decomposition of soil organic matter

In this chapter, the focus is on the third factor, triggered in the rhizosphere and drilosphere (the part of the soil and microflora affected by earthworm activities) by the addition to the soil of specific organic substrates produced by roots or earthworms. A significant proportion of carbon assimilated by plants is translocated directly to the rhizosphere soil as 'rhizodeposition', a mixture of water-soluble exudates and secretions, mucilage and sloughed cells from the root epidermis and cortex (Rovira et al., 1979; Hale et al., 1981). They represent 7-20% of the carbon fixed by photosynthesis, depending upon the plant and soil conditions (Lespinat and Berlier, 1975; Martin, 1977; Haller and Stolp, 1985; Milchunas et al., 1985; Heulin et al., 1987; Trofymow et al., 1987).

Earthworms also produce large amounts of mucus in the anterior gut and add it to the ingested soil. Concentrations vary from 5-7% of the dry weight of the ingested soil in native species of African savannas (Martin et al., 1987) to 15-18% in species with a wide pan-tropical distribution (Barois, 1992) and up to 42% in the Lumbricidae in northern Spain (Trigo et al., 1993). *In vitro* incubations of root mucilage and earthworm intestinal mucus have been conducted to compare the kinetics of microbial responses to the addition of these substrates and assess the priming effects on soil organic matter (Gilot, 1990; Mary et al., 1992). The significance of these mechanisms in soil function is discussed here.

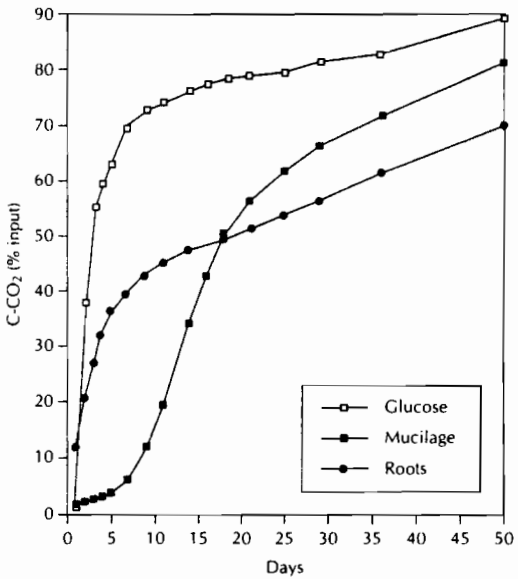
EFFECT OF ROOT MUCILAGE IN THE RHIZOSPHERE

The stimulation of microbial activities by rhizodeposition has been fairly well documented (for example, Samtsevich 1971; Stanghellini and Hancock, 1971; Short and Lacy, 1974; Trofymow and Coleman, 1982; Clarholm, 1985; Guckert, 1985; Billes et al., 1990). Mucilage and root litter are the main sources of carbon in the rhizosphere. In an attempt to assess their mineralisation patterns in the soil, the decomposition of mucilage and fresh maize roots was observed over a 50-day period of laboratory incubation and compared with a control supplemented with equivalent amounts of glucose (Mary et al., 1992) These substrates had been mixed with a sterile sand which had been inoculated with a soil extract to provide microbial populations. The kinetics of mineralisation of the mucilage were clearly different from those of the other two substrates. The decomposition of the maize roots and glucose was rapid during the first 7 days and then proceeded more slowly. The decomposition of the mucilage was slow during the first few days and then accelerated sharply. By the end of the experiment, 81% of the mucilage and 70% of the roots had been mineralised. In a parallel experiment over the same period, 89% of the glucose had been mineralised (*see* Figure 18.1).

An incubation experiment was conducted over 185 days in the presence of soil (orthic Luvisol with 17% clay) (Mary et al., 1991). The organic substrates were the same as those used in the experiment outlined above but their ^{13}C composition differed from the soil carbon; it was possible to discern which parts of the total CO_2 evolved came from the decomposing substrate and from the soil organic matter. All three substrates differed slightly in decomposition patterns. The apparent mineralisation was larger with the addition of glucose than for the other two substrates; after 180 days, the total CO_2-C evolved was equivalent to 67%, 74% and 92% of the carbon introduced with the substrate for roots, mucilage and glucose, respectively. In the first few days of the experiment, the priming effects on soil organic matter were similar, representing 10-15% of the overall mineralisation. After 40% of the substrates had decomposed, significant differences appeared and, after 185 days, the priming effect represented 14%, 19% and 31% of the carbon incorporated as roots, mucilage and glucose, respectively.

In the experiment reported by Mary et al. (1991), no priming effect on nitrogen was observed. Nonetheless, the maximum amount of nitrogen immobilised during the decomposition of the substrates was higher for mucilage (88% of added carbon) than for roots (66%) and glucose (61%). The incubated soil was fumigated to kill the microbial biomass and then further incubated to quantify

Figure 18.1 Kinetics of carbon mineralisation of three substrates added to sand inoculated with a soil extract



Source: Mary et al. (1992)

the amounts of nitrogen and carbon present as microbial biomass. The N/C ratio of the mineral nitrogen and carbon produced was higher in the mucilage (0.28) than in the roots (0.15) and glucose (0.13) treatments. This suggests that mucilage stimulates bacteria rather than fungi, as bacteria have a much higher relative concentration of nitrogen in their biomass than fungi.

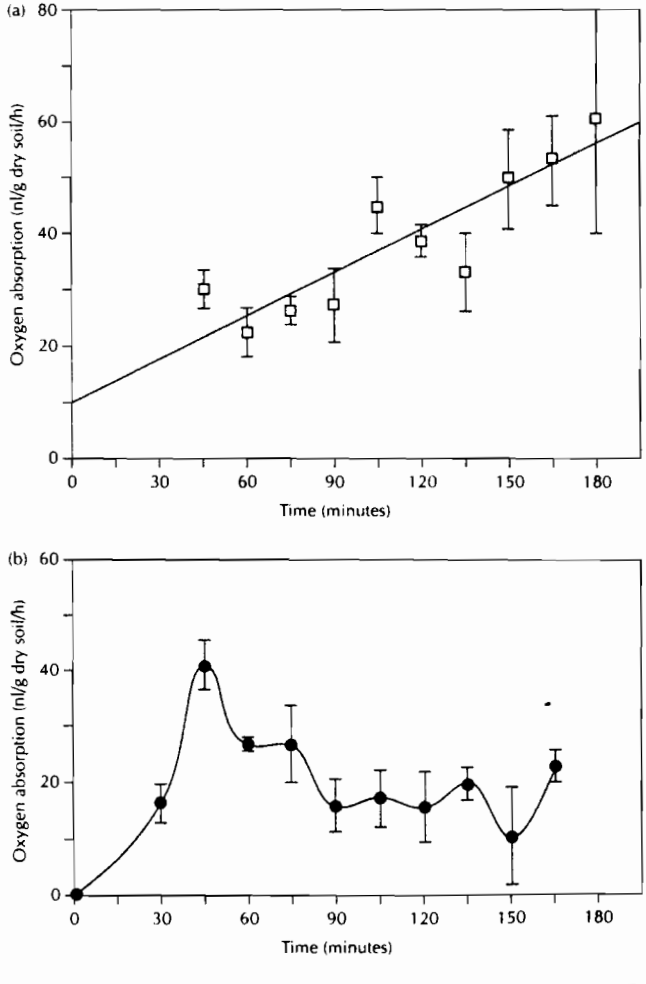
EFFECT OF EARTHWORM INTESTINAL MUCUS IN THE DRILOSPHERE

Some authors have suggested a mutualist relationship between tropical endogeic earthworms and soil microflora in the exploitation of soil organic matter (Lavelle et al., 1980; Barois and Lavelle, 1986; Trigo et al., 1993). The conditions in the anterior part of the earthworm gut suit the activities of soil-free microorganisms: high water content (100-150% of the dry weight of soil), neutral pH and, above all, high concentrations of readily assimilable organic matter as intestinal mucus (5-18% of the dry weight of soil, depending upon the species). This mucus is a mixture of low-molecular-weight (about 200 Da) amino acids and sugars and a glycoprotein of 40 000-60 000 Da (Martin et al., 1987).

Short-term incubations of intestinal mucus were carried out to investigate the response of soil microflora to the addition of these substrates at the same concentration as that observed in the gut (7% for *Millsonia anomala*) (Gilot, 1990) (*see* Figure 18.2 *overleaf*). The intestinal mucus was extracted

with water from the anterior gut of *M. anomala* and freeze-dried. It was then incubated for 3 hours in the chambers of a microrespirometer designed by Verdier (1983). Microbial activity increased sharply, reaching a maximum after 45 minutes and then falling to values similar to those observed for the control

Figure 18.2 Changes over time of oxygen absorption in an African Alfisol supplemented with (a) 7% glucose and (b) 7% intestinal mucus of *Millsonia anomala*



Source: Gilot (1990)

after a further 60 minutes. In the soil supplemented with 7% glucose, a different pattern was observed: respiratory activity increased over the whole period of the experiment (120 minutes).

These results indicate that, in the middle part of the earthworm gut, microorganisms which initially increase their metabolic activity sharply by feeding on the mucus are able to digest soil organic matter at a far higher rate than in bulk soil. Microbial activity at 28°C was 6-10 times higher than in the control (a dry soil sieved at 2 mm, moistened to field capacity) and probably up to 30 times higher than in undisturbed field conditions. The product of the external digestion of microorganisms would be partly reabsorbed with water in the posterior gut to feed the worm. Thus, some 3-19% of the soil organic matter could be assimilated during transit through the gut, which may last from 30 minutes to 2-4 hours (Lavelle, 1978; Barois, 1987; Martin, 1989). Interestingly, when temperature is reduced to 15°C, the increase of microbial respiratory activity in the gut is limited to twice the rate in the control soil.

Zhang et al. (1992) showed that cellulase and mannanase found in the gut content of the earthworm *Pontoscolex corethrurus* were produced by the ingested microflora since they were not found in isolated gut tissue cultures. It is possible, therefore, that in terms of the digestion system there is a mutualist relationship between endogeic earthworms and the ingested soil microflora. It is likely that the efficiency of this digestion system is highly dependent upon temperature. With lower temperatures, the reduction in efficiency would be compensated for by the ingestion of a higher-quality feeding resource and the production of higher amounts of intestinal mucus in the anterior part of the gut to accelerate the activation of the ingested dormant microflora. This may explain why earthworms tend to feed more on litter (easier to digest than soil organic matter) when the average temperature decreases (Lavelle, 1983). On the other hand, Trigo et al. (1993) have found concentrations of intestinal mucus of up to 42% in the anterior gut of temperate earthworm species from northern Spain, much higher than the observed values of 5-18% in tropical species.

Mucus in the drilosphere has a similar effect to that of root exudates in the rhizosphere, or any easily assimilable substrate introduced in the soil.

DISCUSSION

Roots and earthworms produce high amounts of readily assimilable organic substrates in the rhizosphere and drilosphere, respectively. Rhizodeposition may represent up to 10-20% of the total carbon fixed by photosynthesis and earthworms add 5-42% of the dry weight of the ingested soil as intestinal mucus in the anterior part of their gut. In the middle and posterior gut, this mucus is mostly absent. Part of it is metabolised by the ingested soil microflora (Martin et al., 1987); another part is probably reabsorbed by the gut wall and then recycled or simply recirculated in the anterior gut.

Like any readily assimilable resource, root mucilage and earthworm mucus increase microbial activities. Interestingly, microbial response to the addition of these substrates differs from that which occurs after the addition of glucose. The observed priming effect is lower for mucilage than for glucose but the kinetics of microbial response differ. Mary et al. (1992) observed that, after the addition of mucilage, there was a time lag of about 5 days before mineralisation rates started to increase, reaching values which were lower than those obtained with glucose and dead roots. The response of microflora to the addition of earthworm mucus also differed from that obtained with glucose. During the first 45 minutes after the addition of earthworm mucus, respiratory activity increased rapidly, reaching a maximum value of more than 50% higher than that obtained with glucose; there followed a rapid decrease and, after 2 hours, the respiratory activity was the same as that of the control.

There is some evidence that priming effects result from the addition of rhizodeposition to the soil. Sallih et al. (1987) observed the priming effects on nitrogen in a 700-day pot experiment. In pots

supporting the continuous activity of live roots, there was a 25% increase of nitrogen mobilised as mineral nitrogen and root and microbial-biomass nitrogen, compared with a control without plants. A large microbial biomass was maintained by the living plant, clear evidence that soil organic matter mineralisation and the subsequent release of mineral nitrogen was enhanced by the priming effect of root exudates. In contrast, Mary et al. (1991) did not observe a priming effect on nitrogen in laboratory incubations and concluded that the priming effect observed on carbon in the same incubations might only have been an apparent effect as opposed to a real priming effect. In this case, the release of unlabelled microbial carbon by microflora would have resulted from the turnover of biomass and the subsequent replacement of unlabelled material by the labelled carbon introduced in the experiment.

These results indicate that macroorganisms such as roots and earthworms can trigger priming effects on soil microflora in the rhizosphere and drilosphere through the production of assimilable substrates. They also suggest that these substrates have specific effects on microflora. Observations of thin sections in the rhizosphere under the electron microscope have shown that only 30% of the microflora is actually activated by the input of exudates (Foster, 1986). Our results show that the specific composition of these substrates may induce specific temporal patterns of the microbial response. In the drilosphere, the response to the addition of mucus was much faster than with glucose and this pattern may be considered as an adaptation to the specific conditions of earthworm digestion; given that the gut transit in the earthworms used for the experiment generally lasted less than a couple of hours (Lavelle, 1978), a fast activation of microflora is critical for rapidly reaching the level of activity beyond which formerly dormant microorganisms recover their ability to digest soil organic matter. In the rhizosphere, processes leading to the release of assimilable nutrients from soil organic matter are probably much slower. The movements of roots growing into the soil are much slower than soil passing through an earthworm gut. As a result, the soil reached by the growing root tip will be influenced by the production of exudates for a longer period than soil introduced in the highly active microsite represented by the gut of an earthworm. Thus, it may be a selective advantage to produce compounds that will have a prolonged effect on microbial activity and will selectively activate bacteria rather than fungi, as is the case for the root mucilage considered in the experiment of Mary et al. (1992).

We propose calling these substrates 'ecological mediators'. They have a specific role in ecosystem function through activating microflora at specific scales of time and space compatible with the needs of roots or earthworms to reingest the assimilable compounds released by the activated microflora.

These observations lead us to suggest three new research avenues that should be explored:

- Investigate the mechanisms which determine apparent or real priming effects. The metabolic activities of microorganisms involved in this process should be investigated and special attention paid to specific microbial processes such as turnover of microbial biomass and the release of enzymes in the surrounding soil.

- Test the specificity of ecological mediators. This involves studying the assimilable compounds produced by roots, earthworms and other soil invertebrates in terms of their chemical composition and the qualitative and quantitative responses of microorganisms (that is, determine which microflora are activated and the time pattern involved); the implications of different time patterns should be considered. The concepts of synchronisation and synlocalisation of nutrient release for plant uptake should be used in this approach (Swift, 1986; van Noordwijk and de Willigen, 1986).

- Test the functional significance of these processes in terms of strategies for exploiting nutrient resources and the consequences for diversity (for example, Lavelle, 1986). Roots, earthworms and other soil macroorganisms have mutualist associations with microorganisms which allow a better use of soil organic resources by favouring organic matter decomposition and mineralisation and

the uptake of the assimilates thus released by both components of the association. At the higher temperatures of tropical soils, the range of organic resources used is likely to be enlarged as a result of a much faster and stronger response by microorganisms to the addition of exudates or mucus. In evolutionary time, such differences may have promoted increased diversity as a result of the broader and more diverse base of the organic resources made available.

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