Plant and Soil **129**: 1–10, 1990.
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PLSO BARC02

Substrate flow in the rhizosphere

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Key words: arrested metabolism, biomass, carbon budgets, cryptic growth, dormancy, oligotrophy, products, rhizodeposition, substrates

Abstract

The major source of substrates for microbial activity in the ectorhizosphere and on the rhizoplane are rhizodeposition products. They are composed of exudates, lysates, mucilage, secretions and dead cell material, as well as gases including respiratory CO_2. Depending on plant species, age and environmental conditions, these can account for up to 40% (or more) of the dry matter produced by plants. The microbial populations colonizing the endorhizosphere, including mycorrhizae, pathogens and symbiotic N_2-fixers have greater access to the total pool of carbon including that recently derived from photosynthesis. Utilization of rhizodeposition products induces at least a transient increase in soil biomass but a sustained increase depends on the state of the native soil biomass, the flow of other metabolites from the soil to the rhizosphere and the water relations of the soil. In addition, the phenomena of oligotrophy, cryptic growth, plasmolysis, dormancy and arrested metabolism can all influence the longevity of rhizosphere organisms. With this background, microbial growth in the rhizosphere will be discussed.

Introduction

Microbial growth in the rhizosphere is stimulated by the continual input of readily assimilable organic substrates from the root. In ecological terms, the substrate flowing from the roots is the product of photosynthesis and is thus primary productivity and the utilization of these organic substrates by rhizosphere micro-organisms results in secondary productivity. In turn, these micro-organisms can influence the plant as primary producer. Methodology is now available which enables substrate flow in the rhizosphere to be analysed in terms of the balance between the production and utilization of substrates. We will review current evidence on each of these components.

Rhizodeposition

This topic has been reviewed in depth previously (Whipps, 1990; Whipps and Lynch, 1985; 1986) and therefore only a summary will be given here. The term 'rhizodeposition' has been used to describe carbon *loss* from roots (Whipps and Lynch, 1985) and this material generates the rhizosphere effect. However, it should not be forgotten that the living root itself provides a substrate for endorhizosphere colonizers, a consideration often overlooked in the past.

The chemical components coming from roots are conveniently classified into the following four groups, depending on their mode of arrival: 1) water soluble *exudates*, such as sugars, amino acids, organic acids, hormones and vitamins

which leak from the root without the involvement of metabolic energy; 2) *secretions*, such as polymeric carbohydrates and enzymes which depend upon metabolic processes for their release; 3) *lysates*, released when cells autolyse including cell walls, and, with time whole roots; 4) *gases* such as ethylene and CO_2. These groupings cover all stages of plant growth and development with the balance of these various processes changing with age of the plant. This can range from the release of simple sugars during membrane re-organization of the embryo during seed germination (Hale and Moore, 1979) through to root cortical death in the mature plant followed by whole root senescence. It also includes the sloughing-off of root cap cells; approximately 5000 cells per day are lost from maize (Moore and McClelen, 1983). Bearing in mind the complexity of substrate origin, the composition of rhizodeposition products can be expected to include all components of the plant cell.

A range of experiments has been carried out to measure rhizodeposition. Many have involved the growth of plants in nutrient solution culture under gnotobiotic conditions where exudates, sloughed cells and mucilage are collected. Addition of micro-organisms generally increases the carbon loss. These types of rhizodeposition have been analysed quantitatively and qualitatively by chemical analytical techniques (*e.g.* Kraffczyk *et al.*, 1984; Schönwitz and Ziegler, 1982) or by the use of $^{14}CO_2$ feeding experiments, mainly using pulse-chase techniques (*e.g.* Beck and Gilmour, 1983; Lee and Gaskins, 1982). Some experiments have been refined to examine the effect of a whole range of factors on rhizodeposition including textural quality (by addition of glass Ballotini or use of artificial soils), anoxia, water stress, cultivar, nutrient sources, environmental parameters and micro-organisms (see Hale and Moore, 1979; Whipps, 1990). Measured in this way, values for rhizodeposition do not exceed 600 mg g^{-1} root dry weight (Newman, 1985; Whipps, 1990). These values must represent considerable underestimations of rhizodeposition as in many cases respiration from the root and accompanying microflora is ignored. In addition, nutrient solution culture is not suitable for the growth of many perennial plants and this, together with the difficulty of maintaining sterility in axenic cultures has given rise to much data on seedling rhizodeposition, mainly exudation and secretion. Similarly, use of artificial soils and addition of specific micro-organisms can bear little resemblance to the complex structural and functional relationships taking place in the soil.

Due to the complex nature of the soil, two types of tracer techniques have been developed to study rhizodeposition in this milieu. The first, pulse-chase technique, is relatively easy and rapid and is applicable to both laboratory and field experimentation. However, it results in a non-uniform distribution of label in the plant and can thus only provide information on movement and losses of recently fixed carbon and not on the degradation of whole cells or dying older roots. Even repeated pulse feeding cannot evenly label all pools of carbon in the plant. This has led to the development of the second system which is applicable only to the laboratory. Here, plants are grown in controlled environment growth chambers with shoots exposed to an atmosphere containing constant specific activity $^{14}CO_2$. Any $^{14}CO_2$ or ^{14}C-containing material found in the soil must then originate from rhizodeposition. The $^{14}CO_2$ detected in the soil can come from root respiration *per se* or from microorganisms utilizing root derived material. This technique cannot allow for carbon respired from the shoot and so distribution data can only be related to the net fixed carbon rather than total fixed carbon.

Using these constant feeding systems, ^{14}C derived from maize roots has been shown to spread 20 mm from the root after 25 days of growth and act as a primer for microbial degradation of the existing soil organic carbon (Helal and Sauerbeck, 1983; 1986). Except when activity is high during the early stages of decomposition of fresh organic matter, similar results have been found with wheat (Sallih and Bottner, 1988), resulting in an increase in soil biomass (Bottner *et al.*, 1988). Merckx *et al.* (1985) also showed that ^{14}C derived from wheat roots entered the soil microbial biomass and increased throughout a six week experimental period. However, the majority of these studies have examined the amounts and proportions of ^{14}C entering the roots and forming the rhizodeposition. Results from some of these studies are summarised in Table 1.

Table 1. Percentage of net fixed carbon moved to roots and the percentage of net fixed carbon moved to the roots which is lost when shoots of plants are exposed continually to $^{14}CO_2$ (Plants grown in soil). Conditions varied but were *c.* 18°C, 16 h day length, 0.03% CO_2 and none were sterilized

Plant	Age (d)	% of net fixed C transferred to root	% of C transferred to root lost as			Reference
			Respiration	Rhizodeposition in soil	Respiration and rhizodeposition in soil	
		(A)	(B)	(C)	(B + C)	
Wheat	21	38	25	17	42	Barber and Martin, 1976
	21	47	36	25	61	Whipps and Lynch, 1983
	21	59	39	29	68	Whipps, 1984
	28	44	39	11	50	Merckx *et al.*, 1985
	153	?	76	4	80	Johnen and Sauerbeck, 1977
Barley	21	43	21	23	44	Barber and Martin, 1976
	21	54	48	21	69	Whipps, 1984
Maize	14	28	16	52	67	Whipps, 1985
Mustard	73	?	66	13	78	Johnen and Sauerbeck, 1977
Tomato	14	43	20	70	90	Whipps, 1987
Pea	28	44	53	29	82	Whipps, 1987

$$A = \frac{\mu\text{Ci in root + rhizodeposition} + CO_2 \text{ from root and soil micro-organisms}}{\text{net } \mu\text{Ci fixed}} \times 100; \ \mu\text{Ci } \Omega \text{ amount of C}$$

$$B = \frac{\mu\text{Ci in } CO_2 \text{ from root and soil micro-organisms}}{\mu\text{Ci in root = rhizodeposition in soil} + CO_2 \text{ from root and soil micro-organisms}} \times 100$$

$$C = \frac{\mu\text{Ci in rhizodeposition in soil} \times 100}{\mu\text{Ci in root + rhizodeposition in soil} + CO_2 \text{ from root and soil micro-organisms}}$$

Overall, between 30–60% of net fixed carbon is transferred to roots in annual plants. These figures agree well with a range of nine different annual plants grown in nutrient solutions under a range of conditions (Lambers, 1987). Between 40 and 90% of the carbon transferred to the root is lost as rhizodeposition in the soil and respiration from the roots and associated micro-organisms. With wheat, and other annual plant species not shown the quantity of rhizodeposition in soil decreased with time, but the proportion lost as respiration increased. Similar experiments with perennial blue grama grass (*Bouteloua gracilis*) showed that over 70% of net fixed carbon was passed to roots during a simulated summer growing period (Dormaar and Sauerbeck, 1983). Subsequent loss of 30–60% of young, suberised roots occurs when root growth ceases (Ares, 1976).

These constant feeding techniques also suffer problems. For example, root growth may be physically restricted in pots and water and gas exchange and nutrient availability may all limit growth. The pots may leak and harvests have to be sequential and destructive. The seedlings often have considerable food reserves and it may take some time for all plant parts to become evenly labelled and, as shoot respiration cannot be measured *in situ*, distribution data always refers to net fixed carbon. Perhaps most frustrating is that it is impracticable to grow perennial species for long periods.

Estimates of rhizodeposition in field experiments have been obtained using $^{14}CO_2$ pulse-chase experiments and by analyzing components of crop growth and some results are given in Table 2. The value for percentage ^{14}C transferred below ground for wheat is lower than that determined for seedlings in constant feeding experiments (Table 1), but as rhizodeposition falls with time (Keith *et al.*, 1986), a lower annual figure is to be expected. The values of 1.3 t C ha^{-1} $annum^{-1}$ for rhizodeposition measured in these field experiments agrees well with the range of values for wheat and mustard (0.5–2.9 t C ha^{-1} $annum^{-1}$) obtained in $^{14}CO_2$ growth

Table 2. Carbon movement to and rhizodeposition from the roots in field experiments

	% net fixed C transferred to root	% of C transferred to root that is lost		Rhizodeposition (root C + soil C + CO_2C) ($t\,C\,ha^{-1}\,annum^{-1}$)	Reference
		Soil	CO_2		
Wheat[a]	15	25	50	1.3	Keith *et al.*, 1986
Agropyron-Koeleria dominated grass mixture	35–50	[b]	20–28	1.3	Warembourg and Paul, 1977
Douglas fir	73		40–47[d]	5.8–7.5[c]	Sanantonio, 1979; Fogel and Hunt, 1983

[a] Mean value of five sampling times (42–154 d)
[b] No value available
[c] Includes carbon in mycorrhizas
[d] Soil and CO_2 not separated

cabinet experiments (Johnen and Sauerbeck, 1977). This represents approximately 15% of total fixed carbon (Keith *et al.*, 1986). Field measurements of root production without allowance for respiration losses range between 0.2–1.6 $t\,C\,ha^{-1}\,annum^{-1}$ (Barber, 1971; Gregory *et al.*, 1978; Sivakumar *et al.*, 1977).

Perennial plants maintain roots throughout the year and rhizodeposition is known to be cyclical depending on periods of active growth. Using $^{14}CO_2$ pulse-feeding techniques, Singh and Coleman (1974) found that carbon was initially found in actively growing roots of blue grama in the top 20 cm of soil but it subsequently moved to lower regions of soil as roots grew deeper. Between 30–50% of net fixed carbon was transferred below gound in *Agropyron-Koeleria* perennial grassland (Table 2), less than that found in growth cabinet experiments with blue grama (Dormaar and Sauerbeck, 1983) and other field experiments (Kucera *et al.*, 1967; Sims and Singh, 1971). But as only 20–28% of the carbon moved to roots was respired, which is low compared with other grassland systems, the value of 1.3 $t\,C\,ha^{-1}\,annum^{-1}$ should be considered a minimum. Tree species pass on even greater quantities of carbon to their roots resulting in an annual rhizodeposition of 5.8–7.5 $t\,C\,ha^{-1}$ (Table 2). In general, perennial plants invest more of their productivity in root material than annuals and this may be related to their need to withstand prolonged periods of stress.

Another aspect of rhizodeposition concerns the involvement of symbiotic root inhabitants such as Rhizobium and mycorrhizae. Legumes benefit from Rhizobium infection by improved N nutrition from N_2-fixation and the majority of plants benefit from mycorrhizae by improved mineral nutrition, particularly of P, and drought tolerance. In return, the heterotrophs gain organic material from the endorhizosphere. Measurement of total rhizodeposition from legumes in soil are scarce, the majority of studies being concerned with the efficiency and cost of N_2 fixation. Over the life of a range of legumes, between 30–50% of net fixed C enters the roots with 25–40% of net fixed C representing 63–79% of C entering the root being lost (Minchin *et al.*, 1981). Mean values of 13.4, 7.1 and 9.2 mg CO_2 respired g^{-1} root h^{-1} have been found for whole nodulated roots, roots (separated from nodules) and roots from non-nodulated nitrate-grown plants (Ryle *et al.*, 1983). The cost (CO_2 lost g^{-1} root) of N_2-fixation by nodulated roots was up to twice that when growing on nitrate-N (Pate *et al.*, 1979; Ryle *et al.*, 1979).

Studies on vesicular-arbuscular (VA) mycorrhizal infections indicated that conditions which increase permeability of roots or exudation increase infection (Graham *et al.*, 1981). Once synthesised, the cost of maintaining VA mycorrhizas in terms of extra carbohydrate moved to the roots is approximately 6–11% net fixed carbon (Snellgrove *et al.*, 1982; Koch and Johnson, 1984). In experiments with *Vicia faba* plants, it was shown that nodulated root systems of VA mycorrhizal plants fixed more N_2 than nodulated root systems of non-mycorrhizal plants. This was

related to an increase in nodule biomass in doubly infected plants. The plants may compensate for the cost of these symbionts by increasing rates of photosynthesis or by changing morphological characteristics to increase specific leaf area (Bayne *et al.*, 1984; Bildusas *et al.*, 1986; Harris *et al.*, 1985).

Greater amounts of C are transferred to roots with ectomycorrhizae compared with non-mycorrhizal plants (12–22% total fixed C in pine) and this is also partially compensated for by increased rates of photosynthesis (Ried *et al.*, 1983). It has been estimated for Douglas fir that ectomycorrhizae comprise 6% of the total tree biomass and, that for a range of host trees, ectomycorrhizae have a biomass between 0.4–10.0 t C ha^{-1}, most of which must have come from the host (Fogel, 1985; Fogel and Hunt, 1983).

With such large inputs of C to the roots, associated micro-organisms and rhizodeposition may seem wasteful but in ecological terms it may enable the plants to grow where they otherwise could not. Photosynthetic compensation can occur, and, in situations where lack of nutrients or environmental conditions limit growth, plants may have excess source potential (Herold, 1980). Thus, when root growth is limited, excess carbohydrate and reducing power can build-up and the roots switch on an 'overflow metabolism' via an alternative, cyanide-resistant respiratory pathway (Barneix *et al.*, 1984; Lambers, 1980).

Carbon budgets

We have indicated previously that whereas the substrate made available by roots to the microbial population of the rhizosphere is apparently very large, there is still a larger microbial biomass present than can be accounted for theoretically (Barber and Lynch 1977; Whipps and Lynch, 1983). The results of this analysis are given in Table 3. The basis of the calculation was to (1) grow rhizosphere bacteria in pure shaken culture in liquid media; (2) take a sample to estimate the cell density microscopically; (3) harvest and weigh the culture; and (4) assess the dry weight per unit cell. The total number of bacteria colonizing the roots was assessed from viable count measurements of the surrounding solution, sand or the roots themselves. The latter estimate was obtained by shaking the roots with glass beads in a surfactant to ensure by microscopic observation that the roots were free from bacterial colonization. This procedure does not allow for bacteria in the endorhizosphere and therefore, if anything, underestimates rather than overestimates the microbial biomass associated with the root.

It is difficult to be certain about the efficiency at which the bacterial biomass associated with roots is produced but it seems reasonable to assume a yield factor of *c.* 0.35 g dry weight g^{-1} substrate (Barber and Lynch, 1977). From this assumption the substrate required to generate the biomass can be calculated and compared with measured carbon flow using the method of continuous $^{14}CO_2$ labelling of the plant referred to above. In the first barley study (Barber and Lynch, 1977), the apparent substrate supplied was only 9% of that required, and in the subsequent barley and wheat studies (Whipps and Lynch, 1983), the figures were 25% and 10% respectively. Unfortunately, it is difficult to comment on the generality of these calculations be-

Table 3. Substrate input and microbial biomass of gnotobiotic cereal plants

	Barley[a]	Barley[b]	Wheat[b]
Microbial biomass			
Mean cell wt ($\times 10^{-12}$ g)	1.9	3.2	3.2
Bacterial biomass ($\times 10^{-4}$ g mg^{-1} dry root)	2.6	2.0	3.4
Substrate input			
Calculated ($\times 10^{-4}$ g C mg^{-1} dry root)	3.7	2.8	7.8
Measured ($\times 10^{-5}$ g C mg^{-1} dry root)	3.4	7.5	7.9

[a] Barber and Lynch (1977)
[b] Whipps and Lynch (1983)

cause no other studies have been undertaken. Certainly the lack of measurement of the endorhizosphere population and the lack of consideration of the maintenance energy (Pirt, 1975) of the microbial population makes the actual discrepancy in the calculation even greater.

The discrepancies could in part be caused by underestimation of substrate production, but this seems rather unlikely because the figures are consistent with those referred to above by several other investigators. It is likely therefore that the microbial biomass has been overestimated in some way. The most obvious interpretation is that the bacteria growing on roots are very much smaller than those produced in pure liquid culture; indeed Arthrobacter cells can change shape at low growth rates (Chapman and Gray, 1981). This concept was investigated using two strains of *Enterobacter cloacae* which colonized lettuce roots readily (Lynch, 1989). There is often a tacit assumption that all Gram negative bacteria associated with roots are pseudomonads but *Enterobacter* spp. are also important colonists, especially in the endorhizosphere (Kleeberger *et al.*, 1983). They are of particular interest because various strains have the capacity to fix N_2 (Neilson and Sparell, 1976), to produce soil stabilizing agents (Chapman and Lynch, 1985) and to protect against damping-off diseases (Nelson *et al.*, 1986). Despite extensive studies of these bacteria in various conditions of starvation and water stress (osmotic and matric) associated with roots and in isolation, one of us (Lynch, unpublished) has found no evidence for a significant change in cell dimensions by light or electron microscopy. However, closer examination of thin sections by electron microscopy indicated that starved cells which, although viable after one year, exhibited considerable plasmolysis both around the periphery of the cell and towards the core. Such cell morphology has been found in other ecosystems, particularly in oil wells (Lappin-Scott *et al.*, 1988). It is unrealistic experimentally to quantify this phenomenon because it can only be observed in the thin sections but it could contribute to the underestimation of microbial biomass. Another consequence of this study was the observation that the Enterobacter cells survived without any apparent substrate input. This may mean that our ideas of maintenance, which were largely derived for pure microbial cultures in chemostats (Pirt, 1975), are not readily applicable to organisms in natural ecosystems. For example, Anderson and Domsch (1985) showed that such low levels of carbon input were required to sustain soil biomass that maintenance energy requirements had to be much smaller than previously thought. Much of the population introduced to roots and that colonizing the rhizosphere from the soil might be sustained in a viable state for long periods in a state of *arrested metabolism*. This could be considered as a state close to *dormancy* while not exhibiting sporulation or other morphological features commonly associated with the dormant state. However, it would be dangerous to extrapolate these ideas too far because other phenomena such as *cryptic growth* and *oligotrophy* may occur. In batch culture, cryptic growth takes place as organisms utilize the cellular contents of both living and dead cells so there is a very protracted death phase (Postgate and Hunter, 1962). It is expected that this state could occur to an extent in the rhizosphere. Oligotrophs as opposed to copiotrophs appear to be able to sustain themselves on very low inputs of carbon which can be derived from the atmosphere (Lynch, 1989). Wainwright (1988) has reviewed the evidence of this occurring in fungi and has even suggested that a part of this carbon could be derived from fixation of CO_2 by chemolithoheterotrophy (organisms can grow as heterotrophs but gain additional energy by the simultaneous oxidation of reduced forms of elements). However, it seems most likely, in our rhizosphere studies, where roots have been forced-aerated that some carbon substrates for bacteria could be derived from traces of organic carbon in the air supply.

It is therefore difficult to produce satisfactory carbon budgets of the rhizosphere until more extensive and precise experimentation in the laboratory has been carried out. It is even more difficult to produce budgets of rhizospheres under field conditions. Lynch and Panting (1980a) have modified the fumigation-respiration technique of biomass determination (Jenkinson and Powlson, 1976) for use with instant cores of soil. Using this method, it was demonstrated that the soil biomass increased with increased rooting density, brought about by crop growth in the

spring and by the greater proliferation of roots in the surface layers of direct-drilled crops as against those seeded after first ploughing the soil. Similarly, the increased root growth in grassland versus arable cropping in the same soil series caused a three-fold increase in soil microbial biomass (Lynch and Panting, 1980b). However, this information can only be obtained by using intact cores of soil because sieving decreases the soil biomass in winter and increases it in summer (Lynch and Panting, 1981). Sieving breaks up roots and allows a rapid proliferation of bacteria on the readily assimilable substrates so released. Although this concept has been questioned by Jenkinson and Powlson (1980), homogenization of soils by sieving must disrupt the soil ecophysiology to a considerably greater degree than the passage of the plough through the soil which has been shown by others to modify the size of soil biomass (Doran, 1987). The limitation therefore in attempting to produce energy budgets in the rhizosphere of field soils seems to be one of reliability of the methodology used. Far from being a problem in estimates of biomass (Martens, 1985; Martin and Foster, 1985; Merckx and Martin, 1987; Sparling *et al.*, 1985) the presence of roots in soil should be a stimulus to the understanding of substrate flowing in the soil. With suitable methodology, it should also prove possible to develop dynamic models of microbial activity in the rhizosphere (Bazin *et al.*, 1990).

Products

As indicated above, the yield of micro-organisms utilizing the products of rhizodeposition can be assumed to be *c.* 0.35 g dry weight g^{-1} substrate consumed. A proportion of remaining substrate consumed will appear as CO_2 from microbial respiration and another as microbial products. A further proportion of these microbial products will be consumed by other microorganisms or be absorbed by the root system, but some products will be recalcitrant and have long half-lives in the soil/plant ecosystem. The range of products has been reviewed (Lynch, 1976; 1990) and the categories of products are summarised in Table 4.

Some of the products formed as primary metabolites make a substantial contribution to the carbon budget in the rhizosphere. Other secondary metabolites are formed in low concentrations whereas they may not make a major direct impact of the carbon budget, they may regulate rhizodeposition.

Methods have only been developed relatively recently to definitively characterize the categories of products. However, it should now be possible to carry out experiments to determine the dynamics of product formation and the effect of environmental and chemical perturbations on steady-state processes which exist. Such analyses are available for pure cultures of micro-organisms (Pirt, 1971) and the complexity

Table 4. Categories of microbial products in the rhizosphere

Class of compound	Function
(*a*) *Likely absorbed by roots*	
Plant growth regulators	*Potentially* growth stimulatory at low concentrations but inhibitory in higher concentrations
Organic acids	Phytotoxic
H_2S, HCN	Phytotoxic
Antibiotics	Disease control but phytotoxic if absorbed
Ionophores	Promotion of nutrient uptake and possible disease control by making Fe unavailable to pathogens
(*b*) *Likely bound by roots*	
Lectins/Agglutinins (Proteins)	Promote specific binding of micro-organisms to roots and other micro-organisms
Polysaccharides	Non-specific microbial binding, stabilization of soil structures
Free enzymes	Various – oxidoreductases, transferases, hydrolases, lysases including involvement in nutrient cycling, pathogenesis and disease control

of mixed cultures in a complex environment is obviously far more of a challenge. In the first instance, it would seem sensible to develop experimental procedures based on microcosms and increase the complexity of experiment thereafter.

A major opportunity is to change the quality and quantity of chemicals produced in the rhizosphere. This could be done either by inoculating the roots with organisms synthesising such compounds or by genetic modification of known rhizosphere component microorganisms. Direct chemical treatment or genetic modification of the plant are also possibilities. However, genetic manipulation should only be pursued in detail after the ecophysiology of the rhizosphere ecosystem is better understood. Only then will the molecular biologists have more precise targets on which to use their skills.

Conclusions

Central to the understanding of substrate flow in the rhizosphere is the analysis of rhizodeposition. A substantial amount of information has been accumulated especially from the continuous ^{14}C labelling experiments. However, most of the studies were carried out with young plants and there is a need to carry the analyses through to maturity. There is also a need to extend the continuous labelling experiments to host/symbiont and host/pathogen interactions. Unfortunately, with the possible exception of the excellent facilities which are retained for these studies in Wageningen, there is very little current experimental work being carried out internationally in this field. The emphasis of rhizosphere studies has shifted strongly towards rhizosphere manipulation and whereas this can be fully justified in strategic and applied science areas, a better understanding of the underlying physiological energetics involved would provide a much better framework on which to base manipulation.

References

Anderson T-H and Domsch K H 1985 Determination of ecophysiological maintenance requirements of soil microorganisms in a dormant state. Biol. Fertil. Soils 1, 81–89.

Ares J 1976 Dynamics of the root system of blue grama (*Bouteloua gracilis* (H.S.K.) Lag.). J. Range Manage. 29, 208–213.

Barber D A and Lynch J M 1977 Microbial growth in the rhizosphere. Soil Biol. Biochem. 9, 305–308.

Barber D A and Martin J K 1976 The release of organic substances by cereal roots in soil. New Phytol. 76, 69–80.

Barber S A 1971 Effect of tillage practice on corn (*Zea mays* L.) root distribution and morphology. Agron. J. 63, 724–726.

Barneix A J, Breteler H and Van de Geijn S C 1984 Gas and ion exchanges in wheat roots after nitrogen supply. Physiol. Plant 61, 357–362.

Bayne H G, Brown M S and Bethlenfalvay G J 1984 Defoliation effects on mycorrhizal colonization, nitrogen fixation and photosynthesis in the *Glycine-Glomus-Rhizobium* symbiosis. Physiol. Plant. 62, 576–580.

Bazin M J, Markham P, Scott E and Lynch J M 1990 Microbial interactions in the rhizosphere. *In* The Rhizosphere. Ed. J. M Lynch. pp 99–127. John Wiley, Chichester.

Beck S M and Gilmour C M 1983 Role of wheat root exudates in associative nitrogen fixation. Soil. Biol. Biochem. 15, 33–38.

Bildusas I J, Dixon R K, Pfleger F L and Stewart E L 1986 Growth, nutrition and gas exchange of *Bromus inermis* inoculated with *Glomus fasciculatum*. New Phytol. 102, 303–311.

Bottner P, Sallih Z and Billies G 1988 Root activity and carbon metabolism in soils. Biol. Fertil. Soils 7, 71–78.

Chapman S J and Gray T R G 1981 Endogenous metabolism and macromolecular composition of *Arthobacter globiformis*. Soil Biol. Biochem. 13, 11–18.

Chapman S J and Lynch J M 1985 Some properties of polsaccharides of microorganisms from degraded straw. Enzyme Microb. Technol. 7, 161–163.

Doran J W 1987 Microbial biomass and mineralizable nitrogen distributions in no-tillage and plowed soils. Biol. Fertil. Soils 5, 68–75.

Dormaar J F and Sauerbeck D R 1983 Seasonal effects of photoassimilated carbon-14 in the root system of blue grama and associated soil organic matter. Soil Biol. Biochem. 15, 475–479.

Fogel R 1985 Roots as primary producers in below-grown ecosystems. *In* Ecological Interactions in Soil. Plants, Microbes and Animals. Eds. A H Fitter, D Atkinson, D J Read and M B Usher. pp 23–26. Blackwell Scientific Publications, Oxford.

Fogel R and Hunt G 1983 Contribution of mycorrhizae and soil fungi to nutrient cycling in a Douglas-fir ecosystem. Can. J. For. Res. 12, 219–232.

Graham J T , Leonard R T and Menge J A 1981 Membrane-mediated decrease in root exudation responsible for phosphorus inhibition of vesicular-arbuscular mycorrhiza formation. Plant Physiol. 68, 548–552.

Gregory P J, McGowan M, Biscoe P V and Hunt B 1978 Water relations of winter wheat. 1. Growth of the root system. J. Agric. Sci. 91, 91–102.

Hale M G and Moore L D 1979 Factors affecting root exudation. II: 1970–1978. Adv. Agron. 31, 93–124.

Harris D, Pacovsky R S and Paul E A 1985 Carbon economy of soybean-*Rhizobium-Glomus* association. New Phytol. 101, 427–440.

Helal H M and Sauerbeck D R 1983 Method to study turnover processes in soil layers of different proximity to roots. Soil Biol. Biochem. 15, 223–225.

Helal H M and Sauerbeck D R 1986 Effect of plant roots on carbon metabolism of soil microbial biomass. Z. Pflanzenernaehr. Bodenkd. 149, 181–188.

Herold A 1980 Regulation of photosynthesis by sink activity – the missing link. New Phytol. 86, 131–144.

Jenkinson D S and Powlson D S 1976 The effects of biocidal treatments on metabolism in soil. V. A method for measuring soil biomass. Soil Biol. Biochem. 8, 209–213.

Jenkinson D S and Powlson D S 1980 Measurement of microbial biomass in intact soil cores and in sieved soil. Soil Biol. Biochem. 12, 579–581.

Johnen B G and Sauerbeck D R 1977 A tracer technique for measuring growth, mass and microbial breakdown of plant roots during vegetation. *In* Soil Organisms as Components of Ecosystems. Eds. V Lohm and T Persson. Proc. VI Int. Soil. Zoo. Coll. Ecol. Bull. Stockholm 25, 366–373.

Keith H, Oades J M and Martin J K 1986 Input of carbon to soil from wheat plants. Soil Biol. Biochem. 18, 445–449.

Kleeberger A, Castorph H and Klingmuller W 1983 The rhizosphere microflora of wheat and barley with special reference to Gram negative bacteria. Arch. Microbiol. 136, 306–311.

Koch K E and Johnson C R 1984 Photosynthate partitioning in split-root citrus seedlings with mycorrhizal and non-mycorrhizal root systems. Plant Physiol. 75, 26–30.

Kraffczyk I, Trolldenier G and Beringer H 1984 Soluble root exudates of maize: influence of potassium supply and rhizosphere micro-organisms. Soil Biol. Biochem. 16, 315–322.

Kucera C L, Dahlman R C and Koelling M R 1967 Total net productivity and turnover on an energy basis for tallgrass prairie. Ecology 48, 536–541.

Lambers H 1980 The physiological significance of cyanide-resistant respiration in higher plants. Plant Cell Environ. 3, 293–302.

Lambers H 1987 Growth, respiration, exudation and symbiotic associations: the fate of carbon translocated to the roots. *In* Root Development and Function. Eds. P J Gregory, J V Lake and D A Rose. pp 125–145. Cambridge University Press, Cambridge.

Lappin-Scott H M, Cusack F, Macleod A and Costerton J W 1988 Starvation and nutrient resuscitation of *Klebsiella pneumoniae* isolated from oil well waters. J. Appl. Bacteriol. 64, 541–549.

Lee K J and Gaskins M H 1982 Increased root exudation of ^{14}C-compounds by sorghum seedlings inoculated with nitrogen-fixing bacteria. Plant and Soil 69, 391–399.

Lynch J M 1976 Products of soil micro-organisms in relation to plant growth. CRC Crit. Revs Microbiol. 5, 67–107.

Lynch J M 1986 Rhizosphere microbiology and its manipulation. Biol. Agric. Hort. 3, 143–152.

Lynch J M 1989 Development and interactions between microbial communities on the root surface. *In* Interrelations Between Micro-organisms and Plants in Soil. Eds. V Vančura and F Kunc. pp 5–12. Academic, Prague and Elsevier, Amsterdam.

Lynch J M 1990 Microbial metabolites. *In* The Rhizosphere. Ed. J M Lynch. pp 177–206. John Wiley, Chichester.

Lynch J M and Panting L M 1980a Cultivation and the soil biomass. Soil Biol. Biochem. 12, 29–33.

Lynch J M and Panting L M 1980b Variations in the size of the soil biomass. Soil Biol. Biochem. 12, 547–550.

Lynch J M and Panting L M 1981 Measurement of the microbial biomass in intact cores of soil. Microbial Ecol. 7, 229–234.

Martens R 1985 Limitations in the application of the fumigation technique for biomass estimations in amended soils. Soil Biol. Biochem. 17, 57–63.

Martin J K and Foster R C 1985 A model system for studying the biochemistry and biology of the root-soil interface. Soil Biol. Biochem. 17, 261–269.

Merckz R and Martin J K 1987 Extraction of microbial biomass components from rhizosphere soils. Soil Biol. Biochem. 19, 371–376.

Merckz R, Den Hartog A and Van Veen J A 1985 Turnover of root-derived material and related microbial biomass formation in soils of different texture. Soil Biol. Biochem. 17, 565–569.

Minchin F R, Summerfield R J, Hadley P, Roberts E H and Rawsthorne S 1981 Carbon and nitrogen nutrition of nodulated roots of grain legumes. Plant Cell. Environ. 4, 5–26.

Moore R and McClelen C E 1983 A morphometric analysis of cellular differentiation in the root cap of *Zea mays*. Am. J. Bot. 70, 611–617.

Nelson A H and Sparell L 1976 Acetylene reduction (nitrogen fixation) by Enterobacteriaceae isolated from paper mill process waters. Appl. Environ. Microbiol. 32, 197–205.

Nelson E B, Chao W-L, Norton J M, Nash G T and Harman G E 1986 Attachment of *Enterobacter cloacae* to *Pythium ultimum* hyphae: possible role in the biological control of *Phythium* pre-emergence damping-off. Phytopathology 76, 327–335.

Newman E I 1985 The rhizosphere: carbon sources and microbial populations. *In* Ecological Interactions in Soil: Plants, Microbes and Animals. Eds. A H Fitter, D Atkinson, D J Read and M B Usher. pp 107–121. Blackwell Scientific Publications, Oxford.

Pate J S, Layzell D B and Atkins C A 1979 Economy of carbon and nitrogen in a nodulated and non-nodulated (NO_3-grown) legume. Plant Physiol. 64, 1083–1088.

Pirt S J 1975 Principles of Microbe and Cell Cultivation. Blackwell Scientific Publications, Oxford, 274 p.

Postgate J R and Hunter J R 1962 The survival of starved bacteria. J. Gen. Microbiol. 29, 233–263.

Reid C P P, Kidd F A and Ekwebelam S A 1983 Nitrogen nutrition, photosynthesis and carbon allocation in ectomycorrhizal pine. Plant and Soil 71, 415–431.

Ryle G J A, Arnott R A, Powell C E and Gordon A J 1983 Comparisons of the respiratory effluxes of nodules and roots in six temperate legumes. Ann. Bot. 52, 469–477.

Ryle G J A, Powell C E and Gordon A J 1979 The respiratory costs of nitrogen fixation in soyabean, cowpea and white clover. II. Comparisons of the cost of nitrogen fixation and the utilization of combined nitrogen. J. Exp. Bot. 30, 145–153.

Sallih Z and Bottner P 1988 Effect of wheat (*Triticum*

aestivum) roots on mineralization rates of soil organic matter. Biol. Fertil. Soils 7, 67–70.

Santantonio D 1979 Seasonal dynamics of fine roots in mature stands of Douglas-fir of different water regimes: a preliminary report: *In* Root Physiology and Symbiosis. Eds. A Reidacker and J Gagnaie-Michard. pp 90–203. Proceedings IUFRO Symposium on Root Physiology and Symbiosis, Nancy France, 1978, Vol. 6. CNRF Champenoux, France.

Schönwitz R and Ziegler H 1982 Exudation of water-soluble vitamins and of some carbohydrates by intact roots of maize seedlings (*Zea mays* L.) into a mineral nutrient solution. Z Pflanzenphysiol. 107, 7–14.

Sims P L and Singh J S 1971 Herbage dynamics and net primary production in certain ungrazed and grazed grasslands in North America. *In* Preliminary Analysis of Structure and Function in Grasslands. Ed. N R French. pp 59–124. Range Science Department Science Series No. 10. Colorado State University, Fort Collins, Colorado.

Singh J S and Coleman D C 1974 Distribution of photoassimulated ^{14}carbon in the root system of a shortgrass prairie. J. Ecol. 62, 359–365.

Sivakumar M V K, Taylor H M and Shaw R H 1977 Top and root relations of field-grown soybeans. Agron. J. 69, 470–473.

Snellgrove R C, Splittstoesser W E, Stribley D P and Tinker P B 1982 The distribution of carbon and the demand of the fungal symbiont in leek plants with vesicular-arbuscular mycorrhizas. New Phytol. 92, 75–87.

Sparling G P, West A W and Whale K N 1985 Interference from plant roots in the estimation of soil microbial ATP, C, N and P. Soil Biol. Biochem. 17, 275–278.

Wainwright M 1988 Metabolic diversity of fungi in relation to growth and mineral cycling in soil – a review. Trans. Br. mycol. Soc. 90, 159–170.

Warembourg F R and Paul E A 1977 Seasonal transfers of assimilated ^{14}C in grassland: plant production and turnover, soil and plant respiration. Soil Biol. Biochem. 9, 295–301.

Whipps J M 1984 Environmental factors affecting the loss of carbon from the roots of wheat and barley seedlings. J. Exp. Bot. 35, 767–773.

Whipps J M 1985 Effect of CO_2 concentration on growth, carbon distribution and loss of carbon from the roots of maize. J. Exp. Bot. 36, 644–651.

Whipps J M 1987 Carbon loss from the roots of tomato and pea seedlings grown in soil. Plant and Soil 103, 95–100.

Whipps J M 1990 Carbon economy. *In* The Rhizosphere. Ed. J M Lynch. pp 59–97. John Wiley, Chichester.

Whipps J M and Lynch J M 1983 Substrate flow and utilization in the rhizosphere of cereals. New Phytol. 95, 605–623.

Whipps J M and Lynch J M 1985 Energy losses by the plant in rhizodeposition. Ann. Proc. Phytochem. Soc. Eur. 26, 59–71.

Whipps J M and Lynch J M 1986 The influence of the rhizosphere on crop productivity. Adv. Microb. Ecol. 9, 187–244.