Chapter 14
Effects of Root Exudates in Microbial Diversity and Activity in Rhizosphere Soils

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14.1 Introduction

The rhizosphere is the soil volume at the root-soil interface that is under the influence of the plant roots and the term was introduced by Hiltner in 1904 (Brimecombe et al. 2001). Microbial population in the rhizosphere has continuous access to a flow of low and high molecular weight organic substrates derived from roots. This continuous flow of organic compounds may affect together with specific physiochemical and biological conditions microbial activity and community structure of the rhizosphere soil (Sorensen 1997; Brimecombe et al. 2001). Current techniques still lack the adequate sensitivity and resolution for data collection at the micro-scale, and the question 'How important are various soil processes acting at different scales for ecological function?' is therefore challenging to answer. The nano-scale secondary ion mass spectrometer (NanoSIMS) represents the latest generation of ion microprobes, which link high-resolution microscopy with isotopic analysis. Recently Herrmann et al. (2007) have described the principles of NanoSIMS and discusses the potential of this tool to contribute to the field of biogeochemistry and soil ecology.

Both microbial activity and microbial diversity of the rhizosphere have been extensively studied as testimonies by numerous chapters and books (Keister and Creagen 1991; Lynch 1990a; Pinton et al. 2001, 2007; Waisel et al. 1991). This interest depends on the important effects that microorganisms inhabiting the rhizosphere have on plant activity. Both beneficial and detrimental interactions occur between microorganisms and plants (Lynch 1990b); among the former symbiotic dinitrogen fixation, association with mycorrhizae, biocontrol against pathogens and production of plant growth promoting compounds by beneficial rhizobacteria have

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been the most studied (Brimecombe et al. 2001). Detrimental interactions are due to the presence of plant pathogens and deleterious rhizobacteria which inhibit plant growth without causing disease symptoms (Brimecombe et al. 2001). However, despite the wealth of information of the effects of rhizodeposition on microbial activity and microbial diversity, there is still considerable debate on the underlying mechanisms and the extent of the relative effects.

This review discusses the state-of-the-art of microbial activity and microbial diversity in the rhizosphere soil. Since it is not possible to prepare an exhaustive review as the complexity and vastness of the treated matter exceeds the limits of a single chapter, we have operated a selection of related topics. In the case of microbial diversity we have mainly discussed the recent advances obtained by using molecular techniques, which allow the detection of unculturable microorganisms. Among the various parameters used to determine microbial activity we have focused the discussion on soil respiration and enzyme activities because the former is strictly linked to organic C mineralization, and thus to oxidation of root exudates, and the latter represent specific reactions involved in the release of plant and microbial available nutrients. The difficulties of interpreting of community-level physiological profiles are also examined. Given the major effects of rhizodeposition on composition and activities of microbial communities inhabiting rhizosphere soil, the initial focus will be on the classification, collection, functions, and factors affecting root exudates.

14.2 Rhizodeposition: Classification, Quantification and Effects on Biotic Processes of the Rhizosphere Soil

Through rhizodeposition, roots introduce into the soil water soluble exudates, polymers such as carbohydrates and proteins, lysates and cell walls, whole cells, whole roots and gases such as CO_2 and ethylene (Morgan and Whipps 2001). Although most root products are C compounds, the rhizodeposited products include ions, sometimes O_2 and even water. Excretions and secretions have a perceived functional role whereas diffusates and root debris do not (Uren 2007). Excretions are the product of internal metabolism such as respiration while secretions are deemed to facilitate external processes such as nutrient acquisition. Both excretion and secretion require energy and some exudates may act as either. For example, protons derived from CO_2 production in respiration are deemed excretions while those derived from an organic acid involved in nutrient acquisition are deemed secretions. Root products differ, not only in their function, but also on the basis of their (a) chemical properties such as composition, solubility, volatility and molecular weight and (b) site of origin. The chemical properties determine in turn the biological activities of these root products and their behaviour in soils; thus the persistence in soil depends on chemical properties, particularly sorption and biodegradability of the root compounds. However, it is important to emphasize, first, that biological activity of some compounds of rhizodeposition such as phytohormones,

exoenzymes, phytoalexins, allelochemicals, and phytotoxins has been studied primarily in solution cultures or under axenic conditions, whereas it is well established that the behaviour of these compounds depends on their survival in the soil (Uren 2007) and, second, that usually specific roles or functions have been assigned to single compounds released from roots (Uren 2007), but it is likely that rhizosphere soil processes are the result of combined effects of more than one root exudate. In addition to attributing specific functions to a root exudate, it is important to calculate the relative amount needed to carry out the specific function. For example, Uren (2007) has calculated that the amount of ascorbic acid released from wheat plants to reduce and dissolve sufficient Mn oxide in the rhizosphere to give the observed Mn concentration in the mature plant should be so high as to be unrealistic. Thus root exudates in addition to resorcinol should be involved in the reduction of Mn.

Amounts and composition of root exudates depend on plant genotype, plant growth stage and environmental conditions such as CO_2, light, pH, temperature, moisture and nutrients (Grayston et al. 1996; Neumann and Römheld 2001). It is well established that plants devote a large proportion of the C fixed to root exudation (0–40% of the total net C assimilated by plants) (Lynch and Whipps 1991). According to Uren (2007), the fixed C committed to roots by the plant is generally divided equally between root tissue and root products. Among the root products, 60% are used in root respiration and the remainder (10% of the net fixed C) is released as border cells, root debris, diffusates and secretions, with the latter being the minor components (Darrah 1996; Lynch and Whipps 1991; Whipps 1990). Of course, caution is required in generalizing these estimates because sick or stressed plants can involve a larger commitment than healthy plants (Farrar et al. 2003).

Root border cells represent root cap cells that are separated from the root apex during root growth (Hawes and Lin 1990; Hawes et al. 2003). In the rhizosphere soil of maize plants these cells remain active among root hairs, secreting mucilage, for up to three weeks after their separation from the root (Foster et al. 1983; McCully 1989, 1995). Then, sooner or later, these cells die and are lysed with release of their content in the external milieu; in this way they contribute to the C transfer from roots to soil (Uren and Reisenauer 1988).

14.3 Methodology for Collecting Root Exudates and Studying the Rhizosphere Effect

Systems used to collect the overall root exudation products such as those based on collection of water soluble root exudates by immersion of the roots into aerated trap solutions (usually solutions with Ca^{2+} so as to stabilize the membrane) yield no information to allow distinguishing the part of the root that has produced the root exudates or determining spatial variability in root exudation (Neumann and Römheld 2001). Thus, these methods do not enable one to determine which section of the root is the source of exudation. It is advisable to avoid long-term exposure of roots to solutions with low ionic strength, as these can stimulate exudation (Jones

and Darrah 1993; Prikryl and Vancura 1980). However, the main inconvenience of these systems is the lack of impedance of solid growth media that stimulate the root exudation (Neumann and Römheld 2001). This limit may be overcome by collecting exudates of plants growing in solid media such as sand or vermiculite and then percolating the media with the trap solution (Johnson et al. 1996) or letting roots grow directly on filter papers (Neumann et al. 1999), resin foil (Kamh et al. 1999) or micro suction cups (Göttlein et al. 1996). The drawback of these methods is the possibility that certain exudates might be adsorbed by the solid medium.

Under field conditions rhizosphere soil samples are usually taken by removing the soil attached to roots and the effect of plant on soil properties is studied by comparing the behaviour of rhizosphere soil with that of the bulk soil. However, this approach presents several drawbacks. In particular, bulk soil can contain root hairs (Norvel and Cary 1992). Furthermore, it is not possible to control the various variables (plant physiology, root age, root section, temperature, moisture, etc.) affecting the root exudation and thus the rhizosphere soil. Microbial community structure assessed by phospholipid fatty acid analysis (PLFA) was more affected by plant species than soil moisture. Community level physiological profiles (CLPP), in terms of diversity of substrate utilization and average well colour development (AWCD), were affected by plant species and soil moisture (Chen et al. 2007).

The study of the rhizosphere is a very complex task and several microcosms have been created to study the dynamics and the interaction between soil, microorganisms and plants in the rhizosphere. Most of these systems are based on the physical separation of rhizosphere and the adjacent bulk soil by porous membranes (Kuchenbuch and Jungk 1982) and may allow sampling soil at different distances from the rhizoplane. These systems are enclosed in so-called rhizoboxes, delimiting vertical or horizontal nets. In some systems the nets are localised in the bottom of the box and the soil below the roots is considered rhizosphere soil (roots mate approach) (Kuchenbuch and Jungk 1982). In the slit system (Hinsinger and Gilkes 1997) the division is made with a 0.2-μm membrane and only the hair roots may grow in a thin layer of soil. In the rhizobox of Li et al. (1991), membranes with different pore diameters can allow penetration of root hairs and hyphae in the soil compartment. The rhizobox set up by Wenzel et al. (2001) allows monitoring of the pH, redox potential and soil moisture during the experiment, through the use of proper microsensors; it also allows the monitoring of root development, distribution and morphology without involving destructive sampling. In spite of the advantages of this rhizobox, which is amenable to dynamic measurements, Wenzel et al. (2001) underlined the importance of combining studies using their rhizobox with field measurements of rhizosphere processes, since any experimental approach based on an in vitro system implies deviations from the indigenous soil-plant system.

Model root systems (MRS) have been used to discriminate between the effect of the various root exudates. In a simple device reported by Badalucco and Kuikman (2001), the soil is pressed to a precise density (1.4 g cm^3) into a plastic ring standing on a Petri dish covered with aluminium foil. The top of the soil is covered with a cellulose paper filter (Whatman 41), which can be wetted with different solutions of root exudates (MRE, model root exudates). This system produces a gradient of

the root exudate at increasing distance from the filter paper (Falchini et al. 2003) and it is possible to sample soil slices at different distances from the filter paper, which simulates the rhizoplane.

When working with model compounds, these should be incorporated in amounts reflecting the daily carbon input to the rhizosphere, ranging usually between 50 and 100 μg C g^{-1} soil (Trofymow et al. 1987; Iijima et al. 2000). Therefore it is unrealistic to apply several mg C g^{-1} of soil (Badalucco and Kuikman 2001; Baudoin et al. 2003).

14.4 Effects of Root Exudates on Microbial Activity of Rhizosphere Soils

Microbial activity of rhizosphere soil varies among different plant species, probably because these differ in root exudates composition (Van der Krift et al. 2001; Warembourg et al. 2003). Plants with a high concentration of root solutes and a rapid growth should stimulate a high rhizosphere microbial activity. This hypothesis was evaluated by Valè et al. (2005) by comparing the rhizosphere microbial activity (in vitro mineralization of a small amount of ^{14}C-glucose) and bacterial abundance (expressed as the number of CFU, or colony-forming units) between six herbaceous species grown in the greenhouse and differing in plant biomass and root C concentrations (including soluble and insoluble C). The microbial activity was positively correlated with root soluble C concentration and shoots biomass and negatively correlated with concentration of insoluble C in roots.

14.4.1 Competition Between Plants and Microorganisms for Soil Nutrients

Since it stimulates microbial growth, rhizodeposition might actually decrease the availability of mineral nutrients in the region of the rhizosphere where it is released. Information on the topology and timing of release of specific compounds from different root districts has been obtained using whole cell biosensors (Jasper et al. 2001; Casavant et al. 2003; see this volume, Chap. 9). However, mineral nutrient immobilization following rhizodeposition does not impair plant growth because the apical regions of the root (i.e. the zone extending from the root hair zone to the root apex) extract most of those nutrients that are available for uptake before extensive rhizospheric colonization by saprophytic microorganisms occurs. If this hypothesis is true, microbial growth should be limited.

On the other hand, microbial growth itself becomes limited following depletion of mineral nutrients in the rhizodeposition zone. This effect was observed in the maize rhizosphere (Merckx et al. 1987). Similarly it was found that microbial respiration was not limited in the rhizosphere of winter wheat by available C

(Cheng et al. 1996) but probably by some other nutrients. Among the major nutrients, N may be the main limiting nutrient for microbial growth due to the high C to N ratio of root products (Marschner 1995); indeed, rhizodeposition by maize plants increased N immobilization but also microbial denitrification due to the presence of easily available organic C (Qian et al. 1997).

Some factors complicate the study of the competition between roots and microorganisms for nutrients. Among these are the fact that: 1) lateral roots explore a soil different from that explored by the main roots; 2) nutrients immobilized by microbial assimilation are likely to be recycled following the death and degradation of microbes and microfauna; 3) root exudation of soluble organic compounds correspond to a net release between efflux and influx; 4) the uptake of any nutrient by plants and microorganisms depends on the form of this nutrient in the rhizosphere soil.

The apices of lateral roots must grow through the rhizosphere of the superior axis from which they originate, and thus they may experience the effects related to the type of exudates produced by the main axis and the exudate using microbial population (Uren 2007).

Nutrients immobilized by microbial assimilation are likely to be recycled following the death and degradation of microbes and microfauna, but the timing and location of such events in relation to the nutrient-absorbing regions of the root are difficult to investigate because of the difficulties in simulating these processes under laboratory conditions. However, Mary et al. (1993) measured the recycling of C and N during the decomposition of root mucilage, glucose and roots by simply mixing low concentrations of these substrates with soil.

The net release between efflux and influx (Darrah and Roose 2001) can be influenced by microorganisms (Phillips et al. 2004). For example, 2,4-diacetylphloroglucinol (DAPG) produced by *Pseudomonas* can block the amino acid influx whereas the fungal product zearalenone can increase amino acids efflux in different plants (alfalfa, *Medicago sativa*, maize, *Zea mays*, and wheat, *Triticum aestivum*). Studies based on the use of the reporter bacteria have highlighted that DAPG is likely to mediate the chemical communication among different populations of rhizobacteria (Maurhofer et al. 2004; see this volume, Chap. 9).

Generally both plants and microorganisms prefer ammonium to nitrate but this is not always so (Badalucco and Kuikman 2001). For example, tomatoes prefer nitrate, white spruce ammonium, and some arctic sedges amino acids (Badalucco and Kuikman 2001). In the rhizosphere soil of pine (*Pine ponderosa*) the plant accounted for 70% of the total NO_3^- consumption whereas it only accounted for 30% of the total NH_4^+ consumption (Norton and Firestone 1996). However, the presence of roots reduced NH_4^+ consumption by both nitrifiers and heterotrophs, and the nitrifiers competed with heterotrophs for NH_4^+, transforming it to nitrate and thus keeping inorganic N in a form available to roots. Using two bacterial biosensors for detecting NO_3^- availability, De Angelis et al. (2005) reported significantly lower NO_3^- availability in the rhizosphere of wild oat than in bulk soil and the competition between roots and the whole-cell bioreporters could be attenuated by soil amendment with NO_3^-.

14.4.2 Soil Respiration

Soil respiration has often been used as an index of microbial activity (Nannipieri et al. 1990). The respiration of rhizosphere soil is greater than that of the bulk soil not located around organic debris, because in the former case CO_2 can originate not only from microbial respiration of soil organic C but also from root respiration and microbial decomposition of rhizodeposition. Raich and Mora (2005) reported that root and rhizosphere soil respiration of an annual crop accounted for 27–30% of the overall soil respiration. Separation between root respiration and CO_2 evolution from rhizosphere soil is methodologically difficult. An interesting approach was followed by Cheng et al. (1993), who saturated soil with unlabelled glucose before the ^{14}C pulse-labelling of plant shoots so as to eliminate the use of labelled substrates released from roots by soil microorganisms. Root respiration and microbial respiration of rhizosphere soil accounted for 40% and 60% of the overall respiration, respectively. Kuzyakov (2002b) suggested that microbial respiration accounted for 50–60% of the total plant-induced respiration. Usually, microbial respiration in the rhizosphere soil is highly dependent on climatic conditions, nutrient availability and root exudation, which is itself controlled by the rate of photosynthesis during light periods (Kim and Verma 1992).

Another interesting approach was followed by Rochette and Flanagan (1997), who grew C_4 (*Zea mays* L) plants on soil developed under C_3 plants (mixture of grass and legumes) to measure the isotope ratio ($\delta^{13}C$) of CO_2 evolved from soil under corn or from soil kept free of vegetation. The $\delta^{13}C$ values of CO_2 from the control soil were significantly lower than those of CO_2 from the corn plots and this allowed to estimate the CO_2 of the rhizosphere soil, which ranged 18–25% of crop net photosynthesis and 24–35% of crop net CO_2 assimilation during most of the growing season.

Recently, CO_2-C evolution from decomposition of root exudates has been studied in systems simulating the rhizosphere zone, by monitoring the mineralization of single synthetic low molecular weight organic compounds commonly present in root exudates (Kozdroj and van Elsas 2000; Badalucco and Kuikman 2001; Baudoin et al. 2003; Falchini et al. 2003; Landi et al. 2005). Thus, Falchini et al. (2003) monitored the diffusion of ^{14}C-labelled glucose, oxalic acid, or glutamic acid into soil from a filter placed on the surface of a sandy loam soil. Glutamate showed a higher mineralization than glucose during the first three days, whereas the mineralization of oxalic acid showed a three-day lag phase. Both glutamate and glucose addition caused a positive priming effect. Hamer and Marschner (2005) reported that fructose and alanine induced a stronger priming effect in forest soil when compared with other root exudates such as oxalic acid and catechol. Oxalic acid induced both negative and positive priming effects whereas catechol always reduced mineralization of soil organic matter. It was not possible to predict the occurrence and magnitude of the priming effect from the chemical and physical soil properties, but it was observed that the priming effect was more pronounced in forest soils containing low biodegradable organic carbon (Hamer and Marschner

2005). Li and Yagi (2004) observed that C inputs by rice (*Oryza sativa*) grown under elevated CO_2 retarded the mineralization of organic matter in the 0–5-cm surface layer of a paddy soil. Several hypotheses have been proposed to interpret positive priming effects. According to Fontaine et al. (2003), addition of easily available organic C stimulates the growth of r-strategists and the successive growth of k-strategists is responsible of the degradation of recalcitrant organic matter. Another hypothesis explains the positive priming effect as due to the increase in the turnover of native microbial biomass (Chander and Joergensen 2001; De Nobili et al. 2001) whereas Kuzyakov et al. (2000) suggested that the activation of soil microorganisms by the addition of the easily available organic C increases enzyme synthesis with higher degradation of soil organic matter. The real and apparent priming effects caused by the addition of ^{15}N labelled fertilizers have been discussed by Jenkinson et al. (1985).

14.4.3 Nutrient Dynamics and Functional Aspects of Rhizosphere Soil

It is well established that root exudation of easily available organic compounds affects nutrient dynamics through the turnover and mineralization of organic compounds (Grayston et al. 1996; Hamilton and Frank 2001; Kuzyakov and Cheng 2001; Kuzyakov 2002a). In this regard the most studied plant nutrients are nitrogen and phosphorus.

The assimilation of root-derived C stimulates microbial N immobilization because the average C/N ratio of rhizodeposition is higher than the C/N ratio of soil microflora (Badalucco and Kuikman 2001). This may result in a temporary reduction of available N to the plant. Only under conditions of low N availability may the rhizosphere be a region of excess C supply where N concentration limits microbial growth (Merckx et al. 1987). Norton and Firestone (1996) suggested that N immobilization rates of rhizosphere soil of *Pinus ponderosa* seedling were limited by NH_4^+ rather than by C availability. The microbial N immobilization promoted by the microbial assimilation of root-derived C can be counterbalanced by the protozoan stimulation of the N mineralization; root exudation promotes bacterial growth with mineralization of organic N and then bacteria are grazed by protozoa with release of NH_4^+ due to the higher C/N ratio of the protozoa than bacteria (Clarholm 1985; Kuikman et al. 1990; Liljeroth et al. 1990, 1994). The higher N mineralization in the rhizosphere soil has been indirectly demonstrated by the higher protease and histidinase activities, both involved in N mineralization processes, in the rhizosphere than bulk soil (Badalucco et al. 1996). It has been postulated that nematodes are the primary consumers of bacteria in the rhizosphere (Griffiths 1990) and they also release ammonia when grazing bacteria because the C/N ratio of the former is higher than that of the latter (Badalucco and Kuikman 2001). According to Jones et al. (2005) the NH_4^+ release as the result of grazing of bacteria by protozoa, nematodes and invertebrates and the slower turnover times of

roots compared to microorganisms (Hodge et al. 2000) counteract the effects due to the fact that microorganisms are superior competitors than plants for both inorganic and organic N sources.

It has been shown that root-derived C stimulates N immobilization-mineralization turnover and denitrification in a greenhouse experiment based on growing maize plants, monitoring ^{13}C natural abundance and ^{15}N added as $^{15}NH_4{}^{15}NO_3$ fertilizer (Qian et al. 1997). The N mineralization-immobilization turnover (MIT) is very important in regulating the amount of N available to plants and it is based on the transformation of organic N to NH_4^+ and the opposite reaction. The alternative pathway is the so-called "direct route" in which microorganisms take up simple organic molecules, such as amino acids, and once these amino acids are inside the microbial cells they are deaminated and the surplus NH_4^+ is released into the extracellular soil environment (Barraclough 1997). Amino acid uptake by rhizobacteria has been proven by using reporter bacteria (Jaeger et al. 1999; Espinosa-Urgel and Ramos 2001; see this volume, Chap. 9).

The amount of available N supplied as fertilizer can affect the type of reactions in the rhizosphere soil. By growing barley plants for 46 days in a sandy loam soil in a cabinet with a ^{14}C-labeled atmosphere and applying either high (169.1 mg N Kg^{-1} wet soil) or low (34.2 mg N Kg^{-1} wet soil) amounts of fertilizer N as ^{15}N labeled ammonium sulphate, it was observed that the proportion of ^{14}C translocated below ground was slightly higher in the high-N than in the low-N treatment and the decomposition of organic matter was reduced in the high-N treatment (Zagal et al. 1993). In contrast, soil microorganisms in the low-N treatment preferred C from soil organic matter over root derived C.

From the global change perspective, increase of atmospheric CO_2 and land cover transformation are among the major impacts caused by human activities. Pinay et al. (2007) determined the effects of two years atmospheric CO_2 enrichment on soil potential respiration (SIR), denitrification (DEA) and nitrification (NEA) activities. Soil microbial activities measured by SIR, DEA and NEA were not sensitive to an increase of atmospheric CO_2 but some of these functions were significantly altered by the type of plant cover, i.e. annuals vs perennials. The relative changes of these microbial activities induced by annual and perennial plants was inversely related to the density and the diversity of the corresponding functional bacterial groups, i.e. change in nitrification (NEA)> change in denitrification (DEA)> change in respiration (SIR). In other words, the functional community with the least diversity and density, i.e. nitrification, was the most affected by the plant cover type and these changes remained after the rain event. In contrast, the respiration process, under the control of a wide diversity and density of microorganisms, did not present any significant change. Denitrification presented an intermediate pattern with significant rate differences after the two year experiment, but functionally converged two months after the rain event.

Increases in bacterial activity were observed in both rhizosphere and bulk soil by Christensen and Christensen (1994) when N was present in limiting concentrations. In addition, Söderberg and Bååth (2004) observed that addition of NH_4^+ but not NO_3^+ decreased bacterial activity (as determined by thymidine and leucine

incorporation) of rhizosphere soil of barley seedlings but not bacterial activity of the bulk soil because plant NH_4^+ uptake decreased the pH of rhizosphere soil due to secretion of H^+ by roots. However, a change in the composition of root exudates in response to the different N source cannot be excluded.

The functional aspects of the microflora inhabiting the rhizosphere soil has been monitored by the community-level physiological profiles (CLPP) generated with sole-carbon-source-utilization tests from BIOLOG (Garland and Mills 1991). In spite of the fact that three closely related legumes, i.e. alfalfa (*Medicago sativa*), common bean (*Phaseolus vulgaris*) and clover (*Trifolium pratense*), showed differences in the composition of the rhizosphere communities as assessed by PCR-SSCP (single strand conformation polymorphism) analysis of 16S rRNA genes, the overall analysis by CLPP indicated that the metabolic potential of all rhizosphere samples was similar (Miethling et al. 2003). Treatment of soil for 14 days with artificial root exudate (glucose, fructose, sucrose, citric acid, lactic acid, succinic acid, alanine, serine and glutamic acid) solution at a rate of 100 μg C g^{-1} day^{-1}, to simulate a daily carbon input to soil by root, markedly changed the BIOLOG oxidation pattern (Baudoin et al. 2003). However, the addition of the exogenous substrates was not followed by an increased oxidation of these compounds in the BIOLOG plates and this does not support the hypothesis that BIOLOG oxidation patterns can be used as an index of substrate availability in situ. It is well established that the CLPP presents several drawbacks. In particular, it is culture-dependent, it does not determine the contribution of fungi and it does not maintain the composition of the microbial communities constant during the incubation (Nannipieri et al. 2003). Degens and Harris (1997) overcame these limitations by measuring the utilization patterns of various substrates by soil microbial communities using short-term responses of soil treated with amino acids, carboxylic acids, carbohydrates and organic polymers.

14.4.4 Enzyme Activity in the Rhizosphere Soil

Enzyme activity is generally higher in rhizosphere than in bulk soil, as a result of a greater microbial activity sustained by root exudates or due to the release of enzymes from roots (Badalucco and Kuikman 2001). The overall enzyme activity of the rhizosphere soil can depend on enzymes localized in root cells, root remains, microbial cells, microbial cell debris, microfaunal cells and the related cell debris, free extracellular enzymes or enzymes adsorbed or inglobated in soil particles. Ultracytochemical techniques have been used with electron microscopy to localize enzymes in electron-transparent components of soil such as microbial and root extracellular polysaccharides, fragments of cell walls and microbial membranes but these techniques cannot be applied in regions of soil with naturally electron-dense particles such as minerals (Ladd et al. 1996). Thus, acid phosphatase has been detected in roots, mycorrhizae, soil microbial cells and fragments of microbial membranes as small as 7×20 nm.

Soil microbes release extracellular enzymes useful for the initial degradation of high molecular weight substrates such as cellulose, chitin and lignin, and mineralise organic compounds to mineral N, P, S and other elements. Enzymes attached to the outer surface of microbial cells, the ectoenzymes, can also carry out the hydrolysis of high-molecular weight substrates (Burns 1982; Nannipieri 1994). In addition to extracellular enzymes, active intracellular enzymes can also be released after cell lysis and remain active in the extracellular soil environment insofar as they do not require cofactors for their activity, extracellular pH and temperature are not denaturing and abiotic inactivation or proteolytic degradation does not occur (Nannipieri 1994). Sorption by soil colloids may protect an enzyme from microbial degradation or chemical hydrolysis and the enzyme can retain its activity if it is not denaturated and its active site is available to the substrates (Nannipieri 1994).

Most extracellular enzymes have a low mobility in soil due to their molecular size and charge characteristics, and thus any secreted enzyme must operate close to the point of secretion and its substrate must be able to diffuse towards it. Acid phosphatase was secreted in response to P deficiency stress by epidermal cells of the main tip roots of white lupin and was present in the cell walls and intercellular spaces of lateral roots (Wasaki et al. 1997). Such apoplastic phosphatase is protected against microbial degradation and cannot be adsorbed by soil colloids, but is effective only when soluble organophosphates, normally present in the soil solution, diffuse in the apoplastic space (Seeling and Jungk 1996). The role of phosphatases in the rhizosphere remains uncertain (Tinker and Nye 2000).

Tarafdar and Jungk (1987) carried out a very interesting study on the relationship between enzyme activity of soil and nutrient cycling in the rhizosphere. They sampled a silt loam soil at different distances from the rhizoplane of either clover (*Trifolium alexandrinum*, 10 days old) or wheat (*Triticum aestivum*, 15 days old) and found that the total P and organic P contents decreased in the rhizosphere soil, whereas the inorganic P content increased in the vicinity of the rhizoplane. Such an increase was probably due to the increase of both acid and alkaline phosphatase activities in the rhizosphere soil and it paralleled the increase in both fungal and bacterial counts, suggesting a probable microbial origin of both enzymes in the rhizosphere soil. Both phosphatase activities increased with plant age, probably as the result of the increase in microbial biomass and/or the increase in total root surface. It has been speculated that plants do not need to secrete phosphatases because the phosphatase activity (mostly of microbial origin) in the rhizosphere soil is generally sufficient to ensure sufficient available P (Tarafdar and Jungk 1987; Tarafdar and Marschner 1994). *Bacillus amyloliquefaciens* FZB45, a plant-growth-promoting rhizobacterium, stimulated growth of maize seedlings under phosphate limitation in the presence of phytate whereas a phytase-negative mutant strain FZB45/M2 did not stimulate plant growth (Idriss et al. 2002). However, the plant origin of phosphatase as of any enzyme of the rhizosphere soil cannot be excluded because plant-borne enzyme can be released in the rhizosphere (Tarafdar and Jungk 1987). Indeed, transgenic *Nicotiana tabacum* (tobacco) or *Arabidopsis thaliana*, which expressed constitutively β-propeller phytase from *Bacillus subtilis* (*168phyA*), secreted

extracellular phytase in much higher amounts than the respective wild-type plants and used sodium phytate as the sole P source (Lung et al. 2005). Similarly, transgenic *Arabidopsis thaliana* with phytase gene (*phyA*) from *Aspergillus niger* was capable of taking up P from a range of organic phosphorus substrates added to agar under sterile conditions (Richardson et al. 2001). However, transgenic *Trifolium subterraneum* L constitutively expressing a phytase gene (*phyA*) from *Aspergillus niger* was capable of exuding phytase and taking up more P than wild-type plant when grown in agar with phytate, but it was not successful when it was grown in soil (George et al. 2004), probably because plant-exuded phytase was adsorbed by soil colloids and/or degraded by soil protease (George et al. 2005).

In a soil-plant (wheat) microcosm, bacterial numbers, protozoan numbers, histidinase and casein hydrolysing activity were monitored after 21 and 33 days of plant growth (Badalucco et al. 1996). Microbial numbers and enzyme activities were higher in the rhizosphere than in the bulk soil; the closer to the soil-root interface, the higher the numbers and the enzyme activities (Badalucco et al. 1996). It was hypothesised that bacteria were the main source of histidinase, whereas protease activity was suggested to be produced by bacteria, protozoa and root hairs.

Using the model rhizosphere system described above, Renella et al. (2005) reported that different root exudates were mineralized to different extents and had different stimulatory effects on microbial growth and on hydrolase activities, mostly localized in the rhizosphere zone. In particular, the rapid increase in the alkaline phosphatase activity could be considered as an indirect evidence of the important role of rhizobacteria in the synthesis of this enzyme in the rhizosphere (Tarafdar and Jungk 1987).

Measurements of enzyme activities have been used to study the effect of transgenic plants on soil metabolism. Both dehydrogenase and alkaline phosphatase activities of soil sampled from transgenic alfalfa, regardless of association with recombinant nitrogen-fixing soil *Sinorhizobium meliloti*, were significantly lower than those of soil sampled from parental alfalfa (Donegan et al. 1999).

Enzyme activities of rhizosphere soil have been measured to assess the perturbation resulting from the introduction of genetically modified microorganisms in the ecosystem (Naseby and Lynch 1998). The inoculation of wheat seeds with a genetically modified strain of *Pseudomonas fluorescens* increased urease and chitobiosidase activities of rhizosphere soil at 0–20 cm depth and decreased alkaline phosphatase but not acid phosphatase activity (Naseby and Lynch 1997). The reduction in alkaline phosphatase activity was attributed to a displacement of the rhizosphere communities producing the enzyme. Opposite changes in the measured enzyme activities were observed when inoculation of wheat seeds with the genetically modified *P. fluorescens* was carried out in the presence of a mixture of urea, chitin and glycerophosphate (Naseby and Lynch 1997).

P. fluorescens F113, which naturally produces the antifungal 2,4-diacetylphloroglucinol (DAPG) and is marked with a *lacZY* gene cassette, increased alkaline phosphatase, phosphodiesterase and arylsulfatase activities of pea rhizosphere

whereas the other inocula reduced enzyme activities compared to the control (without bacterial inoculum) (Naseby and Lynch 1998). It was suggested that increases in enzyme activities were caused by the production of DAPG, which decreased the available inorganic phosphate and sulphate in the rhizosphere being the synthesis of these enzymes controlled by these nutrients (Naseby et al. 1998). However, an opposite trend was found for acid phosphatase activity, which is mostly of plant origin, contrarily to the primarily microbially-determined alkaline phosphatase activity. Therefore, acid phosphatase activity is more dependent upon the nutritional status of the plant. The presence of the F113 strain was associated with low β-galactosidase, β-glucosidase, *N*-acetylglucosaminidase activities and probably this behaviour depended on the increase in available C. On the other hand, no effects on enzyme activities were observed when *Pseudomonas fluorescens* F113 was present in the rhizosphere of field-grown sugar beet (Naseby et al. 1998). It was concluded that the impact of various genetically modified *Pseudomonas* on the rhizosphere populations and functions depended on the nature of the genetic modification (Naseby and Lynch 1998).

The potentialities of enzymes produced by rhizosphere microorganisms, including genetically modified microorganisms, in bioremediation and biocontrol of pests and diseases have been discussed by Naseby and Lynch (2002).

The main problem in interpreting the meaning of enzyme activities in soil are, first, that the current enzyme assays measure the potential rather than the real enzyme activity because the conditions of incubation assays are based on optimal pH and temperature values, optimal substrate concentrations, presence of a buffer and shaking of soil slurries; of course the conditions for enzymes in situ are much different from those used in the assay (Burns 1982; Nannipieri et al. 2002; Gianfreda and Ruggiero 2006) and, second, that the current enzyme assays do not distinguish among different enzymes contributing to the measured total enzyme activity (Burns 1982; Nannipieri 1994; Nannipieri et al. 2002; Gianfreda and Ruggiero 2006). It has been suggested that enzymes can be present in soil in different locations, as intracellular enzymes in active, resting, and dead cells as well as in cell debris and as extracellular enzymes in the soil solution, adsorbed by inorganic colloids or associated in various ways with humic molecules (Nannipieri et al. 2002). It would be important to determine the intracellular enzyme activity of active microbial cells so as to obtain meaningful information on the microbial functional diversity (Nannipieri et al. 2002). Several methods have been proposed to distinguish the extracellular stabilized enzyme activity (activity due to enzyme adsorbed or englobated in soil colloids) from intracellular enzyme activity but all of these have disadvantages (Nannipieri et al. 2002). As discussed above, the situation is more complex in the rhizosphere than in bulk soil, due to the presence of active and still intact root cells detached from the roots, of mycorrhizal cells strictly linked to roots and active bacterial, fungal and faunal cells. All these cells present a broad array of active enzymes.

The source of active enzyme in soil by using the molecular techniques will be discussed in this volume (see Chap. 9).

14.5 Microbial Diversity in the Rhizosphere

Rhizosphere microorganisms are classified on the basis of the interactions with plants, as they can have negative (e.g. phytopathogenic), positive (e.g. plant growth promotion, symbiosis), or neutral (e.g. no benefits) effects on plants (Brimecombe et al. 2001). Symbiotic and pathogenic microorganisms have been well characterized by cultural or direct methods, because of the possible applications of such work in plant protection or crop production, whereas the role of neutral microorganisms in the rhizosphere has been generally neglected. Recently, however, the role of 'neutral' rhizosphere microorganisms in plant nutrition has been re-evaluated (Hirsch et al. 2003; Talbot 2003).

The number of rhizosphere-colonizing microbes has been determined by plate counts or the Most Probable Number (Bakken 1997; Brimecombe et al. 2001; Johnsen et al. 2001). Soil treated with artificial root exudate (glucose, fructose, sucrose, citric acid, lactic acid, succinic acid, alanine, serine and glutamic acid) solution at a rate of 100 μg C g^{-1} day^{-1}, to simulate a daily carbon input to soil by root, markedly increased bacterial counts (Baudoin et al. 2003). Variations of the C/N ratio of the solution added to soil had no effect on the bacterial numbers. It is well established that culture-dependent methods only detect 1–10% of the microorganisms inhabiting the soil (Torsvik et al. 1996). Molecular techniques based on the extraction, purification and characterization of nucleic acids from soil, the BIOLOG technique and phospholipid fatty acid analysis (PLFA) have provided alternative methods for analyses of the microbial diversity in the rhizosphere (Lynch et al. 2004). The limits of the BIOLOG technique have been already discussed. The phospholipid fatty acid (PLFA) technique, which is based on the extraction, fractionation, methylation and chromatography of the phospholipid component of soil lipids, can only be used to estimate gross changes in community structure (Zelles 1999). It is possible to identify species by fatty acid analysis using standard cultural-based media and a suitable database. Molecular methods based on the extraction, purification and characterization of nucleic acids are generally used to study both culturable and unculturable microorganisms in soil. There are a broad variety of these methods for low, intermediate and high resolution analysis (Johnsen et al. 2001; Lynch et al. 2004). Generally, these methods cannot provide the resolution of microbial diversity where it is necessary to identify key microbial species at the community level or to elucidate their role in the ecosystem. These limitations can be overcome to some extent by rRNA gene analysis for microbial diversity studies (see this volume, Chap. 9).

Low-resolution techniques, such as the determination of base distribution in community DNA and the rate at which denatured, single-stranded DNA reanneals, give an estimate of the total genetic diversity (Torsvik and Øvreas 2002; Lynch et al. 2004). Most of the intermediate-resolution techniques are based on comparative analysis of conserved genes such as those coding for ribosomal RNA (rRNA), the so-called rDNA (Johnsen et al. 2001; Lynch et al. 2004). They usually involve polymerase chain reaction (PCR) amplification of rRNA genes from soil DNA

samples, combined with fingerprinting techniques, such as denaturing gradient gel electrophoresis (DGGE), temperature gradient gel electrophoresis (TGGE), terminal restriction fragment length polymorphisms (T-RFLP), amplified rDNA restriction analysis (ARDRA), cloning and sequencing (Johnsen et al. 2001; Torsvik and Øvreas 2002; Lynch et al. 2004). The DGGE (which separates chemically-denatured PCR products of the same size but of different sequences) and T-RFLP (which distinguishes between PCR products by recognizing only the terminal fragment of restriction digestion), have been generally used to study rhizosphere–microbe interactions.

By using DGGE, it was revealed that different plants support different bacterial (Marschner et al. 2002), fungal (Gomes 2003) and archaeal (Nicol et al. 2003) communities and that the structure of microbial communities was affected by root architecture, plant age and various perturbations (Marschner et al. 2002; Nicol et al. 2003). The effect of plant roots on the composition of archaeal communities has been confirmed by PCR single stranded conformation polymorphism (PCR-SSCP) with significant differences in the composition of the Crenarchaeota populations between the rhizosphere soil of different plant species and their respective bulk soil (Sliwinski and Goodman 2004). The effect of root architecture on the composition of bacterial communities from the rhizosphere of grassland species has also been shown by T-RFLP analyses (Kuske et al. 2002). Marschner et al. (2001) found that the composition of bacterial rhizosphere community from three plant species (chickpea, rape and Sudan grass), as determined by PCR-DGGE of 16S rDNA, depended on the complex interaction between soil type, plant species and root zone location. Bacterial diversity was higher in mature root zones than at the root tips in the sand and clay soils but not in the loamy sand soil. They also showed that N fertilization had no significant effect on the composition of bacterial community of the rhizosphere soil whereas both fertilization and soil type influenced plant growth. Stark et al. (2007) recently demonstrated that the addition of green manures improved soil biology by increasing microbial biomass and activity irrespective of management history, that no direct relationship existed among microbial structure, enzyme activity and N mineralization, and that microbial community structure (by PCR–DGGE) was more strongly influenced by inherent soil and environmental factors than by short-term management practices.

Plate counts, but not TGGE fingerprintings, showed an effect of root age on bacterial community structure of rhizosphere soil of *Zea mays* (Gomes et al. 2001). Bacterial community composition on the cluster roots and in the rhizosphere soil, determined by DGGE, differed among three species of *Banksia* (*B. attenuata* R. Brown, *B. ilicifolia* R.Brown and *B. menziesii* R.Brown), and depended on sampling times and cluster root age, as young, mature and senescing roots were distinguished (Marschner et al. 2005). No changes were observed in both acid and alkaline phosphatases, whereas both β-glucosidase and protease activities increased with time. The three species differed in asparaginase activity. Smalla et al. (2001) showed a plant-dependent shift in the relative abundance of bacterial populations by comparing DGGE-fingerprinting of 16S rDNA fragments amplified by PCR from soil or rhizosphere. This shift was more pronounced in the second than in the

first year. DNA sequencing showed that most of the dominant bands from the rhizosphere patterns corresponded to Gram-positive bacteria. The study concerned rhizosphere communities of field grown strawberry (*Fragaria ananassa* Duch), oilseed rape (*Brassica napus* L.) and potato (*Solanum tuberosum* L.), those three plants being host to the pathogenic fungus *Verticillium dahlae*. Both the abundance and composition of *V. dahlae* bacterial antagonists were plant species dependent (Berg et al. 2002). While most studies to date have focused on a single functional gene, analysis of a more complex suite of genes would enable us to better address the role of the community structure in controlling various processes in soil. In the recent years there has been a growing interest in genes and transcripts coding for metabolic enzymes. Besides questions addressing redundancy and diversity, more and more attention is given on the abundance of specific DNA and mRNA in the different habitats. Sharma et al. (2007) have recently reviewed several PCR techniques that are suitable for quantification of functional genes and transcripts such as most probable number (MPN)-PCR, competitive PCR and real-time PCR. These new quality of data is of high relevance to improve mathematic models of turnover processes.

The T-RFLP analysis showed that composition of eubacterial community of rhizosphere of conventionally managed continuous corn and organically managed corn was similar to that of soil light fraction, which includes plant debris of soil, but differed respect to that of heavy fraction, which includes the mineral particles and associated humic matter (Blackwood and Paul 2003). Nunan et al. (2005) have studied the rhizoplane bacterial community rather than the rhizosphere communities, after hypothesising that plant effects on microbial community should be more pronounced on the rhizoplane than in the rhizosphere soil. Neither T-RFLP nor DGGE fingerprints of PCR-amplified 16rDNA did not show any effect of grassland plant species on the bacterial community of rhizoplane.

Neither DGGE nor T-RFLP analyses provide information on key microbial species nor elucidate their role in the rhizosphere if cloning and sequencing are not carried out. In addition, it should be stressed that the selected method for extraction and purification of nucleic acid from rhizosphere soil can markedly affect observations on bacterial community structure (Niemi et al. 2001).

It was found that there was a higher diversity of *amo*A genes (Briones et al. 2003) and *nif*H genes (Cocking 2003) in rhizospheres of rice cultivars and nonlegumes than in bulk soils, respectively. The higher diversity of these two genes encoding key functions in N cycling might suggest that through rhizodeposition, plants select functional groups rather than taxonomic groups of microbes.

Many factors (root architecture, root age, perturbation, stability of soil microflora, etc.) can interfere with the recognition of the effects of plant species on the composition of microbial communities inhabiting rhizosphere soil. In addition, soil microflora appears very stable, since changes due to perturbations are transitory (Nannipieri et al. 2003). An ingenious approach for studying the effects of plant species on composition of microflora by eliminating the problem of the presence of a stable microbial community was carried out by Bardgett and Walker (2004), who studied the effect of colonizer plant species on microbial growth and composition

on recently deglaciated terrain in south-east Alaska by analysing PLFA. Bacterial biomass was increased by *Rhacomitrium*, *Alnus* and *Equisetum* and fungal biomass was increased by *Rhacomitrium* and *Alnus* with respect to bare soil.

The relative importance of specific plant properties vs soil characteristics in determining the composition of bacterial communities of the rhizosphere soil was examined in an innovative experiment, in which *Carex arenaria*, a non-mycorrhizal plant species, was chosen so as to eliminate the confounding factor represented by different levels of mycorrhizal colonization; this plant was grown in 10 different sites with soils presenting different properties (De Ridder-Duine et al. 2005). Bacterial diversity of rhizosphere and bulk soil was analysed by DGGE. It was observed that the diversity of a particular rhizosphere community was more similar to that of the bulk soil community of the same site rather than to that of rhizosphere communities from other sites.

Better insights on the effects of plant root in modifying the structure of soil microbial communities can be obtained by studies in which the rhizosphere effect is simulated by adding specific compounds occurring in root exudates. Both oxalic and glutamic acid changed the DGGE profiles of soil bacterial communities, causing the appearance of few extra-bands in the 0–2-mm soil layer of the model root system (Falchini et al. 2003). Microbial diversity, as determined by ribosomal intergenic spacer analysis (RISA), was changed when soil was treated with a mixture of root exudate compounds (glucose, fructose, saccharose, citric acid, lactic acid, succinic acid, alanine, serine and glutamic acid) at a rate of 100 μg C g^{-1} day^{-1} for 14 days whereas the C/N ratio of the added solution had no effect (Baudoin et al. 2003).

In recent years molecular tools have been developed to analyze the structures of the rhizosphere-associated fungal communities from several crops (Gomes et al. 2003; Kowalchuk 1999; Smit et al. 1999), and also the function and possible role of the observed fungal diversity associated with plant roots, especially their antagonistic potential (Kowalchuk et al. 1997; Vandenkoornhuyse et al. 2003). Gomes et al. (2003) showed a rhizosphere effect of two maize cultivars grown in tropical soils on fungal communities analysed by DGGE of 18 S rDNA amplified by an universal primer. Plant growth development had an effect, whereas no difference was observed between fungal communities of the rhizospheres of the two cultivars. Cloning and sequencing of the dominant bands showed a dominance of members of Ascomycetes and Pleosporales families in young maize plants and a dominance of Ascomycetes and Basidiomycetous yeast in the rhizosphere of senescent plants.

14.6 Effect of Transgenic Plants on Microbial Diversity in the Rhizosphere Soil

Few studies have been conducted to investigate the effect of transgenic plants on soil microbial communities in spite of the several thousands field releases of transgenic crop plants (Kowalchuk et al. 2003; Lynch et al. 2004). Two possible effects

can occur in the rhizosphere soil. With the first, bacterial population inhabiting the rhizosphere soil can capture and stably integrate transgenic plant DNA. In this case it may be risky the acquisition of antibiotic resistance genes, generally used as markers in transgenic crops, because it may change the composition of microbial communities. With the second, both composition and activity of microbial communities of rhizosphere soil can be changed as a consequence of altered root exudation or root morphology in transgenic plants. For example, in the case of transgenic modifications made to improve the plant resistance against microbial pathogens, the composition of rhizosphere microbial communities should be monitored. Indeed, the introduced resistance trait is based on the release of transgenic products such as cell-wall-attacking enzymes or molecules like T4-lysozyme, chitinase or cecropine, which can affect not only bacterial and fungal pathogens but also non-pathogenic microorganisms. Lynch et al. (2004) suggested that a requirement to an accurate monitoring of the effect of transgenic plants on soil microbial diversity is the careful collection of baseline data so as to take natural variations into consideration. In addition, Kowalchuk et al. (2003) have recommended to study effects of transgenic plants on soil microflora in small-scale field experiments since greenhouse conditions can markedly differ from field conditions. The parent variety of the transformed crop should also be included in the experimental design. For example, Dunfield and Germida (2001) observed that the microbial rhizosphere community of the transgenic glyphosate-tolerant oilseed, as determined by fatty acid methyl ester (FAME), was different from those of the three glufosinate ammonium-tolerant oilseed varieties. However, since the parental non-transgenic variety was not compared with the transgenic glyphosate-tolerant oilseed it was not possible to determine if the observed changes were due to the transgenic modification or not (Lynch et al. 2004). Both rhizosphere samples and bulk soil were sampled six times in a two-year, multi-site field study involving a transgenic canicola variety and a conventional variety and the composition of microbial communities as affected by the use of transgenic plants was investigated by community-level physiological profiles, fatty acid methyl ester profiles and terminal amplified ribosomal DNA (Dunfield and Germida 2003). Changes in the composition of microbial communities associated with the introduction of the transgenic variety were temporary and did not persist throughout.

A transgenic T4-lysozyme-expressing potato released detectable amount of T4 lysozyme (De Vries et al. 1999) with bactericidal activity at the rhizoplane (Ahrenholtz et al. 2000). However, the composition of bacterial communities of rhizosphere of both transgenic and a transgenic control without the T4 lysozyme gene, monitored by the BIOLOG approach, fatty acid analysis, DGGE or cloning and sequencing, were influenced by sampling period, plant developmental stage and field site and not by the T4 lysozyme expression (Heuer and Smalla 1997; Heuer et al. 1999; Muyzer and Smalla 1998).

Lynch et al. (2004) have suggested that relevant effects of transgenic plants on composition of microbial communities inhabiting soil should be more important than those due to season and field site.

14.7 Conclusions

Microbial activity, as determined by CO_2 evolution of rhizosphere soil, is often positively correlated with root soluble C concentrations and negatively correlated with non-soluble C. Enzyme activities are also generally higher in the rhizosphere than bulk soil, probably due to higher microbial sources but the currently used enzyme assays do not make it possible to distinguish the respective contributions of microbial, plant and faunal enzymes to the overall measured enzyme activity. Studies on microbial (mostly bacterial) diversity seem to indicate that plant species do not always affect the composition of microbial communities inhabiting the rhizosphere soil, because other factors, such as root architecture, root age, perturbations, stability of soil microflora, etc, also exert a significant influence. Growth in elevated CO_2 may, however, affect decomposition by changing the amount and dynamics of litter fall by modifying litter quality through changes in plant community composition; and by altering the soil environment and its biological activity (by increase of soil water, C input to soil, rhizosphere activity, etc.) with consequent priming of the decomposition of old stable organic matter. These indirect effects can be tested only by long-term studies on litter decomposition in forests exposed to elevated CO_2, but the current literature comprises results only from short-term incubations (Hyvönen et al. 2007).

Techniques used to determine microbial diversity such as those based on DNA fingerprinting have the potential to reveal genetic diversity but say nothing about the expression of these genes (see this volume, Chap. 9). Therefore, a higher microbial diversity that would be promoted by a higher flux of available C in the rhizosphere, as compared to bulk soil, would not necessarily imply a consequent higher diversity of functional genes. A possible objective of further studies would be to relate the utilization of plant C by microbial species to a particular functional role in the ecosystem (see this volume, Chap. 9). However because of current limitations on our understanding with respect to acclimation of the physiological processes, the climatic constraints, and feedbacks among these processes – particularly those acting at the biome scale – projections of C-sink strengths beyond a few decades are highly uncertain.

Interpretation of experiments examining in situ responses of soil microorganisms should be made with caution as incubation studies represent model systems under optimum conditions that rarely occur in the field. However, assessing soil properties under constant conditions allows variables such as soil moisture levels, temperature, microbial-plant interactions and soil type, to be studied individually (Stark et al. 2007). Due to limitations of the currently used methods, studies on microbial processes in the rhizosphere soil should be based on combined measurements of microbial diversity and microbial activity (Fig. 14.1). In this context, an interesting approach was followed by Kourtev et al. (2003), who worked with two exotic plant species, a Japanese barberry (*Berberis thubergii*, D.C., a hardy shrub forming tickets of multi-stemmed plants) and a Japanese stilt grass (*Microstegium vimineum*, Camus, a C4 annual plant), and with a native under-story plant

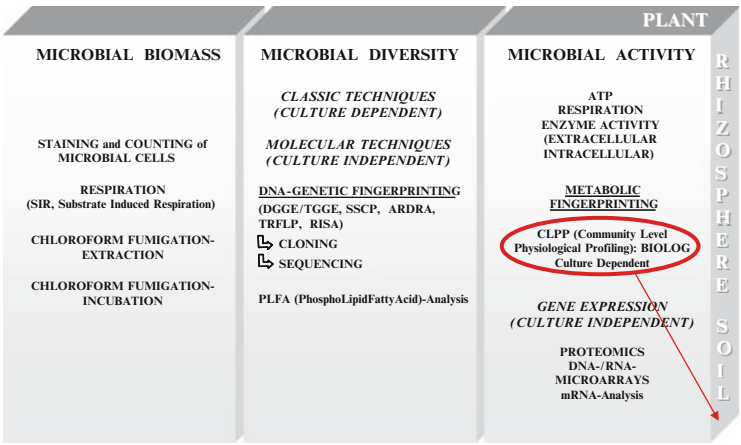

Fig. 14.1 Recommended multidisciplinary approach combining studies on microbial diversity, activity and biomass, to assess the rhizosphere soil microflora and its interactions with plants

(*Vaccinium* spp, a blueberry). The two exotic and the native species were grown in the same soil, and their effect on both microbial diversity and microbial activity was examined. After a three-month incubation period, soils planted with the exotic species differed in PLFA profiles, enzyme activities and SIR profile as compared with the initial soil or with the soil under the native under-story plant. Endocellulase, aminopeptidase, alkaline phosphatase and phenol oxidase activities were higher in stilt grass soil than in barberry or blueberry soils, whereas cellulase, acid phosphatase, peroxidase activity decreased in all planted soils.

There is need for better integration between plant physiology and molecular biology with soil chemistry, physics, and microbial and mesofaunal ecology. Working in isolation can still advance the field; however, the biggest advances will be made when scientific fields are integrated. It has been assumed that each compound released by roots has a specific role or function, but the reality is that very few proposed effects are established (Uren 2007). Probably the reality involves combination of more than one single root exudate compounds and future research should be directed at quantifying the significance of root exudates under realistic plant-soil systems.

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