REVIEW

MICROBIAL DIVERSITY AND MICROBIAL ACTIVITY IN THE RHIZOSPHERE

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INTRODUCTION

It is well established that microbial life only occupies a minor volume of soil being localised in hot spots such as the rhizosphere soil (Nannipieri *et al.*, 2003), where microflora has a continuous access to a flow of low and high molecular weight organic substrates derived from roots. This flow, together with specific physical, chemical and biological factors, can markedly affect microbial activity and community structure of the rhizosphere soil (Sorensen, 1997; Brimecombe *et al.*, 2001). Both beneficial and detrimental interactions occur between microorganisms of rhizosphere soil and plants, and the matter as been extensively studied as shown by several chapters and books (Lynch, 1990a; Keister & Creagen, 1991; Waisel *et al.*, 1991; Brimecombe *et al.*, 2001; Pinton *et al.*, 2001) on the subject.

Rhizosphere is considered the soil volume surrounding the rhizoplane and the term was firstly conied by Hiltner in 1904 (Brimecombe *et al.*, 2001). In his memory a successful (more than 400 participants) meeting was organized in Munich in 2004.

The aim of this review is to discuss both composition and activity of microbial communities of rhizosphere soil, which are affected by rhizodeposition, a term that includes all substances released from roots to soil. Type of compounds released by roots, and systems used to study the rhizosphere effect will be also discussed. Since it is not possible to prepare an exhaustive review as the complexity and the vastness of the treated matter exceeds the limits of this short contribution, the review summarises the main topics without a detailed dicussion of the underlying mechanisms. Relevant reviews are cited more than original reports and the reader may consult cited books and reviews for a better knowledge of the treated matter.

Rhizodeposition and systems to study the rhizosphere effect

Rhizodeposition includes both low and high-molecular weight compounds including monomers such as glucose and amino acids, polymers such as polysaccharides and proteins, root debris and root border cells, root cap cells separated from the root apex during root grwoth (Hawes & Lin, 1990; Hawes *et al.*, 2003). Plants invest a lot of energy in root exudation, which depends on light intensity, temperature, type of plant, nutritional state of plants, stress factors, microbial activity in the rhizosphere and type of soil (for example soil texuture and thus mechanical impedence). Uren (2007) has calculated that 50% of the net carbon fixed by plant is devoted to roots, 15% of the net fixed carbon by plant is respired by roots as CO_2 and 10% is released as root debris including border cells, whereas diffusates and secretions accounted for less than 1%. The organic substances released from roots to the rhizosphere soil support higher microbial biomass and microbial activity in the rhizosphere than in the bulk soil.

Not all compounds released from roots are organic because roots can also release proton, oxygen and water. Root products can be classified according to their perceived function in excretions (CO_2, bicarbonate ions, H^+, electrons, ethylene, etc.) and secretions (mucilage, H^+, electrons, enzymes, siderophores, etc.) with the former being thought to facilate internal metabolism and the latter external processes such as nutrients uptake (Uren, 2007). Protons are excretions when derived from from CO_2 respiration and are secretions when derived from organic acids and used in nutrient uptake. The root products can also be classified according to their chemical properties (composition, solubility, stability, volatility, molecular weight, etc.) and site of origin. Secretions can be classified according to their biological activity (chemical signals, phytoalexins, phytohormones, ectoenzymes, alleloche-micals, etc.). Molecular signals are being increasingly studied due to their role in the microbial and plant interactions (Perry *et al.*, 2007). Chemical properties can markedly affect the persistence of root exudates because several processes (adsorption, biodegradation volatilization, chemical degradation ,etc) can inactivate a root exudate, whose activity is related to the soil volume through it diffuses and to its stability. Low-molecular-weight exudates can difuse to

a longer distance than high-molecular weight compounds but they are more readily assimilated by soil microorganisms. Generally the function of root exudates has been studied by considering the action of a single compound without considering the presence of the right set of circumstances (Uren, 2007). For example, it is well established that organic acids such as citric acid released from roots play an important role in nutrient uptake (see the great amounts released from lupin in calacreous soils) and in the resistance to Al toxicity (citric and malic acid released from wheat and maize root apex). However, these studies have been gene-rally carried out in pure culture without considering all processes listed above that can inactivate the root exudate in soil. There are some evidences that a combined action of at least two compounds released from roots is involved in nutrient acquisition or in molecular signalling.

The mechanisms of plant release are different. The release of low-molecular weight compounds is a passive process along the steep concentration gradient existing between the cytoplasm of root cells and the soil solution whereas the release of high-molecular weight compounds occurs through vesicle transport (Neumann & Römheld, 2007). However, probably the release of high amounts of citrate, maleate, oxalate, phytosiderophores, etc., in response to toxicant such as Al or in response to nutrient deficiencies, is not passive and it may occur in ion-channels under the control of specific mechanisms, not yet understood (Neumann & Römheld, 2007). Root exudation is generally confined to apical root zones. However, root architecture, and thus exudation can change depending on the nutrional status of plants. For example, it is well known that lupin (*Lupinus albus*) can produce cluster roots under P and Fe deficiency (Neumann & Römheld, 2007). It is also well established that low molecular weight exudates are immediately available to microorganisms inhabiting rhizosphere soil and rhizoplane whereas high-molecular wrigth compounds are generally hydrolysed by hydrolases in smaller compounds which can be taken up by microbial cells.

Several methods are available to collect and identify root exudates. They can roughly be divided in methods based on the immersion of roots into aerated and sterile trap solutions with collection of root exudates and methods based on the growth of plants in solid media such as sand or vermiculite (Neumann & Römheld, 2007). The former methods present as drawbacks the absence of impedences, which stimulates root exudation, and the impossibility to detect the source of root exudation. The drawback of the latter methods is the adsorption of root exudates to solid particles of the medium. Today several microcosms are available for studying rhizosphere soil and most of them are based on the physical separation of rhizoplane from the adjacent rhizosphere soil; membranes have porous that allow diffussion of root exudates and the penetration of root hairs and hyphae in the soil compartment; these membranes can be horizontally or vertically located. These systems are usually called rhizo-boxes and some of them, such as the rhizobox set up by Wenzel *et al.*, (2001), are very sophisticated allowing the measurement of the pH, redox potential and soil moisture with proper microsensors.

The study of the effect of root exudates on activity and composition of microbial communties of rhizosphere soil can be done under laboratory conditions with systems that can isolate involved factors. In our laboratory we use the system reported by Badalucco & Kuilman (2001) in which the soil can be pressed to a precise soil density into a plastic ring standing on a Petri dish covered with aluminium foil. The top of soil is covered with a cellulose paper filter (Whatman 41), which can be wetted with different solutions of root exudates so as to create a concentration gradient of the root exudate(s) under study at increasing distance from the filter paper, which simulate the rhizosplane. It is possible to sample soil slices at increasing distance from the filter paper so as to have an idea of the extent of the rhizosphere effect In addition, it is possible to study the effect of a single or a mixture of root exudates, whose amount can reflect the daily carbon input into rhizosphere.

Effect of plant roots on the composition of microbial communities in the rhizosphere soil

It is well established that the number of microorganisms is higher in rhizosphere than bulk soil (Brimecombe, 2001) and this has been assessed by plate counts or the Most Probable Number analysis; however, both techniques only determine 1-10% of the total microflora inhabiting soil (Torsvik *et al.*, 1996; Bakken, 1997; Johnsen *et al.*, 2001). The use of techniques based on phospholipid fatty acid analysis (PLFA) and extraction of nucleic acids from soil has allowed to determine the unculturable microorganisms (Johnsen *et al.*, 2001; Lynch *et al.*, 2004). The PLFA technique is based on extraction, fractionation, methylation and chromatography of the phospholipid component and it can estimate gross changes in community structure (Lynch *et al.*, 2004). Usually the use of PLFA technique has detected differences in the composition of microbial communties when rhizosphere and bulk soils were compared. Composition of microbial communties of rhizosphere soil under *Populus grandidentata* differed from those of the bulk soil but they were not affected by

elevated atmospheric CO_2 (Zak *et al.*, 1996). Both PLFA analysis and plate counts were able to detect differences in the abundance of Gram-negative bacteria between the rhizosphere of two wheat cultivars (Diab El Arab *et al.*, 2004). Generally Gram-negative bacteria are stimulated by rhizodeposition wheras Gram-positive bacteria are inhibited (Steer & Harris, 2000; Soderberg *et al.*, 2004; Johansen & Olsson, 2005). The use of molecular tecnhiques, based on the extraction, purification and characterization of nucleic acids from soil, can allow a better resolution of microbial diversity than the PLFA technique (Johnsen *et al.*, 2001; Nannipieri *et al.*, 2003; Lynch *et al.*, 2004). Both denaturing gradient gel electrophoresis (DGGE) and terminal restriction fragment length (T-RFLP) have the most used to study rhizosphere-microbe interactions. They are generally based on sequences differences of the conservative genes coding for riboso-mal RNA(rRNA), the so called rDNA; these genes are amplified by polymerase chain reaction (PCR) using specific primers and the amplicons are separated by DGGE or T-RFLP (Johnsen *et al.*, 2001; Lynch *et al.*, 2004). It has been shown that the structure of microbial communities inhabiting rhizosphere soil can be affected by root architecture, root age and plant age (Gomes *et al.*, 2001; Kuske *et al.*, 2002; Marschner *et al.*, 2002; Nicol *et al.*, 2003) but the complex interaction between soil type, plant species and root zone location probably is the main factor (Marschner *et al.*, 2001). The type of soil management as well as the type of fertilization can also be important. The composition of eubacterial community of rhizosphere of conventionally managed cotinuous corn was similar to that of the light fraction, which includes plant debris, but differed with respect to that of heavy fraction, which includes mineral particles and the associated humic fractions, of bulk soil (Blackwood & Paul, 2003). Both T-RFLP and DGGE fingerprintings of PCR-amplified 16 rDNA did no show any difference between the composition of bacterial communities of rhizosphere and that of rhizoplane, where the effect of rhizodepositon should be more pronounced (Nunan *et al.*, 2005). Higher diversity of functional genes suche as *amo*A and *nif*H genes was present in rhizosphere than bulk soil (Briones *et al.*, 2003; Cocking 2003). It is obvious that many factors (root architecture, root age, perturbation, stability of soil microflora, etc.) can interfere with the effects of plant species on the composition of microbial communities of rhizosphere soil. In addition, soil microflora appears very stable, since changes due to perturbations are transitory (Nannipieri *et al.*, 2003). Thus the plant effect can be more easily studied in young soils without a stable microbial community. Bardgett and Walker (2004) 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 *Rhacomi-trium*, *Alnus* and *Equisetum* and fungal biomass by *Rha-comitrium* and *Alnus* with respect to bare soil.

An important experiment concerned the effect of plant properties versus soil characteristics in determining the composition of bacterial communities (analysed by DGGE) of the rhizosphere soil was conducted with *Carex arenaria* (de Ridder-Duine *et al.*, 2005). This non-mycorrhizal plant species was chosen so as to eliminate the confounding factor represented by different levels of mycorrhizal colonization and it was grown in 10 different sites with soils presenting different properties. 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 rhizos-phere communities from other sites.

The number of studies concerning the diversity of fungal communities by molecular techniques in the rhizosphere soil is lower than that on bacterial diversity because molecular tools for fungi has been devoloped later. Analysis of on fungal communities by DGGE of 18S rDNA amplified by an universal primer showed a rhizosphere effect of two maize cultivars grown in tropical soils and plant growth development was more important than cultivar type (Gomes *et al.*, 2003). 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.

Effects of single compounds of root exudates on the composition of microbial communities of rhizosphere soil have been studied by using the system reported by Badalucco and Kuikman (2001). Composition of bacterial communities as determined by DGGE profiles were temporarily affected by oxalic acid or glutamic acid but not by glucose (Falchini *et al.*, 2003). A mixture of root exudates compounds was also effective in affecting microbial diversity as determined by ribosomal intergenic spacer analysis (Baudoin *et al.*, 2003).

Effect of plant roots on the activity of microbial communities of the rhizosphere soil

Nannipieri *et al.* (2003) have reported that microbial activity can be evaluated in soil by measuring different parameters. Of course it is not possible to list here all bibliography concerning the effects of plant roots on these different parameters due to the limited space of this contribution. It will be only discussed the bibliography relative to respiration and enzyme activities of rhizos-

phere soil because these two parameters have been the most used. In addition, problems in interpreting the effect of root exudates and environmental conditions on these two parameters as well as the relative methodological problems are representative of problems of any measurement of microbial activity and of interpretation of the relative data.

Soil respiration. Usually respiration of rhizosphere soil is higher than respiration of bulk soil because, in addition to microbial respiration of soil organic C there is the contribution of root respiration and microbial decomposition of rhizodeposition. Separation between root respiration and CO_2 evolution from rhizosphere soil is methodologically difficult. Cheng *et al.* (1993) estimated that root respiration and microbial respiration of rhizosphere soil accounted for 40 and 60% of the overall respiration, respectively. They distinguished the contributions of these two processes to rhizosphere respiration after soil saturation with unlabelled glucose before the ^{14}C pulse-labelling of plant shoots so as to eliminate the use of labeled substrates released from roots by soil microorganisms. Kuzyakov (2002) suggested that microbial respiration accounted for 50-60% of the total plant-induced respi-ration. 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 & Verma, 1992). The amount of soluble and available organic C but not of concentration of insoluble C can immediately stimulate microbial activity in the rhizosphere soil (Valè *et al.*, 2005).

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 3 d, whereas the mineralization of oxalic acid showed a 3 d lag phase. Both glutamate and glucose addition caused a positive priming effect. Several hypotheses have been proposed to interpret positive priming effects. According to Fontaine *et al.*, (2003) addition of easily available organic C can stimulate 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 increased 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).

Enzyme activities 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 & Kuikman, 2001). The overal enzyme activity of the rhizosphere as well as bulk 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 cells 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 x 20 nm, but not associated to clay particles in soil.

Soil microbes release extracellular enzymes 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 be also be released after cells 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.

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.

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. *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 & Jungk, 1987). Indeed, transgenic *N. 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 were capable of using 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 pro-tease (George *et al.*, 2005).

Using the model rhizosphere system described by Badalucco and Kuikman (2001), 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. The rapid increase in the alkaline phosphatase activity could be considered as an indirect evidence of the important role of rhizo-bacteria in the synthesis of this enzyme in the rhizosphere (Tarafdar & 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 nitrogenfixing 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 & 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 & 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 & 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 & 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 phos-phatase 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 & 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 & Lynch (2002).

The main problem in interpreting the meaning of enzyme activities in soil are: i) the current enzyme assays measure the potential rather 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 & Ruggiero, 2006) and ii) 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 & 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 arrays of active enzymes.

CONCLUSIONS

Measurements of microbial activities by classical assays (for example, by measuring enzyme activities) combined with mesurements based on molecuar techniqeus can improve our knowledge on rhizosphere processes and conduct to a better understanding of the meaning of measurements of microbial activity in soil. Sludge application to a pasture soil increased chitinase activity but decreased the diversity of chitinases contributing to the measured enzyme activity with prevailence of actinobacterium-like chitinase sequences, as determined by the analysis of clone libraries constructed from 18 subgroup A chitinases, amplified by using community DNA extracted from soil and analysed by restriction fragment length polymorphism (Metcalfe *et al.*, 2002).

Studies on gene expression in the rhizosphere soil can permit a better understanding of processes such as biological control, stimulation of microbial activtiy by root exudates, competition between microorganisms and roots for nutrients, moecular colloquia between microorganisms, between roots and between roots and microorganisms. Techniques for extracting and characterising mRNA from soil are now available (Nannipieri *et al.*, 2003) whereas soil proteomic is still in its infancy, even if the relative methodological problems and the potential applications have been discussed (Nannipieri, 2006; Ogunseitan, 2006). An advancement in linking between functional activity to community structure has been obtained by applying stable isotope probe (SIP) to soil (Radajewski *et al.*, 2000; Manefield *et al.*, 2006). According to Manefield *et al.*, (2006) it can be more rewarding to use labelled root exudate compounds and monitoring microorganisms of rhizosphere soil involved in the assimilation of the target compound by the use of any SIP technique, than to pulse the whole seedlings and then monitoring labelled nucleic acids or PLFA of microorganisms of rhizosphere metabolising the labelled root exudates.

Reporter technology has been used to assess several functions in the rhizosphere soil including gene epression even at the single cell level (Sórensen & Nybroe, 2006). The ever increasing knowledge of the promoter and regulator gene along with the refinement of reporter gene insertion techniques will allow using the reporter gene technique for monitoring induction, expression and regulation of virtually any gene in the rhizosphere. In addition, also in this case the methodological improvement of the technique will allow designing new reporter bacteria to respond to specific root exudates, as it already occurs for specific signal involved in molecular colloquia (Sørensen & Nybroe, 2006) .

REFERENCES

Badalucco, L & PJ Kuikman. 2001. Mineralization and immobilization in the rhizosphere. *In*: R Pinton; Z Varanini; P Nannipieri *(eds)*. The Rhizosphere. Biochemistry and Organic Substances at the Soil-Plant Interface. Marcel Dekker, New York, pp. 141-196.

Badalucco, L; PJ Kuikman; P Nannipieri. 1996. Protease and deaminase activities in wheat rhizosphere and their relation to bacterial and protozoan populations. *Biol Fertil Soils* 23: 99-104.

Bakken, LR. 1997. Culturable and nonculturable bacteria in soil. *In:* JD van Elsas; JT Trevors & EMH Wellington *(eds)*. Modern Soil Microbiology. Marcel Dekker, New York, pp 47-61.

Bardgett, RD & LR Walker. 2004. Impact of coloniser plant species on the development of decomposer microbial communities following deglaciation. *Soil Biol Biochem*. 36: 555-559.

Baudoin, E; E Benizri & A Guckert. 2003. Impact of artificial root exudates on the bacterial community structure in bulk soil and maize rhizosphere. *Soil Biol Biochem*. 35: 1183-1192

Blackwood, CB & EA Paul. 2003. Eubacterial community structure and population size within the soil light fraction, rhizosphere, and heavy fraction of several agricultural systems. *Soil biol Biochem*. 35: 1245-1255.

Brimecombe, MJ; FA De Lelj & JM Lynch. 2001. The Rhizosphere. The Effect of Root Exudates on Rhizosphere Microbil Populations. *In:* R Pinton; Z Varanini & P Nannipieri *(eds.)*. The Rhizosphere. Biochemistry and Organic Substances at the Soil-Plant Interface. Marcel Dekker, New York, pp 95-140.

Briones, AM; O Satoshi; U Yoshiaki; R Niels-Birger; R Wolfgang & O Hidetoshi. 2003. Ammonia-oxidising bacteria on root biofilms and their possible contribution to N use efficiency of different rice cultivars. *Plant Soil* 250: 335-348.

Burns, RG. 1982. Enzyme activity in soil: location and a possoble role in microbial ecology. *Soil Biol. Biochem.* 14: 423-427.

Chander, K & RG Joergensen. 2001. Decomposition of ^{14}C glucose in two soils with different amounts of heavy metal contamination. *Soil Biol Biochem*. 33: 1811-1816.

Cheng, W; DC Coleman; CR Carrol & CA Hoffman. 1993. *In situ* measurement of root respiration and soluble C concentrations in the rhizosphere. *Soil Biol. Biochem*. 25: 1189-1196.

Cocking, EC. 2003. Endophytic colonisation of plant roots by nitrogen-fixing bacteria. *Plant Soil* 252: 169-175.

De Nobili, M; M Contin; C Mondini & PC Brookes. 2001. Soil microbial biomass is triggered into activity by trace amounts of substrate. *Soil Biol Biochem.* 33: 1163-1170.

De Ridder-Duine, AS; GA Kowalchuk; PJA Klein Gunnewiek; W Smant; JA van Een & W de Boer. 2005. Rhizosphere bacterial community composition in natural stands of *Carex arenaria* (sand sedge) is determined by bulk soil community composition. *Soil Biol Biochem*. 37: 349-357.

Diab El Arab, HG & RA Vilich Sikora. 2004. The use of phospholipid (PLFA) in the determination of rhizosphere specifric microbial communities (RSMC) of two wheat cultivars. *Plant soil* 228: 291-297.

Donegan, KK; RJ Seidler; JD Doyle & LA Porteous. 1999. A field study with genetically engineered alfalfa inoculated with recombinant *Sinorhizobium meliloti*: effects on the soil ecosystem. *J Appl Ecol* 36: 920-936.

Falchini, L; N Naumova; PJ Kuikman; J Bloem & P Nannipieri. 2003. CO_2 evolution and denaturing gradient gel electrophoresis profiles of bacterial communities in soil following addition of low molecular weight substrates to simulate root exudation. *Soil Biol Biochem.* 36: 775-782.

Fontaine S; A Mariotti & L Abbadie. 2003. The priming effect of organic matter: a question of microbial competition? *Soil Biol Biochem*. 35: 837-843.

George, TS; AE Richardson; PA Hadobas & RJ Simpson. 2004. Characterization of transgenic *Trifolium subterraneum* L which expresses *phyA* and release extracellular phytase: growth and P nutrition in laboratory media and soil. *Plant, Cell and Environment* 27: 1351-1361.

George, TS; AE Richardson & RJ Simpson. 2005. Behaviour of plant-derived extracellular phytase upon addition to soil. *Soil Biol Biochem*. 37: 977-988.

Gianfreda, L & P Ruggiero. 2006. Enzyme activities in soil. *In:* P Nannipieri & K Smalla *(eds)*. Nucleic acids and proteins in soil. Springer Verlag, Heidelberg, Germany, pp 257-311.

Gomes, NCH; H Heuer; J Schonfeld; R Costa; L Hagler-Mendoca & K Smalla. 2001. Bacterial diversity of the rhizosphere of maize (*Zea mays*) grown in tropical soil studied by temperature gradient gel electrophoresis. *Plant Soil* 233: 167-180.

Gomes, NC; O Fagbola; R Costa; NG Rumjanek; A Buchner; L Mendonc; A Hagler & K Smalla. 2003. Dynamics of fungal communities in bulk and maize rhizosphere soil in the tropics. *Appl. Environ. Microbiol.* 69: 3758-3766.

Hawes, MC & HJ Lin. 1990. Correlation of pectolytic enzyme activity with the programmed release of cells from root caps of pea (*Pisum sativum*). *Plant Physiol.* 94: 1855-1859.

Hawes, MC; G Bengough; G Cassab & G Ponce. 2003. Root caps and rhizosphere. *J Plant Growth Regul* 21: 352-367.

Idriss, EI; O Makarewicz; A Farouk; K Rosner; R Greiner; H Bochow; Ritcher & R Borriss. 2002. Extracellular phytase activity of *Bacillus amyloliquefaciens* FZB45 contributes to its plant-growth-promoting effect. *Microbiology* 148: 2097-2109.

Jenkinson, DS; RH Fox & JH Rayner. 1985. Interactions between fertilizer nitrogen and soil nitrogen-the so-called "priming" effect. *J Soil Sci* 36: 425-444.

Johnsen, A & S Olsson. 2005. Using phospholipid fatty acid technique to study short-term effects of the biological control agent *Pseudomonas fluorescens* DR54 on the microbial microbiota in barley rhizosphere. *Micro Ecol.* 49: 272-281.

Johnsen, K; CS Jacobsen; V Torsvik & J Sørensen. 2001. Pesticide effects on bacterial diversity in agricultural soils-a review. *Biol Fertil Soils* 33: 443-453.

Keister, DL & PB Creagan. 1991. The Rhizosphere and Plant growth. Kluwer Academic Publisher, Dordrecht, The Netherlands.

Kim, J & SB Verma. 1992. Soil surface CO_2 flux in a flux in a Minnesota peatland. Biogeochem 18: 37-51.

Kuske, CR; OT Lawrence; EM Mark; MD John; AD Jody; MB Susan & B Jayne. 2002. Comparison of soil bacterial communities in rhizosphere of three plant species and the interspaces in an arid grassland. *Appl. Environ. Microbiol.* 68: 1854-1863.

Kuzyakov, Y. 2002. Separating microbial respiration of exudates from root respiration in a non-sterile soil: a comparison of four methods. *Soil Biol Biochem.* 34: 1621-1651.

Kuzyakov, Y; JK Friedel & K Stahr. 2000. Review of mechanisms and quantification of priming effects. *Soil Biol Biochem* 32: 1485-1498.

Ladd, JN; RC Foster; P Nannipieri & JM Oades. 1996. Soil structure and biological activity *In:* G Stotzky & JM Bollag *(eds).* Soil Biochemistry vol 9. Marcel Dekker, New York, pp 23-78.

Lynch, JM. 1990a. The Rhizosphere. John Wiley and Sons, New York.

Lynch, JM; A Benedetti; H Insam; PM Nuti; K Smalla V Torsvik & P Nannipieri. 2004. Microbial diversity in soil: ecological theories, the contribution of molecular techniques and the impact of transgenic plants and transgenic microorganisms. *Biol Fertil Soils* 40: 363-385.

Lung, SC; WL Chan; W Yip; L Wang; EC Yeung & BL Lim. 2005. Secretion of beta-propeller phytase from tobacco and *Arabidopsis* roots enhances phosphorus utilization. *Plant Sci.* 169: 341-349.

Manefield, M; RI Griffiths; A Whiteley & M Bailey. 2006. Stable isotope probing: a critique of its role in linking phylogeny and function. *In:* P Nannipieri & K Smalla *(eds).* Nucleic Acids and Proteins in Soil. Springer Verlag, Heidelberg, pp 205-255.

Marschner, P; CH Yang; R Lieberei & DE Crowley. 2001. Soil plant specific effects on bacterial community composition in the rhizosphere. *Soil Biol Biochem.* 33: 1437-1445.

Marschner, P; N Günter; K Angelika; W Laure & L Reinhard. 2002. Spatial and temporal dynamics of the microbial community structure in the rhizosphere of cluster roots of white lupin (*Lupinus albus* L.). *Plant Soil* 246: 167-174.

Metcalfe, AC; M Krsek; GW Gooday; JI Prosser & EM Wellington. 2002. Molecular analysis of a bacterial chitinolytic community in an upland pasture. *Appl Environ Microbiol.* 68: 5042-50505.

Nannipieri, P. 1994. The potential use of enzymes as indicators of productivity, sustainability and pollution. *In:* CE Pankhurst; BM Doube; VVSR Gupta & PR Grace *(eds).* Soil Biota- Management in Sustainable Farming Systems. CSIRO, East Melbourne Australia, pp 238-244.

Nannipieri, P. 2006. Role of stabilised enzymes in microbial ecology and enzyme extraction from soil with potential applications in soil proteomics. *In:* P Nannipieri & K Smalla *(eds).* Nucleic Acids and Proteins in Soil. Springer, Heidelberg, pp 75-94.

Nannipieri, P; E Kandeler & P Ruggiero. 2002. Enzyme activities and microbiological and biochemical processes in soil. *In:* RG Burns & RP Dick *(eds).* Enzymes in the Environment. Marcel Dekker, New York, pp 1-33.

Nannipieri, P; J Ascher; MT Ceccherini; L Landi; G Pietramellara & G Renella. 2003. Microbial diversity and soil functions. *Eur J Soil Sci* 54: 655-670.

Naseby, DC & JM Lynch. 1997. Rhizosphere soil enzymes as indicators of perturbations caused by enzyme substrate addition and inoculation of a genetically modified strain of *Pseudomonas fluorescens* on wheat seed. *Soil Biol Biochem.* 29: 1353-1362.

Naseby, DC & JM Lynch. 1998. Impact of wild-type and genetically modified *Pseudomonas fluorescens* on soil enzyme activities and microbial population structure in the rhizosphere of pea. *Mol Ecol.* 7: 617-625.

Naseby, DC & JM Lynch. 2002. Enzymes and microorganisms in the rhizosphere. *In:* RG Burns & RP Dick *(eds).* Enzymes in the environment. Activity, ecology and applications. Marcel Dekker, New York, pp 109-123.

Naseby, DC; JP Moënne-Loccoz; F O'Gara & JM Lynch. 1998. Soil enzyme activities in the rhizosphere of field-grown sugar beet inoculated with the biocontrol agent *Pseudomonas fluorescens* F113. *Biol Fertil Soils* 27: 39-43.

Neumann, G & V Römheld. 2007. The release of root exudates as affected by the plant physiological status. *In:* R Pinton; Z Varanini & P Nannipieri *(eds).* The Rhizosphere. Biochemistry and Organic Substances at the Soil-Plant Interface. Taylor and Francis Group, Boca Raton, FL, pp 23-72.

Nicol, GW; LA Glover & JI Prosser. 2003. Spatial analysis of archaeal community structure in grassland soil. *Appl Environ Microbiol.* 69: 7420-7429.

Nunan, N; TJ Daniell; BK Singh; A Papert; JW mcNicol & JI Prosser. 2005. Links between plant and rhizoplane bacterial communities in grassland soils, characterized using molecular techniques. *Appl. Enviro Microbiol* 71: 6784-6792.

Ogunseitan, OA. 2006. Soil proteomics: extraction and analysis of proteins from soil *In:* P Nannipieri & K Smalla *(eds).* Nucleic Acids and Proteins in Soil. Springer, Heidelberg, pp 95-115.

Perry, LG; ER Alford; J Horiuchi; MW Paschke & JM Vivano. (2007) Chemical signals in the rhizosphere: root-root and root-microbe communication. *In:* R Pinton; Z Varanini & P Nannipieri *(eds).* The Rhizosphere. Biochemistry and Organic Substances at the Soil-Plant Interface. Taylor and Francis Group, Boca Raton, FL, pp 297-330.

Pinton, R; Z Varanini & P Nannipieri. 2001. The Rhizosphere. Biochemistry and Organic Substances at the Soil-Plant Interface. Marcel Dekker, New York.

Radajewski, S; P Ineson; NR Parekh & JC Murrell. 2000. Stable-isotope probing as a tool in microbial ecology. Nature 403: 646-649.

Renella, G; M Michel; L Landi & P Nannipieri. 2005. Microbial activity and hydrolase activities during decomposition of model root exudates released by a model root surface in Cd-contaminated soils. *Soil Biol Biochem.* 37:133-139.

Richardson, AE; PA Hadobas & JE Hayes. 2001. Extracellular secretion of *Aspergillus* phytase from *Arabidopsis* roots enables plants to obtain phosphorus from phytate. *The Plant J.* 25: 641-649.

Soderberg, Kh; A Probanza; A Jumpponen & E Baath. 2004. The microbail community in the rhizosphere determined by community-level physiological profiles (CLPP) and direct soil-and cfu-PLFA technique. *Appl Soil Ecol.* 25: 135-145.

Sørensen, J. 1997. The rhizosphere as a habitat for soil microorganisms. *In:* JD van Elsas; JT Trevors & EMH Wellington. *(eds).* Modern Soil Microbiology. Marcel Dekker, New York, pp 21-45.

Sørensen, S & O Nybroe. 2006. Reporter genes in bacterial inoculants can monitor life conitions and functions in soil. *In:* P Nannipieri & K Smalla K *(eds).* Nucleic Acids and Proteins in Soil. Springer, Heidelberg, pp 375-395.

Steer, J & JA Harris. 2000. Shifts in the microbial community in rhizosphere and non-rhizosphere soils during the growth of *Agrostis stolonifera* 32: 869-878.

Tarafdar, JC & A Jungk. 1987. Phosphatase activity in the rhizosphere and its relation to the depletion of soil organic phosphorus. *Biol. Fertil Soils* 3:199-204.

Torsvik, V; R Sørheim & J Gorksøyr. 1996. Total bacterial diversity in soil and sediment communities-a review. *J Ind Microbiol.* 17: 170-178.

Uren, NC. 2007. Types, amounts and possible functions of compounds released into the rhizosphere by soil-grown plants. *In:* R Pinton; Z Varanini & P Nannipieri *(eds).* The Rhizosphere. Biochemistry and Organic Substances at the Soil-Plant Interface. Taylor and Francis Group, Boca Raton, FL, pp 1-21.

Valè, M; C Nguyen; E Dambrine & JL Dupouey. 2005. Microbial activity in the rhizosphere soil of six herbaceous species cultivated in a greenhouse is correlated with shoot biomass and root C concentrations. *Soil Biol Biochem*. 37:2329-2333.

Waisel, Y; A Eshel & U Kafkafi. 1991. Plant roots. The Hidden Half. Marcel Dekker, New York.

Wenzel, WW; G Wieshammer; WJ Fitz & M Puschenreiter. 2001. Novel rhizobox design to assess rhizosphere characteristics at high spatial resolution. *Plant Soil* 237: 37-45.

Zak, Dr; DB Ringelberg; KS Pregitzer; DL Randlett; DC White & PS Curtin. 1996. Soil microbial communities beneath *Populus grandidentata* grown under elevated atmospherich CO_2. *Ecol Appl* 6: 257-262.