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Review Article

Micromorphology of the Rhizosphere with Focus on Plant Nutrition

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Abstract

The paper highlights the historic background of Rhizosphere investigations focusing on Root-Soil Interface activities in terms of micro-morphology, using advanced micro-morphological investigation techniques and tools such as high-resolution microscope, scanning electron microscope, electron-probe micro-analyser analysis, X-Ray Diffraction Technique, Thin Micro-Autoradiography using Kodak thin myler films onto thin sections with optical polish < 1.0 micron. The details of the research findings are summarized in the relevant sections of the paper. The second part of the paper refers to a number of factorial pot experiments with French beans (*Phaseolus vulgaris* L) as test crop, involving three nitrogen sources (viz. ammonium phosphate, choline phosphate and calcium chloride) at 2 N levels (500 ppm n and 1000 ppm N), 2 initial soil pH levels adjusted at pH 7 & pH 8 and two growth (G) stages (21 days and 42 days) were conducted under a standard growth chamber condition (temperature 28°C, light intensity 10, 000 LUX, R. H. 60%). Following dry ashing (475°C) shoots and roots were analysed for $H_2PO_4^{-2}$ (Phospho- molybdate method (Jackson, 1964) Ca^{2+}, Mg^{2+}, K^+, Na^+, NH_4^+, NO_3-, Cl^-), dithinate-citrate extractable Fe^{2+}, Mn^{2+}, Zn^{2+} (Mehra and Jackson, 1960). The analytical values of soil (rhizosphere and non-rhizosphere) and root and shoot parameters were subject to Analysis of Variance Tests (ANOVA Test) to determine significant differences of based on single factors of factorial combinations and reveal 'main effects' and interactions. The summary of results of these experiments is briefly summarized in the relevant sections of the paper that follows.

Keywords: Micromorphology, Rhizosphere, Soil-root interface, Plant nutrition, Factorial experiment.

INTRODUCTION

Definitions of Rhizosphere

Rhizosphere is the narrow region of soil or substrate that is directly influenced by the root secretion (exudates) and associated microorganisms known as the root microbes. Lorentz Hiltner (1904) defined the rhizosphere as the area around a plant root that is predominantly inhabited by a unique population of microorganisms and postulated by the chemicals released from plant roots. observed an abundant and preferential colonization of the soil in the vicinity of plant roots by microorganisms. He described the area immediately adjacent to the roots as the 'rhizosphere'. Since then many attempts have been made to describe and define the zone of enhanced microbial activity with greater accuracy and this has led to the introduction of such terms as 'inner and outer rhizosphere', 'rhizocylinder', 'rhizoplane' etc. however, intrinsic heterogeneity of soil in the zone makes such categorization extremely difficult and often ambiguous (Sarkar and Jones,1980a).

Hence, the rhizosphere is subject to the influence of chemicals extracted by roots of living plants and the microbial community in this microzone. In the rhizosphere, competition between microorganisms and plants occurs for both iron and phosphorus demand, being microorganisms more competitive for their capacity to break down plant-chlators and plants more able to counteract direct competition with microorganisms. Having large surface area, the active uptake of water and minerals through root hairs is highly efficient. Root hair cells secret acid (H+ malic acid) which exchanges and helps solubilise the minerals into ionic forms, making ions easier to absorb.

All processes and functions taking place in the rhizosphere are dominated by the activities of plant roots, rhizosphere micro-organisms interactions and enzymes are recognized as the main actors of all activities occurring in the rhizosphere environment. The production and activity of rhizosphere enzymes is controlled by several factors, in turn depending on soil-plant-microorganism interactions. In general, higher activity of rhizosphere enzymes can be interpreted as a general fuctional diversity of the microbial community. The lack of satisfying methodologies for accurately measuring the location of and activities of rhizosphere enzymes have often hampered clear knowledge and understanding of their properties and functions. Sophisticated technologies now available will be helpful to reveal the origins, locations and activities of enzymes in the rhizosphere.

Organisms found in the rhizosphere include bacteria, fungi, comycetes, nematodes, protozoa, algae, viruses, archaea, arthropods etc. Rhizosphere organisms that are deleterious to plant growth and health include pathogenic fungi, comycetes, bacteria and nematodes- briefly termed as soil borne pathogens. Rhizosphere Effects (REe) are defined as biological, chemical, and physical changes in soils that occur because of root exudates and rhizo-deposition. Biological processes are related to the parasitism of host plant microorganisms that promote nutrient and water uptake from soils. The rhizosphere inhabiting microorganisms often compete for water, nutrients, space and sometimes improve their competitiveness by developing an intimate association with plants (Hartmann et al, 2009). These microorganisms play important roles in growth and ecological fitness of their host.

LITERATURE REVIEW

Micro-morphology of Rhizosphere

Micro-morphological investigations have been used in wide range of studies for both characterization and classification of fabric (Brewer, 1964; Stoops & Jongerius, 1975) including that of humic microfabric (Barrat, 1969; Babel, 1965, 1975) and of specific features such as oriented clay cutans, nodules and voids. Specific micro-morphological techniques namely transmission (Jenny and Grossenbacher, 1963) and scanning electron microscopy (Dart, 1971; Campbell and Rovira, 1973) have also been used to illustrate the special relationship of soil-root-microorganisms in undisturbed rhizosphere. The physical and nutritional environment is highly dependent on the very nature of the fabric around the root gave a detailed physical description of fabric at the tree-root-soil interface.

Micro-morphology is the branch of soil science that is concerned with the description, interpretation and to a certain extent, the measurement of soil components, unique features and fabrics (i.e., micro-pedons) in soils at microscopic level. The first thin section studies of soils date from the beginning of the 20th century. Since then, soil micromorphology has gradually gained importance in research, several systems of thin section description were developed, each using specific concepts and terminology, and new methods were applied. In the beginning its main use was in the field of soil genesis, as well as soil classification, although its practical application in determining soil types is hindered by many factors inherent to the present classification systems. Micromorphology is a precious tool in palaeopedogy to disentangle polygenetic processes pointing to changes in climatic/environmental conditions over time. For the same reason it became an important tool in geo-archaeological research. There is a need for more extensive correlation with physical and chemical data, especially as a contribution to pedometric studies and soil management. It can also be a great help in monitoring field and laboratory experiments.

On a similar line used an innovative methodological approach based on the chemical analysis of different portion of soil horizons (alluvial pedofeatures, pedogenic matrix and skeletal parent rock fragments) by laser ablation inductively coupled plasma mass spectroscopy (LA-ICP-MS) associated with traditional micro morphological techniques such as: optical and scanning electron microscopy. Validation of LA-ICP-MS techniques provided in situ accurate and reproducible RSD: 13-18% analysis of low concentration trace elements and rare earth elements (REE) in soil samples of 0.001-0.1 p.p.m. concentrations.

Microscopic bodes of various forms, dimensions and morphology have been observed in the soil suspension under electron microscope (Nitin, 1964). Some of the limitations of direct electron microscopy have been minimized by the introduction of ‘Scanning electron Microscopy’ (S.E.M.). The S.E.M. has several advantages including those of minimal preparation, considerable depth of focus, high magnification, and high resolution with three dimensional effects. The technique has been used with varying degrees of success, by several workers (Marchant, 1970; dart, 1971; Campbell and Rovira, 1973; Rovira and Campbell, 1974, Sarkar and Wyn Jones, 1980b, Sarkar and Jenkins, 1980) to observe microbial association of the root surface and their distribution in the rhizosphere.

Soil chemistry and plant nutrition of rhizosphere

The works of Barber (1962), Nye and Tinker (1969) have emphasized the importance of the movement of nutrients, either by mass-flow of diffusion process to the root surface. The various experimental observations generally indicate that the major portion of Ca and mg reach root surface by mass-flow, whereas diffusion is the mechanism for supplying plants with K and P (Barber et al, 1963). Root interception and mass-flow are the significant mechanisms for uptake and supply of Fe, B, Cu and Sr to plant roots.

Specific effects of various fertilizer applications on the availability of nutrients have not received adequate attention so far as it rightly deserves. All the same, it has been well established that the application of nitrogen from various sources benefits the availability of macronutrients and micronutrients in soil, either by way of increase of decrease in their availability to growing plants, depending on the ionic forms present in fertilizer sources (Blair et al, 1970, miller et al, 1970, Riley and Barber, 1969; Sarkar and Wyn Jones, 1980a). A pH drop in the root environment applying ammonia (Sarkar and Wyn Jones, 1980) and choline and a pH increase following application of nitrate (Sarkarkar and Wyn jones, 1980) have been observed in classical studies. The Rhizosphere effect of ph on the availability of various nutrients in soil is widely known (Denahue, 1965). Effects of plant-microbe interactions (Rhizosphere effect) on nutrients uptake have also been reviewed (Sarkar and Wyn Jones, 1980).

A number of workers have shown that that the availability of micronutrients to plants depends to a large extent on the soil pH (Loneragan, 1975; lutz et al, 1972; Olomu et al, 1973). Interdependence with soil has also been observed (Mukhopadhya et al, 1967; Olomu et al, 1973; Patrick and Dalaune, 1972). The manipulation of rhizosphere pH by using NH_4^+ - N and NO_3^-N was pioneered by relatively recently by Sarkar and Wyn Jones (1982) have elaborated on this method by using choline as well as ammonia salts to produce low rhizosphere pHs. In this and other studies, including studies relating to Micro-morphology of rhizosphere; Sarkar et al, (1976, 1979); Sarkar and Jenkins, (1980), Sarkar and Wyn Jones (1981) have made a significant contribution on Micromorphology of the Rhizosphere with special reference to plant nutrition. The paper highlights some of their findings along with other workers working on similar lines.

MICROMORPHOLOGY OF THE RHIZOSPHERE: METHODOLOGY AND FINDINGS

Part I of the Study

Sarkar et al. (1976), Sarkar and Jenkins (1979) and Sarkar et al. (1980& 1980) are some of the early among the research workers to conduct research studies, among other themes, on the "Micromorphology and Biochemistry of Soil Science (Sarkar,1977) on dwarf French beans grown in controlled greenhouse conditions. Since then, soil micro-morphology with specific focus on the rhizosphere has gradually gained importance, particularly using thin sections with optical polish < 1 micron, drawn out of rhizosphere soil core embedded into epoxy resin material.

Refinement of this technique has been tried and tested with varying degree of demonstrative success in different research laboratories in the world; each of course using different concepts, approaches, objectives and terminologies. For instance, in the beginning, its main use was in the field of soil genesis, as well as soil survey and classification. Micro-morphology is a precious tool in paleopedology to disentangle polygenetic processes pointing to changes in climatic/environmental conditions over a certain period of time horizon. And precisely for the same reason, over a period of time, it became an important tool in geo-archological research investigations.

Reverting to the original work of Sarkar et al. (1976) on "Modifications to Mechanical and Mineralogical composition of soil within Rhizosphere' it was found during factorial experiment study of rhizosphere of rhizosphere development around French bean roots, the mechanical and mineralogical modifications were found obvious and recorded. Whereas, the former entailed in relative decrease of material ranging from 63-6.3 Micro-m e.s.d. in size; probably due to disaggregation of polymineralic shale particles, the latter was more obvious in the finest (i.e. <0.063 micro m.) fraction and involved a decrease of both regularly and irregularly interstratified 10/14 Angstrom material such as; "amorphous" (extractable) Al, Fe and C accumulated in the rhizosphere; whilst Si was depleted. These effects were interpreted as being due to "weathering "of soil materials in the vicinity of the plant root i.e. rhizoplane.

Sarkar et al. (1979) had pursued their study a step further by conducting research on "Detection of Elemental concentration change in the Root environment by employing Contact Micro-Autoradiography Technique". This particular technique involved contact exposure with irradiated (45Ca and 32P). Thin sections prepared from impregnated soil blocks, and subsequent development and fixing of the films were carried out. Relative effectiveness of the detection of elemental concentration changes of Ca and P in the vicinity of roots were tested using X-ray films (i.e. industrex fine grade film and Kodak stripping film).

Research Findings

Of the two, stripping film was found to be most suitable. Thus, using this film, gradient in concentration of Ca in the vicinity of roots was detected in case of dwarf French beans receiving choline phosphate treatment. However, when dwarf French bean received calcium nitrate treatment, Ca was shown to have accumulated on the soil-root interface i.e. rhizoplane. No depletion zone of P was detected as such with either of the fertilizer treatments. However, in case of Choline phosphate treatment P was found to have migrated to endodermal layer (i.e. endodermis). General association of P with organic debris (mostly humic substances) of root or microbial origin or even a mix of the two as well as sesquioxidic materials was also visibly apparent.

A further follow-up of the above mentioned study was subsequently carried out by Sarkar and Jenkins (1979) titled "Micromorphology of the Rhizosphere' revealed that thin sections (<25 micron) prepared from the resin impregnated blocks- both of the rhizosphere and control soil were examined under microscope. The fabric of control soil was found to be characterized by the presence of large grain and irregular 'orthovughs', sporadically distributed in the fabric with high grain/plasma ratio. The S-matrix of the rhizosphere fabric was described as integrated “Humicol-Agricol.” Insepic plasma fabric extending upto 250 micron from the rhizosphere was detected. No preferential orientation of the fine grains were noticed on the root surface, although very fine fabric components of organic and inorganic sources intermixed with root hairs (fluoresced brown), adhered with partially decayed cortical cells forming “micro-aggregates”/ micropedons or simply “clusters”. This special fabric feature was described as “spongy mullicol”.

Part II of the study

Sarkar and Jenkins (1980a) conducted further studies on the theme of micro-morphology of the rhizosphere titled “Detection of Elemental concentration change in the root environment using Electron-Probe Microanalysis (EPMI) Technique”. In this study, a representative thin section prepared from the undisturbed and homogenized block of rhizosphere soil, receiving choline phosphate treatment and subjected to micro-probe x-ray analysis. Three selected sites in the vicinity of roots were studied. Maintaining the sample field stationary, the electron beam was allowed to scan along a selected line across rhizospheric fabric perpendicular to root. Independent scanning profiles relative to concentrations of P, Ca, Fe, Mn, Al and Si were presented superimposed. Similarly, between the scanning profiles of Ca and P in selected 2-3 fields were studied; which suggested a possible mutual association of these elements in the root interior as well as in the rhizosphere. A relatively high concentration of P in the root and on the rhizoplane compared to the neighbouring matrix suggested a possible depletion of this element (i.e. P) in the vicinity of root i.e. rhizoplane. Trace elements like Fe, Al and Mn tended to concentrate remotely from the root surface and did not show association with phosphate in the root environment.

As a follow-up of the earlier study by Sarkar and Jenkins (1980), Sarkar and Wyn Jones (1982a) conducted a study on “Effect of Rhizosohere pH on the availability and uptake of Fe, Mn and Zn”. In this study, a number of pot experiments on finding the relationship between Rhizosphere pH, the extractable levels of Fe, Mn and Zn in the soil and their uptake into the roots and shoots of dwarf French beans were studied. Variations in the rhizosphere pH were induced by applying three different sources of nitrogen- choline phosphate, ammonium phosphate and calcium nitrate to an initially homogenized soil (pre-adjusted to either pH 7 or pH 8). The rhizosphere ph was found to be significantly lower following the application of either ammonium or choline phosphates and to be increased by calcium nitrate treatment. The Fe and Zn contents of both shoot and root were found to be inversely proportional to Rhizosphere pH. The Mn contents also increased with increasing pH; but a sharp increase was apparent below pH 5.5. The shoot Fe, Mn and Zn contents were significantly correlated with the extractable levels determined in the Rhizosphere and non-Rhizosphere (control) soil.

Another study by Sarkar and Wyn Jones (1982) followed soon after the previous study. This study was on “Influence of Rhizosphere on the nutrients status of dwarf French beans”. In this study, French bean seedlings were grown on choline, ammoniacal and nitrate forms of nitrogenous fertilizers, together with equivalent basal applications of P as KH_2PO_4 were tested for nutrients uptake from the Rhizosphere soil. Statistical tests on soil (both Rhizosphere and non-Rhizosphere and plant (root and shoot) revealed that with the exception of P, levels of all other estimated macro (Na^+, K+ Ca^{2+}, Mg^{2+}) were significantly changed after 42 days plant growth as compared to 21 days growth period in the pot experiment. The higher uptake into shoots of Na^+, K^+ ,Fe^{2+} Mn^{2+}, Zn^{2+} and $H_2PO_4^-$ and higher biomass accumulations in the Rhizosphere were associated with lower Rhizosphere pH.

The uptake of Ca^{2+} and Mg^{2+} increased with higher Rhizosphere pH, Whilst, ammoniacal and choline forms of fertilizer applications decreased Rhizosphere pH and increased P uptake; nitrate form on the contrary reversed the trend showing significant inverse relationship between shoot phosphate and Rhizosphere pH, Ca and Fe were associated with an inhibition of translocation of P form from root to shoot. However no causal relationships could be established in such relationship. Both shoot weight and shoot phosphate contents were closely associated with a number of Rhizosphere soil parameters and much less so with the non-Rhizosphere soil parameters.

DISCUSSION AND CONCLUSION

A systematic review of research investigations and pioneering work done by Sarkar and associates in Bangor, U.K. Stanley A. Barber and associates in Purdue, U.S.A., Nye and Tinker and associates in Oxford, U.K. as well as other research workers in different parts of the world in the 20th Century, mainly relating to micro-morphological investigations needs special recognition as it deserves. These experiments have often been combined with parallel pot and/or field experiments; and closely corroborated with analysis of plant and shoot samples (tissues) as are derived from test crop (s) at different stages/period of growth. These experiments have given interesting; and to a great extent, conclusive results: the details of which are given in the paper, which are self-explanatory. It would appear from the ongoing trends of research studies in different laboratories that there is a clear need to evolve, develop, try, test and adapt some of the some of the recently developed and advanced techniques such as the use digital sensors, IoT techniques, Artificial Intelligence (AI) techniques etc. to help increase the proper sampling methodology of drawing Rhizosphere samples for testing, enhancing depth of focus, higher resolution for micro-morphological investigation, relying analytical and statistical data which are drawn from *in situ* samples rather than *ex-situ* samples. Electrochemical Sensors now-a-days being widely used under AI can help provide key information/data required for precision agriculture: pH and soil nutrients levels in particular. Sensors electrodes work by detecting specific ions in soil, Currently, sensors mounted to specifically designed “sleds” help gather, process, map soil chemical data. Such AI techniques will have accuracy, precision and reliability over and above the current techniques of nutrients movement, concentration, depletion and competition scenario analysis with fair degree reproducibility and validity.

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