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CHAPTER

Ecological Agriculture: Strategy for Sustainable Development

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INTRODUCTION

The microorganisms that promote Biological Nitrogen Fixation (BNF) have great importance, since nitrogen is an essential component of proteins, nucleic acids and other nitrogen compounds, and therefore of life for all living beings [1]. Ecosystems in climax, the microbiote is in balance in the soil, maintaining its biodiversity and sustainability, but this balance can be easily broken down by man or natural phenomena [2]. Even though the greatest contributions of the BNF have been detected in oceans and leguminous plants, some plants of the family Gramineae have shown a very significant potential in obtaining nitrogen by the action of nitrogen fixing bacteria [3, 4].

Initially the endophytic microorganisms were considered harmless to plants, but from ’ 70s onwards their importance was realized. [5]. There are several positive effects attributed to endophytic bacteria, such as the promotion of plant growth [6–8], biological control of pests and diseases in plants [9], biological nitrogen fixation [10–13], induction of systemic resistance [14], production of siderophores [15, 16] and production of antibiotics [17].

The promotion of plant growth occurs mainly by the production of phytohormones such as auxins, cytokinins, gibberellins, abscisic acid and ethylene by the endophytic bacteria. The production of these phytohormones has been reported in bacteria such as *Gluconacetobacter, Azospirillum, Herbaspirillum, Erwinia, Pseudomonas* and *Pantoea* [18]. The indoleacetic acid is a naturally occurring important auxin that causes physiological effects on the plant, such as increased growth [19–21]. Mycorrhizal infection of roots of legumes has been reported to stimulate root growth, nodulation and N_2 fixation, especially in soils low in available P [22]. The progress to date in using rhizospheric and endophytic microorganisms, arbuscular mycorrhizal fungus and role of microbial biomass and enzyme activities in sustainable agriculture soil in a variety of applications is summarized and discussed here.

IMPROVEMENT OF BIOLOGICAL NITROGEN FIXATION BY RHIZOSPHERIC AND ENDOPHYTIC MICROORGANISMS

Undoubtedly, after carbon, oxygen and hydrogen, nitrogen is quantitatively the most important element required by plants and animals for growth both in water and or land, reaching about 1.5% of the dry weight of innumerable agricultural crops [23]. So, this was one of the nutrients that contributed most to the so-called Green Revolution. But its indiscriminate use led to many environmental problems [24]. Some of the adverse environmental effects of excessive use of nitrogenous fertilizers are: (i) methemoglobinemia in infants due to NO_3 and NO_2 in waters and food; (ii) cancer due to secondary amines; (iii) respiratory illness due to NO_3, aerosols, NO_2 and HNO_3; (iv) eutrophication due to N in surface water; (v) material and ecosystem damage due to HNO_3 in rainwater; (vi) plant toxicity due to high levels of NO_2 and NH_4 in soils; and (vii) excessive plant growth due to more available N, depletion of stratospheric ozone due to NO and N_2O [25].

Biological Nitrogen Fixation (BNF) is the process by which atmospheric nitrogen gas (N_2) is converted into ammonia (NH_3), which is subsequently available to plants. In agricultural settings, perhaps 79% of this biologically fixed N_2 comes from symbiosis involving leguminous plants and bacteria of the family Rhizobiaceae. The family Rhizobiaceae currently includes six genera: *Rhizobium, Ensifer (Sinorhizobium), Mesorhizobium, Allorhizobium, Azorhizobium* and *Bradyrhizobium*, which are collectively referred to as rhizobia [26]. In recent years, however another β-proteobacteria have been showed to produce nodules in the legume [27] *Methylobacterium* [28]; *Blastobacter* [29], and *Devosia* [30] as well as β-proteobacteria such as *Ralstonia* [31] and *Burkholderia* [32].

The agronomic implications of symbiosis have promoted research on biological nitrogen fixation and the characterization of rhizobia [33], as well as success in constructing better inoculants that requires a two-step approach. First, the strains need to be improved in order to compete successfully with indigenous strains for root nodulation of legumes. Several loci have been identified to date that affect competitiveness for strains nodule occupancy. Usually mutations in these loci affect the ability of a strain to form nodules rapidly and efficiently. Other loci, such as those that confer antibiotic production, can be added to strains to enhance nodulation competitiveness when co-inoculated with antibiotic-sensitive strains. Second, the inoculum strains must be improved with respect to symbiotic nitrogen fixation [34].

Even though the greatest contributions of the BNF have been detected in oceans and leguminous, some plants of the family Gramineae have shown a very significant potential in obtaining nitrogen, by the action of nitrogen fixing bacteria [3, 4]. These plants have a fascicule root system, taking advantage of the leguminous' pivoting system to extract water and soil nutrients, and because they are widely used as food by humans. Therefore, even if only part of N could be provided by the association with fixing bacteria, the economy in nitrogen fertilizers would be equal to or higher than that observed with leguminous plants that can be self-sufficient in nitrogen [2, 35].

PLANT GROWTH PROMOTING RHIZOBACTERIA: INCREASE CROP PERFORMANCE

Biological Nitrogen Fixation (BNF) by associative diazotrophic bacteria is a spontaneous process where soil N is limited and adequate C sources are available. Yet the ability of these bacteria to contribute to yields in crops is only partly a result of BNF. A range of diazotrophic plant growth-promoting rhizobacteria (PGPR) participate in interactions with C_3 and C_4 crop plants (e.g., rice, wheat, maize, sugarcane and cotton), significantly increasing their vegetative growth and grain yield. The mechanisms involved have a significant plant growth-promoting potential, retaining more soil organic-N and other nutrients in the plant-soil system, thus reducing the need for fertilizer N and P. According to Kennedy et al. [36], this diversity will need to be carefully considered in the future design of the most efficient inoculant biofertilizers. For example, an important question is whether inoculants should be restricted to a single strain of bacterium, such as *Azospirillum* or not. If all of the plant growth promoting (PGP) mechanisms can be well expressed in a single strain of bacterium, this would simplify the design of inoculants products. However, it is unlikely that a single strain of bacterium would be capable of optimal activity. A list of some diazotrophs promoting crop production is provided in Table 3.1.

Table 3.1 Biology and potential role of some diazotrophs promoting crop production (adapted by Kennedy et al. [36]).

Diazotrophs	*Condition for BNF*	*Habitat*	*Energy source*	*Mechanism of effect*
Azotobacter chroococcum	Aerobic	Rhizosphere	Organics in soil	BNF
Clostridium spp.	Anaerobic	Soil saprophyte	Organics in soil	BNF
Azospirillum spp.	Microaerobic	Rhizosphere, mildly endophytic in roots, stems and leaves	Organics in soil, root exudates and plant tissue Root exudates	BNF*, PGP**
Herbaspirillum seropedicae	Microaerobic	Endophytic, Rhizosphere		BNF, PGP
Azoarcus sp.	Microaerobic	Endophytic	Root exudates	BNF
Burkholderia vietnamiensis	–	Rhizosphere, Endophytic	Organics in soil and root exudates	BNF, PGP
Rhizobium leguminosarum bv. phaseoli	–	Endophytic in roots	Root exudates	PGP
Rhizobium etli bv. phaseoli	–	Endophytic in roots	Root exudates	PGP
Gluconacetobacter diazotrophicus	Microaerobic	Endophytic in roots, stems and leaves	Root exudates and plant tissue	BNF

*Biological Nitrogen Fixation (BNF); **plant growth promoting (PGP).

Bacterial mediated increase in root weight is a commonly reported response to PGPR inoculations [37–40]. More importantly, increases in root length and root surface area are sometimes reported [41, 42]. The reporting of root length and root surface area is important because increase in these parameters is more reflective of an increase in the volume of soil explored, than that which would be indicated by just increase in root weight. For example, treatment of clipped soybean roots with *Azospirillum brasilense* Sp7 caused a 63% increase in root dry weight, but more than a 6-fold increase in specific root length (root length per unit root dry weight), and more than a 10-fold increase in total root length [43].

The production of phytohormones has been implicated in the growth promotion by biofertilizing-PGPR [44]. Most commonly, IAA producing PGPR are believed to increase root growth and root length, resulting in greater root surface area, which enables the plant to access more nutrients from soil. Gutierrez-Manero *et al*. [45] provided evidence that four different forms of Gibberellin (GA) are produced by *Bacillus pumilus* and *B. licheniformis.*

POTENTIAL APPLICATIONS OF ENDOPHYTIC MICROORGANISMS

Plants can be considered complex microecosystems where different niches are exploited by a wide variety of microorganisms. Such niches include not only the external surfaces of plants, but also the internal tissues in which all microorganisms that inhabit, at least for one period of their life cycle, the interior of a vegetable, without apparent harm to the host or external structures may be considered as an endophyte [5, 14].

Endophytic bacteria and fungi form complex and fascinating associations with their host plants. These associations are often considered as mutualistic, because these bacteria and fungi help to protect their hosts from biotic and abiotic stresses, and the plants supply the endophytes with nutrients. In the latest decades, a great deal of information on the role of endophytic microorganisms in nature has been collected [5, 14, 46–49]. The capability of colonizing internal host tissues has made endophytes valuable for agriculture as a tool to improve crop performance [5]. However, the utilization of endophytic microorganisms in agricultural production depends on our knowledge of the microorganism-plant interaction and our ability to maintain, manipulate and modify beneficial microbial populations under field conditions.

At the moment, endophytic bacteria and fungi have been isolated from a large diversity of plants (Table 3.2), and cultivation-independent analysis showed that a high number of uncultivable species also colonize plants endophytically [50–52], but most likely, there is not a single plant species devoid of endophytes [46]. So, the plant-associated habitat is a dynamic environment in which many factors may affect the structure and species composition of the microbial communities that colonize plant tissues. Some of these factors are seasonal changes, plant tissue [18, 53], plant species and cultivar, soil type [18, 52, 54–56] and interaction with other beneficial or pathogenic microorganisms [51, 57].

Table 3.2 Examples of reported endophytic bacteria, fungi and plants harboring them.

Endophytes	*Host Plant*	*References*
Bacteria		
Methylobacterium, *Bacillus*, *Curtobacterium*	Citrus	[51]
Burkholderia, *Pantoea*,	Sugar cane	[73]
Gluconacetobacter diazotrophicus		[74]
Brevundimonas, *Enterobacter*, *Pantoea*	Rice	[75]
Clavibacter, *Cellulomonas*, *Mycobacterium*	Wheat	[76]
Flavobacterium, *Agrobacterium*, *Pseudomonas*	Potato	[77]
Ralstonia, *Enterobacter*, *Acinetobacter*	Soybean	[18]
Fungi		
Pestalotiopsis	Medicinal plants	[66]
Acremonium, *Aspergillus*, *Colletotrichum*, *Fusarium*	Soybean	[78]
Colletotrichum, *Xylaria*, *Botryosphaeria*	Apple trees	[79]
Xylaria, *Colletotrichum*, *Cordana*	Banana trees	[80]
Fusarium	Atriplex	[81]
Dothiorella, *Phomopsis*	Mango	[82]

Therefore, the role of endophytes in ecosystems has been shown to involve: i) inducing plant protection against pathogens and insects [5, 58, 59]; ii) altering plant community structure by impacting on the herbivory on specific plant species [60, 61]; and iii) increasing plant fitness [62]. Furthermore, endophytes are potential sources of novel natural products for exploitation in medicine, agriculture, and industry [17, 63–66].

So, the main biotechnological applications of endophytes are plant growth promotion and biological control. The plant growth promotion by endophytic bacteria may result from indirect effects such as the biocontrol of soilborne diseases through competition for nutrients, antibiosis, the induction of systemic resistance in the plant host, or siderophore-mediated competition for iron [47, 48, 55, 67, 68]. Bacterial siderophores are low-molecular-weight compounds with high Fe^{3+} chelating affinity responsible for the solubilization and transport of this element into bacterial cells [69]. In a state of iron limitation, the siderophore-producing microorganisms are also able to bind and transport the iron-siderophore complex by the expression of specific proteins [70]. The production of siderophores by microorganisms is beneficial to plants, because it can inhibit the growth of plant pathogens [69]. In this context, Cao et al. [71] observed the potential of developing siderophore-producing *Streptomyces* endophytes for the biological control of Fusarium wilt disease of banana, and Rajendran et al. [72] showed enhanced growth and nodulation of pigeon pea by inoculation of siderophore-producing endophytic *Bacillus* strains.

Endophytes can also produce the enzyme 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase. This enzyme has no function in bacteria but cleaves ACC, the precursor of ethylene in plants, and thus modulates ethylene levels, which contributes to plant growth promotion [83, 84].

The plant growth promotion can also result from the production of phytohormones, such as auxins, cytokinins and gibberellins [85]. The most related phytohormone produced by endophytic bacteria is the auxin indole-3-acetic acid (IAA), such as *Gluconoacetobacter*, *Azospirillum, Herbaspirillum, Methylobacterium, Erwinia, Pantoea* and *Pseudomonas* [12, 18, 86-91]. At least, there are three pathways for IAA biosynthesis— two tryptophan-dependent pathways [indole-3-acetamide (IAM) and indole-3-pyruvate (IPyA)] and one tryptophan-independent pathway [19, 85]. Moreover, the endophytes can supply the host plant with fixed nitrogen or the solubilization of soil phosphorus and iron [12, 47, 48, 55, 92].

The recombinant DNA technology has been applied as a tool to improve endophytic microorganisms, aiming for the introduction of new characteristics of agronomic interests as biological control of pests. The commercial product results due to the introduction of a heterologous gene in an endophytic microorganism with the purpose of insect control [93]. Dowing et al. [94] obtained the biocontrol of the sugarcane sorer *Eldana saccharina* by the expression of *Bacillus thuringiensis cry1Ac7* and *Serratia marcescens chiA* genes in *Herbaspirillum seropedicae.* The use of an endophytic bacterium was also seen as a possible solution to the problem of inaccessibility of conventional *B. thuringiensis*-based products to the interior regions of the plant.

Other important application of endophytes is the search for new natural products. In this context, some endophytic fungi have been found to produce commercially important natural products that were previously found only in plants. Some renowned examples include paclitaxel, camptothecin, podophyllotoxin, and hypericin, although it is far from clear to what extent endophytes actually contribute to the biosynthesis of these compounds within their host plants [65]. Therefore, the study of the structure and species composition of plant-associated microbial populations is fundamental to understanding how plant-associated biological processes are influenced by environmental factors and, consequently, have important biotechnological implications.

ARBUSCULAR MYCORRHIZAL FUNGI IN NATURAL ECOSYSTEMS

Among the microorganisms found in soil, mycorrhizal symbionts stand out because they interact directly with plants, participating in the processes of nutrient absorption, water uptake, growth, and reproduction [95]. Most terrestrial plant species form symbiotic mycorrhizal associations, known as mycorrhizae, with certain soil fungi. In this type of mutualistic association, the host plant receives nutrients from the mycelium, while the fungus receives carbohydrates produced by the plant [96]. Mycorrhizae have been classified into seven categories based on structural characteristics and the location of the hyphae in the root cells of the host [97]. The seven categories are as follows: ectomycorrhiza, arbuscular mycorrhiza, ericoid mycorrhiza, arbutoid mycorrhiza, monotropoid mycorrhiza, ectendomycorrhiza, and orchid mycorrhiza. Arbuscular mycorrhiza (AM) and ectomycorrhiza (ECM) have been studied the most because they are associated with the majority of vascular plants and are economically important compared to the others, which are less common [98].

The arbuscular mycorrhizae association, characterized by the formation of arbuscules in the cortex of the host roots, is probably the most widespread and predominant type of symbiosis found in terrestrial ecosystems [99], and occurs in the majority of important agricultural plants [100]. It is estimated that arbuscular mycorrhizal fungi (AMF) colonize species in more than 80% of the plant families. Recently these fungi were classified in a new Phylum called Glomeromycota based on the sequence of the small subunit of the rDNA (SSU) [101].

The main effect of the arbuscular mycorrhizae fungi, in the relationship, is supplying the host plant with nutrients that are relatively immobile in the soil, particularly phosphorous (P) and trace elements, by increasing the surface area of the colonized roots of the plant [102, 103]. In addition, the fungi can contribute to nutrient cycling [96]; increasing the plants tolerance to biological stress, such as root pathogens [104], or abiotic stress, such as excess of metals [105], drought, and salinity [106, 107]; improving the quality of the soil [108] and the processes of particle agglomeration by producing glomalin, a hydrophobic glycoprotein produced by the hyphae and spores [109]; and help maintain plant diversity [110]. Therefore, it is essential to understand mycorrhizal symbiosis in order to develop techniques that contribute to sustainable ecosystems [111]. Smith and Read [103] discussed the importance of identifying the best plant-fungus combination that promotes, within an ecosystem, the efficiency of the transfer of nutrients between the symbionts.

Arbuscular mycorrhizal fungi are not host specific. A single plant species can be colonized by any species of AMF, but the infectivity potential and the efficiency of the relationship can vary based on the plant-fungus combination [112]. Environmental conditions can also influence the compatibility of a relationship, which is controlled by the genome of each organism [113, 114].

Attention has been given to studying arbuscular mycorrhizal associations that involve the production of inoculum AMF because of the benefits these fungi provide to the plants. However the lack of technology that allows the introduction of these fungi into axenic cultures makes it difficult to produce inoculum at an industrial scale and, consequently, the use of inoculum AMF in agronomic operations is currently limited [100]. Probably the main reasons that inoculum AMF are not used in the cultivation of seedlings are the need to constantly care for the inoculum and the difficulty of providing standard high-quality inoculum to the consumer [115]. Procedures used to select AMF inoculants should involve the identification of the factors that limit the plants growth in a particular environment. For example, the inoculation of a certain AMF is necessary in phosphorous deficient soil that lacks or has a low number of native infective propagules [116]. The mycorrhizal propagules are comprised of glomerospores [117] (Fig. 3.1), colonized root fragments, and hyphae from the soil [118]. The hyphae have a fundamental role in the formation, function, and perpetration of mycorrhizae in natural and altered ecosystems. In the absence of active roots, the glomerospores serve as durable propagules, responsible for the colonization of new roots, and are thus an important aspect of AMF ecology in the soil [119].

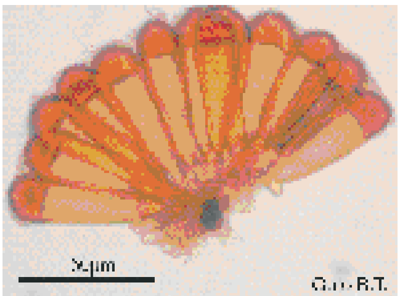

Fig. 3.1 Glomerospores of *Glomus clavisporum from* Caatinga areas in the Brazil Northeast (Courtesy of Prof. Bruno T. Goto, Federal University of Pernambuco, Recife-PE-Brazil).

Arbuscular mycorrhizal fungi are influenced by the physical-chemical factors of a soil, such as pH, heavy metals, salinity, temperature, water stress, erosion, organic matter, and aeration, among others [120]. Conventional agronomic techniques and human activity can affect the community and the efficiency of these fungi, interfering with the quality and availability of the inoculums [121], as well as the productivity and stability of the soil-plant system [122].

In natural ecosystems, the diversity of arbuscular mycorrhizal species is maintained by the survival of the fittest [123]. These fungi are widely distributed in most ecosystems, from forests [124] to deserts [125], in tropical [126, 127], temperate [110], and arctic regions [128]. However, the occurrence of arbuscular mycorrhizal associations has been cited more in the tropics than in temperate environments [103]— a fact that can be related to the optimal temperatures for the growth of these fungi, which are higher than the temperatures in temperate regions [129].

The diversity of AMF in the soil can be related to the ecological conditions of each ecosystem [130], leading to the establishment of different patterns of distribution. More than 205 species of AMF have been described [131], mostly based on spore morphology. *Glomus* is predominant in most ecosystems [126, 132-134]. Various studies have been done in Brazil about the occurrence and AMF diversity. Fifty-two species were cited from regions in the Northeast within Caatinga areas [135], and a new species (*Racocetra intraornata*) was recently described [136]. Thus, Souza et al. [127] identified 24 AMF taxa, mostly within Glomaceae and Acaulosporaceae, while Silva et al. [137] found 21 AMF species, the majority belonging to the genus *Glomus*. Thirty-eight AMF species were recently identified from southern Brazil in areas with conventional and organically cultivated apple trees and pastures. The majority of the species were in the genera *Glomus* (15) and *Acaulospora* (15).

Arbuscular mycorrhizal fungi induce a distinct growth response on the plants they are associated with and can influence the structure and composition of the vascular plant community [110]. Further, plant species can influence the sporulation pattern of AMF in the rhizosphere and significantly affect the fungal diversity [138]. Decreasing AMF diversity can be explained by the loss of host diversity and an unstable ecosystem [139], normally caused by some kind of stress. Knowledge about native AMF species adapted to local environmental conditions can constitute the additional element that guarantees the establishment of a plant species, for example, during revegetation.

ROLE OF MICROBIAL BIOMASS AND ENZYME ACTIVITIES IN SUSTAINABLE AGRICULTURE

The biological activity in soils is largely concentrated in the topsoil, the depth of which may vary from a few to 30 cm. In topsoil, the biological components occupy a tiny fraction (< 0.5%) of the total soil volume and make-up less than 10% of the total organic matter in soil. These biological components consist mainly of soil organisms, specially, microorganisms [140].

The microorganisms present important functions in the soil, such as nutrient cycling and the degradation of pollutants (pesticides, urban and industrial wastes) [141-145]. Additionally, soil microorganisms promote other important functions for environmental sustainability. A list of the role of soil microbial biomass in agriculture system is provided in Table 3.3. According to Powlson et al. [146], the main function of microorganisms is to mediate soil processes and present high rates of turnover, being a sensitive indicator of changes in soil organic matter.

Table 3.3 The role of soil microbial biomass in agriculture systems (adapted by Kennedy and Doran [147]).

Decomposition of organic residues with release of plant nutrients
Formation of beneficial soil humus
Solubilization of inorganic forms of plant nutrients
Promotion of plant growth
Improved plant nutrition through symbiotic relationships
Conversion of atmospheric N_2 to plant available N
Improvement of soil aggregation, aeration and water infiltration
Biological control in the rhizosphere
Pesticide and pollutant degradation

Microorganisms of soil represent the fraction of the soil responsible for the energy and nutrient cycling and the regulation of organic matter transformation (Fig. 3.2). The organic residues are, in this way, converted to biomass or mineralized to CO_2, H_2O and mineral nutrients representing an important pool of nutrients (N, P and S), which are continually assimilated during the growth of microorganisms. Thus, microbial biomass is considered an important source and drain of nutrients in the soil, promoting mineralization of organic matter and inorganic nutrients (NH_4^+, NO_3^-, $H_2PO_4^-$, SO_4^{2-} and CO_2), making them avilable for the growth of plant, or immobilizing the nutrients in microbial tissues for their maintenance and growth. Consequently, soils that maintain a high content of microbial biomass are capable of accumulating and cycling nutrients in the soil system [148].

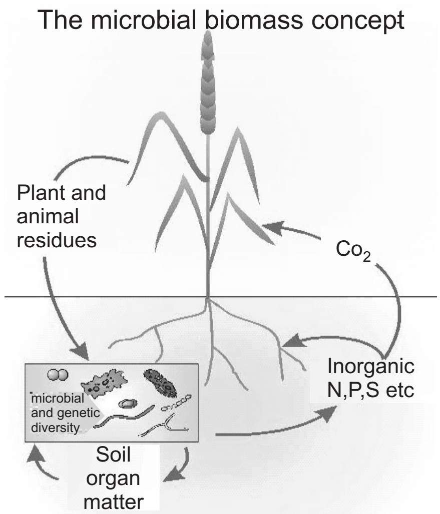

Fig. 3.2 Role of microbial biomass in the cycling of plant nutrients (Courtesy of Prof. Philip Brookes, Rothamsted Agricultural Station, UK).

In addition to the effect on nutrient cycling, microorganisms also affect the physical properties of soil, as stability of aggregates. Production of extracellular polysaccharides and other cellular debris by microorganisms helps in maintaining soil structure, as these materials function as cementing agents that stabilize soil aggregates. Thus, they also affect water holding capacity, infiltration rate, crusting, erodibility, and susceptibility to compaction [149].

The soil microbial biomass comprises all soil organisms with a volume of less than about 5×10^3 um^3, other than plant tissue, and can be considered as the living part of soil organic matter [150]. The proportion present as living microbial cells (microbial biomass C in mg per kg of soil) typically comprises 1 to 5% (w/w) of total organic C, and microbial N forms 1 to 6% (w/w) of total organic N [151, 152].

Microbial biomass is strongly related to organic matter content of soil. Systems with high organic matter inputs and available soil organic matter tend to have higher microbial biomass contents because they are preferred energy sources for microorganisms [153]. Thus the addition of readily decomposable C sources such as glucose to the soil results in a rapid rise in microbial growth and activity. A high content of easily decomposable organic C can lead to fast growth of soil microbes, likely resulting in higher microbial biomass and activity. In this way, Chowdhury et al. [154] observed that manure compost with high easily decomposable C was more effective than saw-dust and rice husk composts in enhancing soil microbial biomass C.

The behavior of soil microbial biomass is also governed by the ratio of carbon and nitrogen (C/N ratio). Fresh residues are relatively rich in carbon compared with nitrogen (C/N>20). During decomposition, soil microbes utilize reduced carbon as a respiratory substrate while accumulating carbon in the walls of their bodies (microbial biomass), a process called immobilization. In this process, N from organic residues, ammonium and nitrate ions from soil are also immobilized to microbial biomass. Immobilization is particularly evident during the decomposition of residues with high C/N ratio, thus favoring increase in microbial biomass, conform reported by Tu et al. [155], which examined how different regimes of organic management impact microbial biomass and activities, and determined how the resulting changes in microbial activities influence nutrient (N) availability for plants. The organic substrates used included composted cotton gin trash, animal manure and rye/vetch green manure. The results showed that microbial biomass and activity were generally higher in organically than conventionally managed soils with cotton gin trash (higher C/N ratio) being most effective. Straw mulching further enhanced microbial biomass, activity, and potential N availability by 42, 64, and 30%, respectively, relative to non-mulched soils, likely via improving C and water availability for soil microbes [155].

Soil microbial biomass produces different enzymes that play important roles in different metabolic pathways. Some enzymes are extracellular. They break down large molecules such as starch, cellulose, proteins into small molecules such as glucose that can be absorbed. Others are intracellular enzymes. A bacterial cell contains about 1000 enzymes [156]. When the soil organisms die, and the cell membrane disrupts, or when changes occur in the cell membrane permeability, the intracellular enzymes are put into the soil matrix and may form complexes with soil colloids [157]. The enzyme-colloid complex protects the enzymes against the effects of pH, temperature, desiccation, and attack by other enzymes and microorganisms. Consequently, they stay active for a period of time. They are called abiotic enzymes [157]. Soil aggregates and their constituent clays influence the interaction of enzymes with their substrates. The clay particle with its large external and internal surface areas is capable of adsorbing enzymes such as urease and protease. Enzymes adsorbed or intertwined with humic constituents are protected from hydrolysis by other enzymes. Adsorption also makes the catalytic sites less available. A small molecule such as urea can readily diffuse to a urease site and undergo decomposition. But a large molecule, such as protein, would not diffuse as readily to a protease site and consequently would be broken down at a much lower rate than urea [156]. In the soil matrix, adsorption of enzymes to the soil humate moves their optimum pH and temperature to higher values [158].

Enzymes are mainly proteins and consequently their structure is affected by factors such as temperature, pH, salt concentration, heavy metals, and other contaminants. Considering that sewage sludge contains heavy metals in different concentrations, it may affect the growth of soil organisms and soil enzyme activity [159].

As enzymes are involved in cell metabolism or may be active even as abiotic enzyme, they are the first soil property that show alteration when the system is disturbed. Soil enzyme activity has been considered as an indicator of soil quality since a long time, because it controls the supplying of nutrients to plants and the microbial growth [160]. Some individuals or group of soil enzymes have correlated to plant growth in different soils and soil management.

CONCLUSION

Microorganisms are potential tools for sustainable agriculture and the trend for future. The Biological Nitrogen Fixation (BNF) process offers an economically attractive and ecologically sound means of reducing external nitrogen input and improving the quality and quantity of internal resources. Endophytic microorganisms form complex and fascinating associations with host to improve plant growth promotion and biological control. Arbuscular mycorrhizal fungi are widely distributed in most ecosystems and actively participate in essential processes of the environment. Currently, there is a need to develop greater knowledge of soil ecosystems, and their biological diversity and ecological functions to build a broad basis for sustainable agricultural development, which should rely as much as feasible on renewable resources.

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