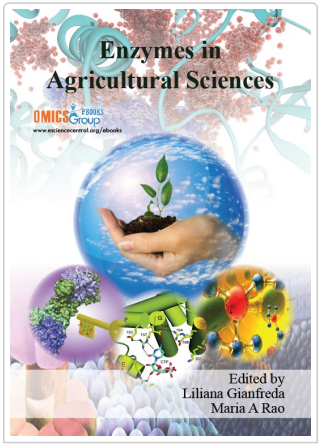

Enzymes and Soil Fertility

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1. Abstract

Soil is a fundamental resource in the agricultural production system and monitoring its fertility is an important objective in the sustainable development of agro-ecosystems. In order to evaluate soil fertility, changes in its physical, chemical and biological properties must be taken into account. Among the biological features, soil enzymes are often used as index of soil fertility since they are very sensitive and respond to changes in soil management more quickly than other soil variables. Thus, the objective of this work was to review some of the aspects that are connected with using soil enzymes as indicators of agricultural practices impact (e.g., soil fertilization, crop rotation, tillage) and soil fertility. The results that are discussed in the works listed in the bibliography showed no consistent trends in enzymatic activity as being dependent on farming management practices that have stimulated, decreased or not affected this activity. The influence of inorganic fertilization and organic amendments on the soil enzyme activities depended on the dose of this amendment, the time of its application, the content of harmful substances (e.g., heavy metals), the soil type and climatic conditions. Soil fertility indicators including soil enzymes as well as some advantages and shortcomings concerning the use of these indices are also discussed. Single enzyme activities are often used as indicators of soil fertility, which is considered to be a conceptual mistake, since they usually take part in only one specific process and therefore they cannot reflect the rate of all of the metabolic processes in soil. Complex expressions, in which different properties are combined, are thought to be more suitable for estimating soil fertility, although their use is limited to the area and the conditions in which they have been described.

2. Introduction

Soil is an important component of all terrestrial ecosystems as well as a main source of production in agriculture. Proper soil functioning is essential for the maintenance of the global biochemical cycles for all required nutrients and thus, the processes in soils affect many other biotic and abiotic components of ecosystems [1]. To understand the functioning of soils and to prevent soil damage due to both natural and anthropogenic factors, it is important to have suitable tools for predicting and assessing soil changes that are caused by environmental factors and management practices. Strategies based on biological indicators would be a suitable tool to evaluate the sustainability of the soil ecosystem. Studies of soil enzymes are important since they indicate the potential of the soil to support the biochemical processes that are essential for the maintenance of soil fertility [2]. Soil enzymes regulate the functioning of the ecosystem and play key biochemical functions in the overall process of organic matter transformation and nutrient cycling in the soil system [3-6]. The overall enzyme activity in soil consists of various intracellular and extracellular enzymes that originate from microorganisms (e.g., bacteria, fungi) or from plants and animals (e.g., plant rots or residues, digestive tracts of small animals) [6]. The same enzyme can originates from different sources and the exact origin as well as the temporal and spatial variability of the activity is difficult to identify [7]. Intracellular enzymes exist in different parts of living and proliferating cells, while extracellular enzymes are produced and secreted by living cells and act outside the parent cells as free enzymes in a soil solution or as enzymes that are still associated with the external surface of the root epidermal or microbial cell wall (so-called ectoenzymes). When secreted outside the cell, enzymes can be free in a soil solution or they can be adsorbed by soil mineral constituents or complexed with humic substances or both. The amount of free extracellular enzymes in soil is very low compared with that in the adsorbed state due to their short life span in an inhospitable environment such as [8]. Adsorbed enzymes are resistant to proteolysis, thermal and chemical denaturation [9], but immobilization usually protects enzymes against degradation at the cost of some loss of activity. Although bounded enzymes reveal less activity than free enzymes, the most important part of their activity is being

responsible for the transformation of organic matter and the availability of nutrients.

Numerous factors can influence enzyme activity in soil. Natural parameters (e.g., seasonal changes, geographic location, in situ distribution, physical-chemical properties, content of organic matter and clay) usually affect the enzyme activity level by influencing both the production of enzymes by plants and microorganisms and their persistence under natural conditions. The physical and chemical properties of a soil are involved in the immobilization and stabilization processes of most extracellular enzymes. A high content of clay or humus colloids is usually associated with stable but less active enzymes. Agricultural activities and environmental pollution (e.g., fertilizers, pesticides, tillage, heavy metals, PAHs) may affect the chemical composition and structural characteristics of soil, which in turn will influence the species composition and abundance of soil microorganisms and/or their metabolic activity, the enhancement or suppression of enzyme production and the overall activity of an enzyme in soil [6].

Soil enzymes are important in soil functioning because of the following features: 1) they play a critical role in the decomposition of organic materials and the transformation of organic matter, 2) they release available nutrients to plants, 3) they participate in N2 fixation, nitrification and denitrification processes, and 4) they take part in the detoxification of xenobiotics, such as pesticides, industrial wastes, etc. [10].

Soil enzyme activities have been suggested as sensitive indicators of soil fertility since they catalyze the principal biochemical reaction that involves nutrient cycles in soil, are very sensitive and respond to changes caused by natural and anthropogenic factors easily and can be easily measured since large number of samples can be analyzed within a few days using a small amount of soil [3,4,11,12]. 3. Soil Fertility – Definitions and Evaluation of Concepts The quality and fertility of soils play an important role in the sustainable development of the terrestrial ecosystem. Soil quality has been defined as ‘the continued capacity of soil to function as a vital living system, within ecosystem and land use boundaries, to sustain biological productivity, promote the quality of air and water environments and maintain plant, animal and human health’ [13]). Soil fertility is an integral part of soil quality that focuses more on the productivity of the soil, which is a measure of the soil’s ability to produce a particular crop under a specific management system. All productive soils are fertile for the plant being

grown, but many fertile soils are unproductive because they are subjected to same unbeneficial natural factors (e.g., drought) or management practices [14].

A variety of definitions and approaches have been proposed for the term ‘soil fertility’ [15,16]. In 1931 Waksman [17] wrote that ‘the measure of soil fertility is the crop itself’ but he did not succeed in distinguishing between the concept of soil fertility and that of soil productivity. The biological concept of the term ‘soil fertility’ was, however, presented in his consideration about the nature of this phenomenon ‘soil fertility and the rate of oxidation were found to be influenced by the same factors and to the same extent so that it was suggested that the later could be used as a measure of the former’. The importance of humus in the assessment of soil fertility was emphasized in the definition given by Howard in 1940 [18] ‘soil fertility is the condition of the soil rich in humus in which the growth processes are getting on fast and efficiently’ Nearly 50 years later, a definition of soil fertility connected with plant nutrition was proposed by Foth and Ellis [14], who stated that soil fertility is ‘the status of a soil with respect to its ability to supply elements essential for plants growth without a toxic concentration of any element’. Thus, soil fertility focuses on an adequate and balanced supply of elements or nutrients to satisfy the needs of plants under different climatic and soil conditions. A few years later, Ştefanic’s [19] definition again dealt with the most fundamental biological feature of soil fertility: ‘fertility is the fundamental feature of the soil, that results from the vital activity of micro population, of plant roots, of accumulated enzymes and chemical processes, generators of biomass, humus, mineral salts and active biological substances. The fertility level is related with the potential level of bioaccumulation and mineralization processes, these depending on the programme and conditions of the ecological subsystem evolution and on anthropic influences’. This complex definition was often replaced by the one proposed by Persson and Otabbong [20], who simply wrote that soil fertility is ‘the long-term capacity of a soil to produce good yields of high quality on the basis of chemical, physical and biological quality factors’. Furthermore, they discussed the concept of soil fertility thoroughly and specified three main components of soil fertility – physical, chemical and biological. These components continuously interact with each other under the influence of climatic factors, soil type and management practices [21]. The fertility of soil can be improved, maintained or decreased, depending on the cultivation practices that are used. For example, soil potassium or magnesium content can be increased or decreased. However, some fertility factors cannot be modified because of cultivation managements, e.g., soil type and topography. For instance, soil pH and the susceptibility of the soil to compaction are dependent on the constituents of the original parent rock. Subsequent events, including the growth of plants and the addition of fertilizers, modify the soil’s characteristics and alter its fertility. For instance, the original soil pH can be modified by legumes, which increase soil acidity (i.e. decrease soil pH). In relation to the statements above, actual and potential soil fertility can be distinguished. Potential fertility is when all variable fertility factors are optimized. In this situation, the unchangeable factors alone manage soil fertility. Persson and Otabbong [20] themselves concluded that yield level is an imprecise definition of soil fertility because of the complexity of the soil and soil processes.

Soil fertility cannot be assessed directly; it must be determined on the basis of changes in soil properties. Soil fertility is significantly affected by physical, chemical, microbiological and biochemical properties, which are sensitive to changes in the environment and land management. When soil fertility is considered in terms of the highest level of productivity, the emphasis is mainly on the physical and chemical properties of the soil. Among the chemical properties, total carbon and nitrogen content, soil reaction and the content of available nutrients are the most important in evaluating soil fertility, while as regards to the physical properties, the most important are bulk density,

porosity, water retention, soil temperature,, etc. Recently, concern regarding the long-term productivity and sustainability of agro-ecosystems has been concentrated on various bio-indicators and the application of biological methods, particularly in the development and protection of soil resources [22]. Biological indicators are used to assess soil quality or fertility because of their central role in nutrient transformations and their rapid response to changes in management practices. The biological properties of soil such as microbial biomass (C) [13], ecophysiological quotients [23], specific biochemical properties such as the activity of hydrolytic soil enzymes related to C, N and P cycles [10] and the composition of the microbial community [24] have been proposed as indicators of soil fertility.

4. Potential Role of Soil Enzymes in Maintaining Soil Fertility

Among the many biological properties that have potential as sensitive indicators of soil quality and fertility, enzyme activities often provide a unique integrative biological assessment of soil function, especially those that catalyze a wide range of soil biological processes, such as dehydrogenase, urease and phosphatase [11]. Soil enzymes play key biochemical functions in the overall process of the transformation of organic matter and soil nutrient cycling in the soil system [4,5]. The agricultural significance of soil enzymes has been progressively expanded since the first report on soil enzymes was written about a century ago [25]. Soil enzymes, which were once used as descriptive parameters, are now appreciated for their multiple functions in microbial activities, soil processes and ecosystem responses to management and global environmental change [26]. Selected enzymes that are of great agricultural significance are presented below and in Table 1.

Class/EC number*	Recommended name	Soil function and agriculture significance	Reaction	References
Oxidoreductases				
1.11.1.6	Catalase	Release oxygen from hydrogen peroxide; has a detoxification function in cells	2H2O2 → 2H2O+O2	[27,28]
1.11.1.7	Peroxidase		S+H2O2 → oxidized S+H2O	[29,30]
1.18.6.1	Nitrogenase	N fixation, catalyses the conversion of atmospheric, gaseous dinitrogen (N2) and dihydrogen (H2) into NH3	N2+3H2 →2NH3	[31]
1.10 1.13 1.14	Phenol oxidases	Oxidize phenolic compounds and are involved in humification of organic matter	e.g. laccase: 2 *p*-diphenol+O2 → *p*-quinone+2H2O	[29,30]
Transferases				
2.8.1.1.	Rhodanese	Performs intermediate step in oxidation of elemental S which is found in small amounts in soil or is added as a S fertilizer	S2O32–+CN → SCN–+SO32–	[32,33]
Hydrolases				
3.1.4.1	Phosphodiesterase	Indicator for P cycle, revealed to be a good index of the soil P availability to the plant	R2NaPO4+H2O → ROH+RNaHPO4	[34,35]
3.6.1.1	Pyrophosphatase	Indicator for P cycle, interest in this enzyme activity derives from the fact that ammonium polyphosphate, an inorganic salt of polyphosphoric acid and ammonia, is one of the frequently used phosphoric fertilizers	Pyrophosphate+H2O → 2PO43-	[36]
3.5.1.1 3.5.1.2.	L-Asparaginase L-Glutaminase	Act on C-N bonds (other than peptide bonds) on respective amino acids releasing NH3, important in N mineralization to provide plant available N	Asparagine → aspartic acid+NH3 Glutamine → glutamic acid+NH3	[37,38]
3.5.1.13	Aryl acylamidase	Hydrolyses propanil, which is use as a component of herbicide	Anilide+H2O → carboxylate+aniline	[39]

3.5.1.4	Amidase	Hydrolysis of C-N bonds other than peptide bond in linear amides releasing NH3, important for N mineralization to provide plant available N form	Monocarboxylacid amide+H2O → monocarboksyl acid+NH3	[37]
3.4.11.2	Arylamidase	Indicator of N cycle, hydrolysis of a N-terminal amino acid from peptides, amides and arylamides, an index of N mineralization in soils		[40,41]
3.2.1.26	Invertase	Indicator for C cycle, catalyzes the hydrolysis of sucrose to glucose and fructose; its substrate, sucrose, is one of the most abundant soluble sugar in plants, partially responsible for the breakdown of plant litter in soil	C12H22O11+H2O → C6H12O6+C6H12O6	[42,43]
3.2.1.8	Xylanase	Responsible for decomposition of xylan, a polysaccharide found with cellulose in soil	Hydrolysis of b-1,4-xylan bonds	[43,44]
Broad spectrum enzymes assay	Fluorescein diacetate hydrolysis	Provides general indicators of soil hydrolytic activity, which is carried out by proteases, lipases, and esterases; energy and nutrients for microorganisms, the measure of microbial biomass, organic matter decomposition and nutrient cycling		[45,46]

Table 1: Agronomically important soil enzymes and their functions. Parts adopted from Gianfreda and Bollag [6], Gianfreda and Ruggiero [7] and Dick [10].

*The enzymes are classified according to the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (NC-IUBMB); ROH: Hydroxylated compound; R: either alcohol or phenol group; S: reduced organic substrate.

Dehydrogenases activity (DHA) (EC 1.1): Soil dehydrogenases are the major representatives of the oxidoreductase enzymes class. Lenhard [47] was first to introduce the concept of determining the metabolic activity of soil microorganisms by measuring the activity of dehydrogenases because of its simplicity as compared to other quantitative methods. The activity of the DHA reflects the total range of the oxidative activity of soil microorganisms and may be considered a good indicator of the oxidative metabolism in soils, and thus, of microbiological activity [48]. Dehydrogenases oxidize soil organic matter by transferring protons and electrons from organic substrates to inorganic acceptors [49]. Many specific dehydrogenases transfer hydrogen on either the nicotinamide adenine dinucleotide or the nicotinamide adenine dinucleotide phosphate. Throughout mentioned co-enzymes hydrogen atoms are involved in the reductive processes of biosynthesis. These processes are part of the respiration pathways of soil microorganisms and are closely related to the type of soil and air-water conditions [50].

Nitrate reductase activity (NR) (EC 1.7.99.4): Nitrate reductase is an important enzyme in the process of denitrification, which catalyzes the reduction of NO_2^- to N_2O under anaerobic conditions. The nitrogen that is present in the structure of this enzyme acts as a terminal electron acceptor by bacteria rather than molecular O_2 and this is irreversible once NO is formed [51]. The systematic name for nitrate reductase is reduced NADP – nitrate oxidoreductase. Flavoprotein (FAD), which contains molybdenum, creates a prosthetic group for this enzyme [52]. Nitrate reductase is an adaptive enzyme and is synthesized only in the presence of NO_3^- ions, while in a soil solution it is repressed not by NH_4^+ per se, which was suggested earlier [53] but by L-glutamine, which is formed by the microbial assimilation of NH_4^+ [54].

The Urease (UR) enzyme is responsible for the hydrolysis of urea fertilizer into NH_3 and CO_2 with the concomitant rise in soil pH and N loss to the atmosphere through NH_3 volatilization [55]. Urease can be produced by bacteria, yeasts, fungi and algae, as well as plants [56]. Urease may be synthesized constitutively in some organisms, but most often urease expression is under N regulation [57]. The enzyme

synthesis is inhibited when cells grow in the presence of a preferred N source such as NH4+ [58]. In contrast, urease production is activated in the presence of urea or alternative N sources [57]. Due to its role in the regulation of N supply to plants after urea fertilization, soil urease activity has received a great deal of attention since it was first reported [59]. Urease has been widely used to evaluate changes in soil fertility since its activity increases with organic fertilization and decreases with soil tillage [60].

Cellulases (EC 3.2.1.4): Cellulose is the most abundant organic compound in the biosphere comprising almost 50% of the biomass that is synthesized by the photosynthetic fixation of CO2 [61]. The growth and activity of soil microorganisms depends on the carbon source that is contained mainly as plant residues that occur in the soil [62]. However, for carbon to be released as an energy source for use by the microorganisms, cellulose in plant debris has to be degraded into high molecular weight oligosaccharides, cellobiose and glucose by cellulase enzymes [63]. Cellulases is a group of hydrolytic enzymes that catalyze the breakdown of □-1,4 linked bonds in cellulose. Complete degradation of cellulose requires at least three enzymes: endo-□-1,4-glucanase, which attacks the cellulose chains at random, exo-□-1,4-glucanase, which removes glucose or cellobiose from the non-reducing end of the cellulose chains and □-D-glucosidase, which hydrolyzes cellobiose and other water soluble cellodextrins to glucose [62]. Since cellulase enzymes play an important role in the global recycling of the most abundant polymer in nature, more research should be done to understand the nature of this enzyme better, so that it may be used more regularly as a predictive tool in the assessment of soil fertility.

Glucosidases (EC 3.2.1.20/21) are the group of C-cycling enzymes that should be investigated as a function of the application of an organic amendment as they play a key role in the breakdown of low molecular weight carbohydrates. The most common and predominant among soil glucosidases is □-glucosidase [64]. This enzyme plays an important role in soils because it is involved in catalyzing the hydrolysis and biodegradation of various □-glucosides that are present in plant debris and which are decomposed in the ecosystem [65]. Glucose, the final product of □-glucosidase activity, is an important carbon source for the growth and activity of soil microorganisms [64,66]. The activity of □-glucosidase is particularly stable and has a low seasonal variability [67] and is very sensitive to soil pH and soil management practices [68,69]; therefore, it can be used as a good biochemical indicator for measuring ecological changes that result from soil acidification and various agricultural practices. Additionally, changes in its activity could be indications of the fungi/bacteria ratio in soil [70].

Phosphatases are a group of enzymes that are of great agronomic value because they catalyze the hydrolysis of organic phosphorus compounds and transform them into an inorganic form of P, which is then assimilated by plants and microorganisms [71]. Agricultural soils contain phosphatases in varying amount depending on the microbial count, the amount of organic materials, mineral and organic fertilizers, tillage and other agricultural practices [72]. The relationship between the available P content and phosphatase activity in soil is complex. A positive, negative or no relationship can be observed between these properties. Generally, a significant and positive relationship between phosphatase activity and P availability [6,73] is obtained in soils that are not fertilized and/or those that have small amounts of nutrients in which a P deficiency occurs. An inverse relationship between these two parameters is usually observed in soils that are fertilized with P and/or those with a sufficient content of available P. There are studies that show that phosphatase activity is inversely proportional to the plant available P content [71,73,74], which confirms the thesis that the production and activity of soil phosphatases is connected with the demand of microorganisms and plants for P. Phosphatases are typical adaptive enzymes and their activity increases when the plant available P content decreases [75]. Kinetics studies indicate that orthophosphate ions, which are the product of the reaction that is conducted by the phosphatases, are competitive inhibitors of their activity in soil [76]. When no relationship is seen, P may not limit the study system and some other factors may influence the enzyme production and activity [77].

Arylsulphatase (EC 3.1.6.1) plays an important role in the mineralization of organic S in soils. This enzyme is involved in the hydrolysis of aromatic sulphate esters (R-O-SO3–) into phenols (R-OH) and sulfate sulfur (SO4–2) by splitting the oxygen-sulphur (O-S) linkage and that is why it is involved in the mineralization of ester sulfate in soil [64]. In most aerobic soils ester sulfates account for up to 70% of the organic S, and therefore are the most important organic S reserve in soil [78]. Additionally, that enzyme can be an indicator of the presence of fungi since only fungi contain ester sulfates, a substrate of arylsulfatase activity [69,79]. Arylsulfatase has been detected in plants, animals, microorganisms and soils [64] and is only one of the many types of sulfatases involved in the mineralization of ester S compounds. Arylsulfatase is a mostly adaptive enzyme and its synthesis by microorganisms may be controlled by the C and S content in the soil environment [10,64].

Proteases: The application of organic nitrogen (N) to soil may be an important economic alternative to the application of fertilizer N due to the reduced cost of the production of inorganic N fertilizers. It has been estimated that about 40% of the total soil N, including proteins, glycoproteins, peptides and amino acids, is proteinaceous material [80]. Organic amendments that are applied in agriculture, such as farmyard manure, municipal solid waste or sewage sludge are characterized by the presence of a high load of organic N forms, such as proteins, nucleic acids and amino-polysaccharides [e.g. 81]. Thus, protein transformation in soil has a considerable influence on soil ecology and agriculture [82].

Protein that is added to soil is readily decomposed by proteases and peptidases into smaller, membrane-permeable peptides and amino acids. The latter are further metabolized with the release of NH4+ [83]. Proteases are ubiquitous and originate from a number of different sources in soil, including microorganisms (bacteria, actinomycetes, and fungi), plants and animal excrements [84]. Many of these enzymes are extracellular, as a large number of native proteins are too large to be absorbed by living cells [83]. Proteolysis is an important process in many ecosystems with regard to N-cycling because it is considered to be a rate-limiting step during N mineralization in soils due to the

much slower primary phase of protease activities during N mineralization compared with amino acids mineralization [85].

Amylases (EC 3.2.11/2): Amylases together with cellulases and invertase form the group of enzymes that are responsible for the rate and course of the decomposition of plant material in soil [86]. Amylase is the name of a group of starch hydrolyzing enzymes in which the most important is □-amylase, which converts starch into glucose and/or oligosaccharides and □-amylase, which converts starch into maltose [87]. The □-amylases are synthesized by plants, animals and microorganisms, while □-amylase is produced mainly by plants [87]. Studies have indicated that the activities of soil amylases may be influenced by different agricultural practices, the type of soil and vegetation [42,86]. Plants may influence soil amylase activity by supplying enzymes directly from their residues or excreted compounds, or by indirectly providing substrates for the synthesis enzymes by microorganisms [86].

5. Soil Enzymes as Indicators of Agricultural Practice

Enzymes play an important role in the cycling of nutrient in nature and because their activity is sensitive to agricultural practices they can be used as an index of soil microbial activity and fertility [88]. Earlier, the emphasis had been placed on the conventional physical and chemical properties as indicators of soil fertility rate, but often these properties responded slowly to management practices and were found to be not sensitive enough to detect changes in soil properties that are caused by agricultural management practices, especially in the short-term [89]. Therefore, there is a need to find suitable tools that reflect the influence of management practices in order to observe possible changes [90]. Biological indicators, such as soil microbiological biomass and enzymatic activity, seem to be better indicators since they respond much more quickly to both natural and anthropogenic factors in comparison with other variables [91]. Thus, they may be useful as early indicators of biological changes in soil [69,92]. Soil enzyme activities are strongly affected by the agricultural management practices and have been used as indicators of irrigation [93], the application of inorganic fertilizers and organic amendments [e.g., 94-98], different management and farming systems [e.g., 37,99,100] and soil tillage [e.g., 40,101-103]. Enzymatic activity was found to be the most strongly influenced soil property under intensive agricultural practices as compared with other biochemical parameters [60].

5.1. Inorganic fertilization, positive and negative feedback mechanisms

Among the different farming practices, the management of mineral fertilizers and organic amendments could have a major impact on soil fertility, thus influencing the quantity and quality of organic residues and nutrient inputs that enter the soil and the rate at which the residues and organic matter are decomposed [104]. The influence of inorganic fertilization on the soil enzyme activity depends on the dose of the fertilizer and the time of its application, the soil type, climatic conditions and the enzyme itself [6]. Studies on the effect of inorganic N fertilization on enzyme activities have led to contradictory results [7]. Some previous studies have shown that N fertilization can accelerate the activity of some C, N and P cycling enzymes, like cellulases [94], urease [105] and phosphatases [98,105] or decrease the activity of urease [106], cellulases [94], peroxidase [107], proteases [108], while some other enzymes are not affected with increasing N fertilizer application [99,109]. More often, however, enzyme activities increased when organic and inorganic N fertilizers were added together [e.g., 110]. Mineral N can directly affect the microbial production of soil enzymes but the effect varies with the type of soil and the enzyme as well as with the kind of enzymatic reaction [97], which is possibly due to changes in the composition of the soil microbial community and, therefore, the enzyme production [4,97]. On the other hand, N fertilization, especially in mineral forms, may have an indirect effect on the

activities of soil enzymes via changes in soil properties, such as soil reaction [7].

Most often soil enzyme activities significantly depend on the dose and the frequency Repeated application of inorganic N fertilizers over a period of 305 days showed no significant effect on the activities of □-glucosidase and proteases [111]. However, the enzyme activities (□-glucosidase, urease, arginine deaminase, acid and alkaline phosphatases) were 10-26% lower at a rate of 160 kg N ha-1 year-1 compared to the highest activity, which was noted in the case of a rate of 40 and/or 80 kg N ha-1 year-1 [90]. Siwik-Ziomek et al. [112] stated that the optimal rate of ammonium nitrate that coincided with the highest activity of arylsulfatase was 100 kg N ha–1, while higher doses (150 and 200 kg N ha–1) decreased this activity. In the study of Šlimek et al. [113], dehydrogenase activity decreased with an increase in the dose of NPK fertilizer and was further decreased in the absence of lime. In the study of Giacometti et al. [108], a significant reduction of dehydrogenase activity was observed at 200 kg ha-1 of mineral N compared to control (N0) plots. This suggested that dehydrogenase activity was highly sensitive to the inhibitory effects that are associated with large additions of mineral fertilizers. According to Iyyemperumal and Shi [97], the activities of cellulase, cellobiohydrolase and acid phosphatase increased with an increasing rate of ammonium nitrate (0-600 kg PAN ha-1 year-1) and were inversely related to the soil microbial biomass. The activities of peroxidase, phenolooxidase, cellobiohydrolase and protease activities were similar at the highest fertilization rate and in the unfertilized control. The above data was similar to that obtained by Sinsabaugh et al. [94], where increasing the available soil N generally stimulated soil cellulase activity but inhibited the activity of oxidative enzymes, such as phenol oxidase and peroxidase. Dick at al. [114] showed that amidase and urease activities decreased with the increased application of ammonia-based N fertilizer. They stated that the addition of the end product of the enzymatic reaction (NH_4^+) suppressed enzyme synthesis.

In fact, a feedback mechanism such as suppressing the production of enzymes whose reaction product was continually supplemented with inorganic fertilizers is generally known for some enzymes, such as urease or phosphatases [76]. Perhaps extracellular enzymes are produced by microorganisms only when the enzymes can help to better use the resource and therefore lead to optimal microbial grow metabolism. This cost-efficient strategy predicts that the production of microbial enzymes can be low when the end products of enz

reactions, e.g., nutrients, are abundant or when the end products of enzymatic reactions, e.g., complex organic substances, are limited [115]. End product suppression on enzymes has been mentioned by many authors and the activity of a nutrient-mineralization enzyme is inversely related to the availability of nutrients [25]. It was shown that negative feedback was indeed the cause of a reduction in phosphatase and chitinase activities after long-term phosphorus and nitrogen fertilization [77]. In the case of high nutrient availability, enzyme activities can be low and thus fertilization will not cause additional suppressive effects [77]. When nutrient availability is low, the nutrient may limit microbial growth and metabolism and then fertilization may stimulate microbial biomass and in turn microbial enzyme production.

Phosphorus fertilization under field conditions has been shown to depress phosphatase activity in agricultural systems [116], although the influence of fertilization on the enzyme was dependent on the content of soil organic matter. A low organic matter content increased phosphatase activity with P fertilization, but soil with a high organic matter content that was amended with P fertilizer showed no changes in the enzyme activity. In the study of Mijangos et al. [89], acid phosphatase did not decrease when P was added as a mineral fertilizer, probably due to the relatively low P dose that was applied (i.e. the highest values of Olsen P were around 30 mg kg-1). According to Chunderova and Zuberts [117], phosphatase activity appears to be inhibited at 100 mg Olsen P kg-1 soil. Similarly, in the study of Criquet and Braud [118], the application of both Na and K phosphate salts at a rate to provide 0.22 g kg-1 of P total quantity (equivalent to 0.5 g P2O5) (supplied as NaH2PO4 and KH2PO4, respectively) did not show any significant effect on acid and alkaline phosphatases and phosphodiesterase activities, although it resulted in a significant increase in the available P content up to 112.2 □g P g-1 soil.

The application of lime to soil most often causes a significant increase in pH and thus significantly affects other physical, chemical and biological soil properties such as microbial biomass and diversity, and therefore, enzyme activities [e.g. 40,66]. According to Haynes and

Swift [119] additions of lime generally increased protease and sulfatase activity, but decreased phosphatase activity. In the study of Ekenler and Tabatabai [120], acid phosphatase decreased with increasing pH because of liming and also found that liming increased the activities of alkaline phosphatase and phosphodiesterase. The different responses of acid and alkaline phosphatases to liming supported the previous finding that phosphatases are inducible enzymes and that the intensity of their release by microorganisms and plants is determined by their requirement for orthophosphate, which is strongly affected by soil pH [3]. The optimum soil pH for crop production and the amount of lime required to achieve this optimum can be investigated by using alkaline (Pal) and acid phosphatase (Pac) activities [121]. The ratio of both of these enzyme activities (Pal/Pac) responded immediately to changes in pH that was caused by the addition of CaCO3 and the ratio of approximately 0:5 divided soils into those with a proper pH and those that still needed an additional lime treatment (Figure 1). In the study of Lemanowicz [122] with long-term nitrogen fertilization (ammonium nitrate at the following rates: 0, 50, 100, 150, 200 and 250 kg N ha-1), the Pal/Pac ratio decreased from 0.44 to 0.21 with increasing doses of the nitrogen ap¬plication. Dick et al. [121] concluded that measuring the Pal/Pac ratio may be preferable to chemical approaches for evaluating the effective soil pH and liming needs.

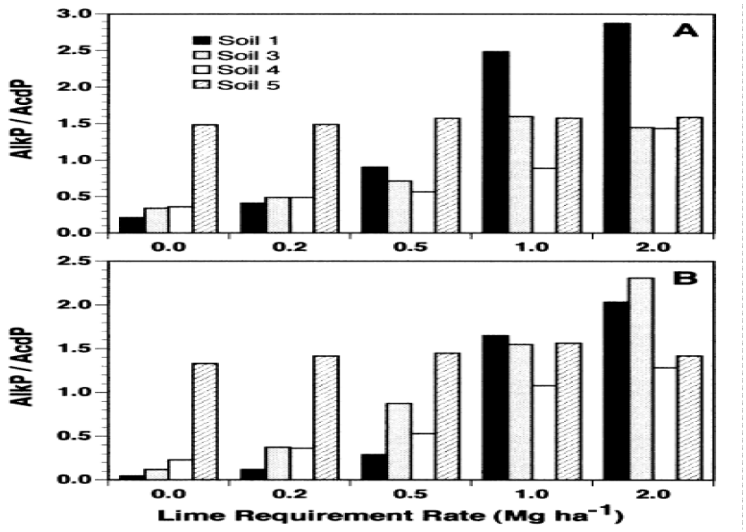


Figure 1: Changes in alkaline phosphatase/acid phosphatase (AlkP/AcdP) activity ratios in soils amended with (A) chicken manure and (B) alfalfa residue after adjusting pH with CaCO3 according to the lime requirement of each soil. LSD0.05 values used for mean comparisons for Soils 1, 3, 4 and 5 are 0.277, 0.236, 0.215 and 0.341, respectively. Adopted from Dick et al. [121].

5.2. Organic amendments

The application of organic amendments (straw, farmyard manure, green manure, sewage sludge, olive mill waste and other waste and by-products) to soil as a nutrient source is a management practice that can increase the soil organic matter content, improve soil biological activity and consequently increase the soil nutrient status with a simultaneous reduction in the dependence on mineral fertilization [e.g. 123-126]. Among the organic amendments that can be used as potential fertilizers, special attention has been paid to urban residues such as municipal solid waste and sewage sludge, the generation of which has been growing rapidly in recent decades and its management has

municipal solid waste and sewage sludge, the generation of which has been growing rapidly in recent decades and its management has become one of the key tasks in environmental policy of many countries [127,128].

Contradictory results have often been observed when the short- and long-term effects of organic amendments on soil enzyme activities were compared [129,130]. The short-term increase in soil enzyme activities after the application of organic amendments can be attributed to a greater microbial biomass due either to the addition of microorganisms and enzymes in the amendment or indirectly to the addition of available organic substrates that promote the growth of indigenous microorganisms [e.g., 88,95]. The long-term effect of organic amendments on enzyme activities is probably the combined effect of a higher degree of the stabilization of enzyme to humic substances and an increase in the microbial biomass with an increased soil C concentration [131] as well as the inhibitory effect of heavy metals and other toxic substances. The increase of enzymatic activity shortly after the addition of organic amendments, probably caused by exhausting the easily degradable substrates, has been noted in many studies [e.g. 70,96,132,133], Perucci [70] found significantly higher values of the activities of eight different groups of soil enzymes within 30 days after the application of compost from municipal residues as well as during the following three years. In the study of Kizilkaya and Bayrakh [96], a sudden and significant increase in enzymatic activity (urease, alkaline phosphatases, arylsulfatase and □-glucosidase) was observed after the addition of sewage sludge in sludge-amended soils followed by a progressive decrease in this activity. Positive influence of manure and vermicompost application on enzyme activity was found by Marinari et al. [126] (Table 2).

Treatments	Dehydrogenase (mg INTF g-1 h-1)	Protease BAA (mg NH3 g-1 h-1)	Acid phosphatase (mg pNP g-1 h-1)
Control	4.1a	75.1a	266a
NH4NO3	6.1b	87.8ab	457b
Manure	20.7c	73.5a	727d
Vermicompost	1.7b	91.8b	613c

Table 2: Enzyme activities in soil fertilized and unfertilized, three months after fertilization Adopted from: Marinari et al. [126]. The values with the same letter are not significantly different ($P \leq 0.05$)

Because olive mill waste water contains a high level of organic matter and nutrient content, its recycling as fertilizer may be an alternative to its disposal that also improves soil fertility and productivity [134]. All nine enzymes that were tested in a ten-year field study in semi-arid Mediterranean areas increased even with the application of the higher de-oiled two-phase olive mill waste (DW) (54 Mg ha-1). Compared to the control, the addition of crude OMW (olive mill wastewater) and dephenolized OMW to the soil increased dehydrogenase activity suddenly and sharply, which was 8 and 4 times higher as compared to the control soil [133]. In the same study [133] diphenol oxidase activity, another enzyme that is involved in redox soil reactions was inhibited by dephenolized OMW as compared with crude OMW. This was probably due to the presence of available diphenol substrates in crude OMW, which could enhance the synthesis of the enzymes by the microorganisms [95].

The rapid development of biogas production is resulting in the increased use of biogas residues as an organic fertilizer. In the study of Chen et al. [135], the extracellular enzyme activities in agricultural soil amended with biogas residues (BGR) versus maize straw (MST) were assessed. The influence of given treatments on enzymatic activity is presented on Figure 2. Conversely, MST significantly increased the activity of these three enzymes. The contrasting effect of treatments of BGR and MST on these enzymes may be attributed to the lower availability of C in biogas residue compared with maize straw. The high lignin content in BGR indicated a low C availability due to the formation of lingo-cellulose or lignin-polysaccharide complexes, which may resist the attack of enzymes [125]. Biogas residue significantly promoted the activities of chitinase and leucine amino peptidase, two enzymes that are related to the N-cycle. These enzymes

were promoted by an N-enriching organic component, e.g., peptidoglucan, which was accumulated as microbial residue during the fermentation of the biogas [136].

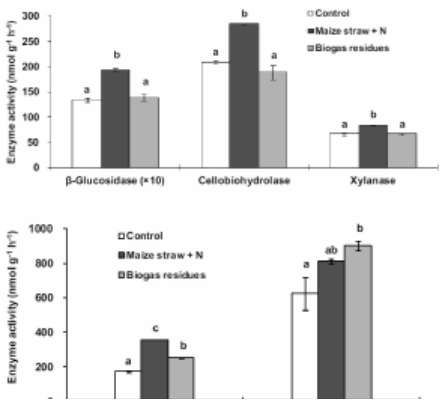


Figure 2: Extracellular enzyme activity as influenced by the addition of biogas residue, maize straw+mineral N. C-cycle related enzymes □-glucosidase, cellobiohydrolase and xylanase are at the top and N-cycle related enzymes chitinase and leucine amino peptidase (LAP) are at the bottom. The values of □-glucosidase activity were divided by ten in order to fit the scale. Data are means of 3 replicates ± standard errors. Different letters show significant differences between the treatments (P<0.05). Adopted from Chen et al. [135].

A decrease of some enzyme activities after the application of organic amendments was also shown [e.g., 133,137] because they may also contain pollutants (e.g., heavy metals and toxic organic compounds) which, if present in an inhibitory concentration, may decrease soil enzymatic activity [6,96]. Sewage sludge may especially inhibit soil enzyme activities drastically due to a higher content of heavy metals [e.g. 138,139]. The inhibitory effect of heavy metals can overcome the stimulatory effects of the addition of municipal solid waste compost to soil [75] and the effect is dependent on the individual sensitivity of enzymes to heavy metals [70,129]. In the study of Moreno et al. [140] no microbial activity, which was indicated by the total lack of dehydrogenase activity, was found with sewage sludge that contained a high Cd content (815 mg kg-1) or when the toxic olive-mill solid waste was applied to the soil. Olive waste shows a high toxicity to microorganisms, directly related to the presence of different types of polyphenols [95]. In fact, in the study of Piotrowska et al. [133], some enzymes (fluorescein diacetate hydrolase: FDAH, nitrate reductase, urease), decreased significantly after the addition of crude olive mill waste water (OMW) compared with dephenolized OMW.

In agricultural practice, the importance of farmyard manure as an organic fertilizer that has great value has gradually decreased in recent years due to new techniques in animal production. On the other hand, green manure or straw residues are still an important source of organic matter and nutrient input into arable soils [124,125,141,142]. According to Perucci and Scarponi [137], the majority of crop residues (wheat straw, maize, sunflower, tobacco, capsicum, sorghum, and tomato) generally caused the activation of phosphodiesterase in untreated soil, while it inhibited acid phosphatase activity. Only the incorporation of tobacco residues caused a significant increase in all of the phosphatases that were tested (neutral, acid and alkaline). In Turkey, it is possible to use tobacco waste as a soil amendment due to its high organic matter and low content of toxic elements [143]. In the study of Okur et al. [143], the highest enzyme activities were observed in soil with 25% of farmyard manure and 75% of tobacco waste compost. Detailed results of enzyme activities are presented in Table 3.

Treatments	DH (mg TPF g-1 16 h-1)	Pal (mg *p*NP g-1 h-1)	UR (mg S g-1 3 h-1)
Control	143.4 (9.1)c	625.3 (78.2)b	50.9 (2.9)b
25% FYM+75% TWC	230.3 (13.8)a	824.2 (82.4)a	71.3 (6.2)a
50% FYM+50% TWC	176.1 (13.8)bc	716.4 (64.4)ab	51.1 (11.6)b
75% FYM+25% TWC	180.1 (14.5)bc	767.0 (62.6)ab	58.9 (4.0)ab
100 % FYM	166.2 (19.5)c	701.2 (79.8)ab	49.5 (7.9)b
100% TWC	208 (8.3)ab	762.2 (73.9)ab	68.2 (10.3)a

Table 3: Dehydrogenase (DH), alkaline phosphatase (Pal) and urease (UR) activity in the soil samples (0-20 cm). Adopted from Okur et al. [143].

Number in parentheses are standard deviation (n=3). Mean value followed by the same letter are not significantly different between different treatments, according to Tukey's test (P<0.05); FYM: farmyard manure; TWC: tobacco waste compost; TPF: triphenyl formazan; pNP: p-nitrophenol; S: saligenian

The increase in enzymatic activities that is observed because of the application of green manure has been mentioned in many studies [69,141,142,144]. Debosz et al. [145] reported that the activities of □-glucosidase, cellobiohydrolase and endo-cellulase in a fertilization system with green manure were significantly higher than in a system with exclusive mineral nitrogen fertilization. Tejada et al. [142] studied the effect of incorporating three green manures originating from the residues of Trifolium pratense L (TP), Brasicca napus L (BN) and a mixture of them (TP+BN). All of the green manures had a positive effect on the biological properties including enzymatic activities. The activities of dehydrogenase, urease, □-glucosidase, phosphatase and arylsulfatase increased more significantly in the TP amended soils (from 79 to 96%), followed by the TP+BN and BN amended sites, thus suggesting the more easily decomposable components of TP, and hence an improvement in their microbial activity. In the study of Kautz et al. [141], the highest dehydrogenase and cellulases activities were observed in treatments with straw and green manure as compared to the control, farmyard manure and mineral N fertilization. It was stated that the higher the dose of the organic amendment the greater the enzymatic activity due to the higher microbial biomass produced in response [123]. As was stated Geisseler et al. [146], plant residue is composed of many polymeric molecules that must be broken down into available units by extracellular enzymes. Therefore, it was no surprise that protease, □-glucosidase and exocellulase activities were significantly increased with the addition of the oats-legume plant residue. According to Pancholy and Rice [86], dehydrogenase activity was influenced by the quality rather than by the quantity of organic matter that was incorporated into the soil. Thus, the stronger effects of green manures on dehydrogenase activity might be due to the more easily decomposable components of crop residues on the soil microorganisms. Under field conditions, however, the decomposition of green manures is complex and is controlled by many factors, such

as carbon and nitrogen content and the C/N ratio, the biochemical nature of the plant residues, soil climatic factors, etc. [142]. The C/N ratio of the organic amendments will largely determine the balance between the mineralization and humification processes [123]. The C/N ratio is considered to be the best parameter for predicting the potential amount of N that can be mineralized from a plant material [147].

Straw incorporation into the soil is considered to be an important strategy to improve soil fertility and to reduce the dependence on mineral fertilization [148]. Although the use of plant residues has long-term positive effects on soil properties, in the short term (several weeks following straw incorporation), this residue may cramp root penetration and cause an N deficiency. The reason for an N deficiency is that more soil N is being immobilized by microorganisms due to the incorporation of the straw [149]. Therefore, it is important to find a way to

enhance the decomposition of straw shortly after its incorporation. One promising method is accelerating straw decomposition through the application of exogenous cellulose to soil [148]. In the study of Han and He [148], 70 U g-1 was considered to be the optimal cellulase concentration for plant growth, while 50 U g-1 was acknowledged as being economically beneficial. Earlier, Fontaine et al. [150] found that exogenous cellulase accelerated the decomposition of cellulose in soil significantly. Similarly, the short-term effects of the application of exogenous protease on soil fertility with the incorporation of rice straw were studied by the same authors [151]. After 120 days of incubation with rice straw, soil protease activity, available N, available P and electrical conductivity of treatments with at least 0.5% added protease were significantly ($P<0.05$) greater than the no-protease control. Protease activities potentially increase the soil available N because they promote the release of amino acids from straw protein through hydrolysis. Protease application increased the available P content because microbial activity can increase as a result of the increase in the available N content. The soil organic matter and pH of treatments with added protease were lower than the non-protease control. Without the incorporation of straw, protease amendments only affected the soil protease activity itself, but not other soil properties. The authors concluded that the addition of exogenous protease can reduce an N deficiency and may be used in fields when straw is applied [151]. Therefore, the application of exogenous cellulase and protease during the incorporation of straw may be a new strategy that will help farmers manage plant residues in the field and increase soil fertility [148,151].

5.3. Bio-fertilizers

Although bio-fertilizers have been known for many years, relatively little research has been done to document the effects (or non-effects) of many bio-fertilizers on crop production, or to provide evidence of their potential effects on soil processes [e.g. 152-214]. Moreover, only a few studies exist on the effects of bio-fertilizers on enzymatic activities [152,155,156]. Valarini et al. [155] concluded that the incorporation of “Effective Microorganisms” (EM) together with animal manure and fresh plant debris significantly improved the soil biological activity measured as alkaline phosphatase, esterases and polysaccharidases activities, due to the quick humification of fresh organic matter. The four-year application of two preparations based on EM in an arable organic farming crop rotation in the temperate climate of Central Europe caused no effects on soil dehydrogenase activity [152]. No clear tendency in changes in dehydrogenase activity was noted in a three-year study with UGmax biofertilizer [156]. The results of the study of Piotrowska et al. [156] showed that the microbiological fertilizer UGmax accelerated the initial phase of the decomposition of post-harvest residues, which was confirmed by a significant decrease in cellulase activity in the soil that had been taken from a field where UGmax was applied as compared with the control field. One of the possible explanations is that the cellulase activity increased directly after UGmax treatment and therefore the post-harvest residues decomposed faster than in the control field. As the result of this fact, five months after the second UGmax application (soil samples were always taken shortly before the autumn UGmax treatment), the post-harvest residue content was lower than in the control soil and simultaneously the cellulase activity had decreased (Figure 3). This suggests that UGmax is probably a medium that determines the decomposition rate of post-harvest residues and that the activity of cellulase was a distinct indicator of soil changes after the application of UGmax. Results presented by Chen et al. [154] suggested that the two agricultural biostimulants used (Z93 which is marketed in USA under the trade name GroZyme® and W91, which is not yet marketed) significantly augmented cellulase activity, which was measured as the rate of filter paper weight loss that systematically increased up to the end of the incubation (56 days).

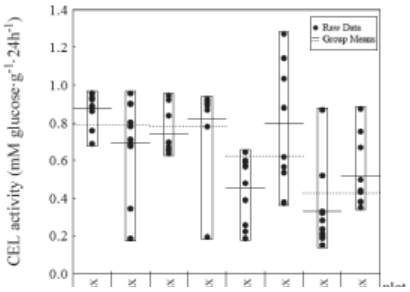


Figure 3: Cellulase activity (CEL) as influenced by UGmax treatment; – mean value for treatment (UGmax vs. control); mean values for both UGmax and control in succeeding years. Adopted from Piotrowska et al. [156].

5.4. Tillage

Tillage may change soil fertility due to altering the physico-chemical [157] as well as microbiological and biochemical properties, and thus the soil enzymes [40]. Tillage influences the soil nutrient level and its availability the distribution of organic matter in the soil profile [158] and the soil water and oxygen content [159]. Tillage causes the acceleration of soil organic matter mineralization and consequently leads to a rapid loss of its content. This in turn, causes a decrease in the soil biological and enzymatic activity [101]. In fact, some authors have reported a decrease in enzyme activities due to the decrease in organic matter content as a result of the mixing of horizons by plowing [e.g. 160].

In some studies, no-tillage system increased soil enzymatic activity as compared to conventional tillage practice [75,161]. Deng and Tabatabai [162] showed that the activity of L-asparaginase, L-glutaminase and urease were generally greater under a zero-till system than under a conventional tillage practice. Mina et al. [102] observed the same trend for soil dehydrogenase, alkaline phosphates and protease activities. The activity of dehydrogenase, urease, protease, phosphatase and □-glucosidase was significantly higher in a no-tillage system with varying percentages of surface residue coverage (0, 3, 66, 100%) and a no-tillage system with 33% residue coverage together with cover crops of Vicia sp. or Phaseolus vulgaris L. as compared with conventional tillage [161]. Usually, the enzymatic activity of the surface layer of a zero-tilled soil are greater than those of the same layer of tilled soils, while the opposite occurs for the deepest soil horizons [e.g. 69,163], which is due to the fact that microbiological activity of surface no-tilled soils is higher than in conventional tillage [6], which is caused by the fact that organic matter is more thoroughly distributed in soil under conventional cultivation compared with that in soils under reduced tillage in which crop residues, which are substrates for soil microorganisms, are concentrated on the soil surface [120].

Some other authors have indicated that plowing causes an increase in enzymatic activity in agricultural soils due to the exposure of new surfaces as soil aggregates are broken [e.g. 164]. In fact in the study of Ulrich et al. [103], the arginine ammonification showed high activity down to a depth of 30 cm of soil profile due to the high aeration in the plow treatment. Similarly, Seifert et al. [165] stated that conventional tillage accelerates the microbial oxidation of organic matter thus stimulating a greater microbial activity. For example, microbial activity, estimated by dehydrogenase activity and by FDA hydrolysis, was higher under conventional tillage than by incorporating plant residues onto surface soil, due to more labile C substrates, which support microbial activity.

In turn, tillage practice (none vs. conventional) had no effect on the enzyme activities in a multi-location field study carried out in Colorado, Kansas and Kentucky (USA) in a loam soil [66]. Similarly, no significant effects of tillage practice on the activities of dehydrogenase, phosphatase and urease activities were noted in soil over four years of spring barley cultivation [166].

The effect of tillage on enzymatic activity in soil depends on the enzyme itself and that is why phosphatase activity was more sensitive to negative changes that were caused by the tillage system [167] than was the urease activity. According to Ekenler and Tabatabai [168], L-glutaminase was the most sensitive N-cycle enzyme followed by amidase, L-asparaginase, L-aspartase, urease, arylamidase for discriminating the effect of tillage (no-till, ridge-till, chise-till). Earlier, the same authors [120] indicated the activity of □-glucosidase and □-glucosaminidase as the most sensitive enzymes that are affected by liming and tillage systems. Moreover, the response of enzyme activity to different tillage can be annually and seasonally dependent [159,163].

5.5. Vegetation cover and cropping system

Soil enzyme activity can be affected by the presence and nature of plant cover [6]. Although most of the soil enzyme activities originate from microorganisms, plant roots are an important source of extracellular enzymes in soil. Juma and Tabatabai [169] showed that sterile corn and soybean roots contain acid phosphatase, but not alkaline phosphatase activity. Although there was no clear relationship between arylsulfatase activity and root distance; enzyme activity tended to be higher close to the root surface (distance 0.25 mm) as compared with the distance of 0.75 mm in all of the crop species (Barssica napus, Sinapus album, Triticum aestivum, Lolium perenne) [170]. Plants actively respond to an insufficient S supply by producing and excreting sulfatases, which may help them to exploit the organic soil S, compounds [170]. The arylsulfatase activity that is found in the root protein extracts cannot originate from soil bacteria, since the seeds and seedlings had no contact with the soil. This could be explained by the fact that higher plants possess their own arylsulfatases that are inducible under an S deficiency or that seed-borne bacteria that colonize the intercellular sites within the root such as endophytes are responsible for the enzyme activity [170].

Plant roots stimulate enzyme activity by creating advantageous conditions for microbial activity [e.g. [171]. Highest higher enzymatic activity in the plant rhizosphere than in bulk soils was found in many researches, which may be explained by the development of a large population of soil microorganisms in the vicinity of roots that metabolize amino acids, sugars, organic acids and other compounds that are

exuded by roots [e.g 170,172]. Leguminous plants have the potential for biological N2 fixation and this could stimulate the activity of the enzymes that are involved in the N cycle (urease and protease-BBA) [161]. In crop systems that involve both leguminous and non-leguminous plants, the leguminous rhizosphere showed a higher activity of acid, neutral and alkaline phosphatase as compared with the non-leguminous plant rhizosphere [172]. The highest catalase activity was recorded in soil under wheat, soybean and winter legume crops, while the lowest activities were found in soil bearing corn and cotton and during the winter fallow period in the rotation system at the Agronomy farm of the Alabama Agricultural Experimental Station (USA) [27]. The highest activity of arylsulfatase among different crop species was with Cruciferae due to their high S demand [170]. Soil that was under permanent grassland had 1.5 times higher dehydrogenase activity and almost a two times higher acid phosphatase activity than no-till and conventionally tilled soils [173]. Higher arylsulfatase activity was noted in soils from a permanent pasture and an alfalfa field than from cultivated wheat fields [174]. Both long-term leguminous cover cropping and the direct incorporation of green manure increase the soil protease activity due to the enhancement of soil organic matter and the stimulation of soil microbial activity [175].

Much research is devoted to the influence of cropping system on enzymatic activities [33,37,100,109]. The type of cropping system may also influence soil enzyme activities. Crop rotation systems, which over time provide greater plant diversity than monoculture systems,

generally have a positive effect on soil enzyme activities [176]. This effect may be due to the stimulation of microorganisms in the rhizosphere and improved physical conditions of soils in crop rotation, due to the high input and diversity of organic materials entering the soil, particularly when the rotations contain legume species, whereas, a monoculture causes the physical degradation of soil, which in turn has a negative effect on soil microbial and enzymatic activities [7,37,109]. Gajda and Martyniuk [100] found higher dehydrogenase and phosphatases activities in organic and conventional systems than in monocultures. In the study of Klose et al. [99], the total intracellular and extracellular arylsulfatase activities in the soils of Iowa (USA) were significantly affected by crop rotation and plant cover. Generally, the highest arylsulfatase activities were obtained in soil under cereal-meadow rotations (corn-soybean, corn-corn-oat-meadow) and the lowest under continuous cropping systems (corn, soybean). As stated by the authors, the results could be due to the positive effects of diversified crop rotations, improved soil structure, a nearly year-round rhizosphere and plant cover, a stabilized microclimate and higher root density. Other studies from long-term field experiments have shown that crop rotations with higher C inputs contribute to higher microbial activities in soils [e.g. 177]. Each rotation produced a different amount of residues, which have different decomposition rates and various contributions to the easily decomposable soil organic matter fraction. Decomposition rates vary among plant materials depending on their content of N, S, soluble C, lignin and carbohydrates [178].

With the development of transgenic crops, there is an increasing concern about the possible adverse effects of their vegetation and residues on the quality of the soil environment. The dehydrogenase and phosphatase activities of soil transgenic alfalfa were significantly lower than those of soil sampled from the parental alfalfa [179]. In the study of Fang et al. [180], the possible effects of the vegetation of transgenic Bt rice lines Huachi B6 (HC) and TT51 (TT) were studied followed by the effect of the return of their straw to the soil on soil enzymes, such as catalase, urease, neutral phosphatase and invertase under field conditions. The results obtained in this study indicated that the vegetation of the two transgenic rice lines and their subsequent straw amendment had few adverse effects on soil enzymes when compared with non-transgenic plants. No different pattern of impact due to plant species was found between the HC and TT rice [180]. The results were in agreement with previous studies in which some significant differences were found in the activity of some enzymes (arylsulfatases, phosphatases, dehydrogenases, urease, invertase and protease) between the soils with Bt and their near-isogenic non-Bt plants [181]. Some studies have reported that there were no significant differences in the activities of phosphatases and catalase between soil cultivated with Bt and non-Bt maize [182]. Similarly, salinity-tolerant MCM6 transgenic tobacco revealed no significant (or only minor) alterations on soil dehydrogenase and acid phosphates as compared with non-transgenic tobacco [183].

6. Soil Enzyme Activities as Possible Indicators of Nutrient Dynamics and Soil Fertility – Advantages and Shortcomings

6.1. Single enzymes

The increasing amount of research on soil enzymes in the 1950s delivered numerous empirical observations of soil enzyme activities with respect to amendments, cultivation practices and responses to environmental and climatic factors. It was hoped that information on extracellular enzymes would provide a suitable tool for determining the total biological activity in soil and consequently a ‘fertility index’ of soils that would be useful for practical purposes in agriculture [3]. Since it was stated that the determination of soil enzyme activity was more important to soil fertility assessment than its microbiological properties, in the 1950s and 1960s the use of single enzyme activities such as invertase, protease, asparaginase, urease, phosphatase and catalase was a common approach that was used in determining soil fertility [3].

As more information on enzyme activities in soils becomes available, it became more difficult to support the correlation with soil fertility and the generalization was more difficult to make [3]. Soil enzymes were investigated because of the more widely differentiated natural and anthropogenic factors and these observations produced many conflicting and confusing data indicating that the use of soil enzymatic

activity as a soil fertility index was limited. More and Russel [184] criticized dehydrogenase activity as a general index of soil fertility because they did not find any useful correlation between that activity and either the soil properties that were known to influence plant growth or plant yield and also because of the poor response of dehydrogenase to the addition of nutrients to soil that was known to be of a

low nutrient status.

The data on the relationship between enzymatic activity and plant productivity, which is closely related to soil fertility, has led to contradictory results as well. Some studies have shown no close relationship between soil enzyme activity and crop yields [e.g. 185], but in many other studies soil enzyme activities were often considered to be the index of soil fertility since they correlated significantly with plant yield [e.g. 186-188]. However, in managed systems this kind of correlation is questionable because other factors may disturb the relationship between enzyme activity and plant productivity. This is likely to be true for agro-ecosystems in which the external input of nutrients and water can greatly increase plant growth and development without a corresponding response in soil microbial and enzymatic activity [10]. The study of Yaroschevich [189] showed that manure-amended soil increased soil enzymatic activity while inorganic fertilization decreased this activity. Crop yields, however, were the same when adequate nutrients were supplied from either inorganic or organic sources. This indicated that the activity of some enzymes is more closely related to plant production under native conditions and highly disturbed landscapes [86,190] than in managed agricultural systems [189]. In the study of de Castro Lopes et al. [188], the enzymatic activities were, among other microbial properties, interpreted as a function of the relative cumulative yields (RCYs) of corn and soybean using linear regression models. Adequacy classes for each enzyme as a function of the RCY were established based on the following criteria – a value of an enzyme that was higher than the relative cumulative yield (RCY) of 80% corresponds to the production of maximum economic efficiency. The values of the enzymes that corresponded to an RCY between 41% and 80% were classified as moderate and values that corresponded to an RCY of ≤ 40% were classified as low. The interpretative classes for enzymatic activities in a clayey Red Latosol of the Cerrado region as a function of the RCY are presented in Table 4.

Enzyme indicator	Classes as a function of RCY		
	Low	Moderate	Adequate
Cellulase activity (mg glucose kg-1 soil d-1)	≤ 70	71-105	>105
b-glucosidase (mg glucose kg-1 soil d-1)	≤ 65	66-115	>115
Acid phosphatas (mg glucose kg-1 soil d-1)	≤ 680	681-1160	>1160
Arylsulfatase (mg glucose kg-1 soil d-1)	≤ 40	41-90	>90

Table 4: Interpretative classes for enzymatic indicators in a clayey red Latosol of the Cerrado region (Brazil) as function of the relative cumulative yield (RCY). Adopted from de Castro-Lopes et al. [188].

Despite its many drawbacks, the use of soil enzymes as a soil fertility index still remains an elusive goal and it has not been abandoned [e.g. 191-194]. There has been growing interest in the application of soil enzymes as early indicators of changes in soil fertility under contrasting agricultural management practices [195, Chapter 1 of this book]. According to Gil-Sotres et al. [193], dehydrogenase, FDA hydrolyzing capacity, urease and phosphatase were the enzymes that are most often used as soil fertility indicators. Masciandaro et al. [196] proposed an index expressed as the ratio between dehydrogenase activity and the water-soluble C content. This ratio was initially used to get quantitative information about soil degradation that is caused by intensive soil use [196]. Later, this index was used by several authors to assess the effects of different crops or management practices on soil fertility [60,197]. Perucci [70] proposed the Hydrolyzing Coefficient as the quantity of fluorescein diacetate that is hydrolyzed after incubation divided by the total quantity before the hydrolysis. This index was mainly used to test the activity of a soil that was amended with compost, although the author did not establish the minimal level for this ratio [194]. Two hydrolases, namely, urease and phosphates, have been widely used in the evaluation of changes in soil fertility due to soil management. Their activity increased due to organic fertilization [198,199] and after the addition of cattle slurry to the soil and decreased as a consequence of plowing [60]. Because both practices are normally carried out at the same time in agricultural soils, it is clear that the use of urease as the fertility index is limited [193]. Soil phosphatase activity is widely used as an indicator of inorganic P availability for plants and microorganisms [71] and is also considered to be a good index of the quality and quantity of organic matter in soils and can be very high in arable soil as long as the content of organic matter is maintained [200] Among the enzymes that are involved in the carbon cycle, □-glucosidase has been the most widely used in the evaluation of soil quality and fertility indicators in soil that has been subjected to different management practices. □-glucosidase activity was significantly lower in arable soils than in woodland and meadow soils [60,69]. Some agricultural practices, such as organic fertilization can increase the activity of this enzyme [69,198].

As was mentioned earlier, the use of individual properties such as soil fertility indicators has led to conflicting and confusing data and conclusions, not only because the adopted methodologies were sometimes questionable, but also because it is conceptually incorrect to use a single enzyme activity to determine plant productivity or soil fertility, what has been widely criticized [e.g. 3,132], and summarized by Nannipieri [75] and more recently by Nannipieri et al. [12]:

1. Soil enzyme activities catalyze a specific reaction and therefore, they cannot be related to the overall soil microbiological activity, which includes a broad range of different enzymatic reactions. The synthesis of a particular enzyme can be repressed by a specific compound, while the overall microbiological activity of soil or crop productivity is not affected.
2. Since a given enzyme is substrate specific, it cannot reflect the total nutrient status of the soil. However, an individual soil enzyme may answer questions regarding a specific decomposition process in the soil or questions about specific nutrient cycles. For example, cellulase complexes and other carbohydrates might indicate the decomposition rate of plant materials, whereas urease is

important agriculturally as an enzyme that might limit the content of nitrogen that is available to plants from fertilizers or natural sources [3]. The urease activity is often considered to be an indicator of organic N mineralization although the enzyme is involved in urea hydrolysis and urea is not an important component of soil organic nitrogen, particularly when urea is not used as a fertilizer. An enzyme activity that is frequently used as indicator of C mineralization is □-glucosidase. Also in this case, as was stated by Nannipieri et al. [12], the assumption is conceptually incorrect because the mineralization of plant residues involves the mineralization of cellulose and lignin, which are the main components of plant residues. □-glucosidase is only one of the enzyme complexes that are responsible for cellulose degradation.

3. Soil enzyme activities show a high degree of spatio-temporal variability that is due to climate, season, geographical location and pedogenetic factors. Usually, enzyme activities fluctuate with the seasons, decreasing in summer and winter with moisture and temperature, respectively, being the limiting factors for their activities.
4. It is difficult to compare the data obtained in one experiment with those from another because they are usually obtained using different protocols, either because there are no standardized methods or because the soil samples have been subjected to different pretreatments (sample collection and storage) prior to the analysis [193].
5. Some chemical compounds in soil can inhibit or activate the synthesis and the activity of an individual enzyme without having any influence on total microbial activity.
6. The overall activity of any single enzyme in soil depends on enzymes in different locations including extracellular enzymes that are immobilized by soil colloids. The activity of immobilized enzymes may not be as sensitive to environmental factors as are those directly associated with microbial activity. On the other hand, this could be an advantage since bound enzymes are less variable, for example in the vegetation period.

These methodological problems along with the inherent complexity of soil systems indicate that no estimation of soil fertility using simple indicators can be considered to be reliable. Efforts to use soil biochemical properties as indicators of soil fertility should be focused on the search for complex expressions that are capable of describing the complexity of soil much more accurately [193].

6.2. Simple and complex indicators

Due to the complexity of soil structure and function, a good soil quality and fertility indicator must be the integrative combination of a number of measurements into an easily understood and quantitative measure [195]. Moreover, since soil enzymes differ in origin, function and location in soil and respond differently to environmental factors, it would be useful to condense the information they give into one single numerical value. That is why, complex indicators, which are calculated with algebraic operations of different soil biochemical properties [11,193] or multivariate analysis such as Principal Component Analysis (PCA) and factorial analysis [201,202] are frequently used. This approach might better reflect both the release of nutrients during organic matter decomposition and the relative availability of inorganic nutrients compared to the activity of a single enzyme.

A few attempts have been made to integrate different enzyme activities in single and complex indexes that can be used in the assessment of agricultural soils. The biological index of soil fertility (BIF) [203] and Enzyme Activity Number [204] was proposed and widely discussed earlier. Both indexes have been tested by other authors to show the effect of soil management on quality [60,70,75,197]. A serious limitation to the use of EAN as an index of microbiological activity is that the alkaline phosphate assay can be used only in neutral or alkaline soils [75]. In the study of Perucci [70] using municipal refuse in loamy soil, the EAN index was correlated with amylase, arylsulphatase, deaminase, dehydrogenase, alkaline phosphatase and protease activity but not with catalase and phosphodiesterase activity. The BIF was only correlated with catalase activity. Thus, it appears that EAN can give a more realistic indication of the microbial activities of soil than BIF. Saviozzi et al. [60] observed lower values of these indexes for cultivated soils than for other soils (Table 5). Similarly, Riffaldi et al. [197] observed an increase in EAN and BIF in untilled management systems compared to tilled systems in southeastern Sicily (Italy). According to Nannipieri [75] the indexes used by Stefanic et al. [203] and Beck [204] was not as sensitive as those that are currently used to determine enzyme activities in soil.

Type of management	BIF	EAN
C	1.2 (0.08)	1.1 (0.10)
G	10.2 (1.00)	4.1 (0.33)
F	8.8 (0.97)	3.3 (0.27)

Table 5: Empirical indexes for comparing quality of cultivated (C) and adjacent native grassland (G) and forest (F) soils. Confidence limits (P=0.05) are reported in brackets. Adopted from Saviozzi et al. [60]. BIF: biological index of soil fertility, EAN: enzyme activity number

A more accurate and focused selection of enzyme activities was carried out by Sinsabaugh et al. [205] who measured six enzyme activities that work in a cascade in the degradation of lignocelluloses material, which is quantitatively the most important component of plant debris. Using principal component analysis (PCA), Sinsabaugh et al. [205] obtained the lignocelluloses factor (LF), which was calculated using the following enzyme activities: β-1,4-glucosidase, β-1,4-endoglucanase or endocellulase, β-1,4-exoglucanase, β-xylosidase, phenolxidase

and peroxidase activities. The LF factor was significantly correlated with the percentage of weight-loss of the plant remains over time. The cascade of enzyme activities approach (i.e. the LF factor) has been considered by some authors to be one of the best among those using biochemical properties as indicators of soil quality due to its accurate and focused selection of enzyme activities [11]. The only criticism

that could be made about this approach is that it only considers the enzymes that are involved in the C cycle, and not those of the N, P or S transformation. That is why the factor can be a good indicator of a soil's capacity to degrade lignocellulosic material, but not as an indicator of the global capacity of the soil to degrade organic compounds. A similar approach was that of Monreal and Bergstrom [206], who obtained a decomposition factor that was able to explain that 96% of the variation in soil enzymatic activity was due to the cropping system and tillage using PCA and that it was mainly caused by changes in □-glucosidase, dehydrogenase and L-glutaminase activities. Fioretto et al. [207], who studied decomposition factors, preferred to consider only the determination of □-amylase and □-amylase among all of the enzymes that are implicated in the latter decomposition process.

Other indicators that consist exclusively of enzyme activities were those proposed by Puglisi et al. [191]. The authors proposed three indexes of soil alteration using different enzymatic activities to establish an index of soil degradation that was the result agricultural practices, including crop density and the application of organic fertilizers in different parts of Italy. These indexes were developed using a data reduction technique (canonical discriminant analysis, CDA). The first index (AI 1) was developed by considering seven enzyme activities (arylsulfatase, β-glucosidase, phosphatase, urease, invertase, dehydrogenase and phenoloxidase). The second index (AI 2) was constructed with β-glucosidase, phosphatase, urease and invertase activities that had been automatically selected by CDA as the most capable of discriminating between altered and non-altered soils. Finally, the third index (AI 3) was developed by considering the enzyme activities that were most studied (β-glucosidase, phosphatase and urease) according to the bibliography and it was tested on several published data sets. The Al 3 index was able to discriminate soils that had been subjected to irrigation with brackish waste, intensive agriculture, contamination by a tannery, landfill effluents and heavy metals [191].

The above-presented indexes, which are based exclusively on enzymatic activities, do not take into account the content of the main nutrients in soil, and thus its nutrient-supplying capacity, which is important for plant growth. That is why Kang et al. [192] proposed a trigonometric approach that is based on three sub-indexes (a nutrient index, a microbial index of the soil and a crop-related index) to establish a Sustainability Index in soil under wheat that had been amended with manures in Punjab (India), and noted that the quality increased with the amendment. The microbial index (MIi) was based on the microbial biomass C and N, potentially mineralizable N, soil respiration, bacterial population, mycorrhizal infection of corn roots and finally dehydrogenase and phosphatase activities. In one of the experiments that were carried out by authors, the treatment with farmyard manure (100% NPK+FYM), which had been added to the soil for 29 years, was the most sustainable for the corn-wheat system. Conversely, the 100% NPK treatment was unsustainable for the same cropping system. The lack of sustainability of the inorganic fertilizer-treated plots was due to the low microbial (0.91) and crop (0.71) indexes. In contrast, the application of FYM gave higher nutrient (1.25), microbial (1.22) and crop (1.66) indexes than the application of inorganic fertilizers, thus making the system more sustainable (sustainability index of 2.43).

The following equation, which was developed by Paz-Ferreiro et al. [208] for native grassland soils in Galicia, was used to assess the biochemical equilibrium of different grassland soils under contrasting management systems [organic slurry (25 kg N ha-1 and 8 kg P ha-1) and inorganic fertilizer (80 kg N ha-1 and 20 kg P ha-1)] [209]:

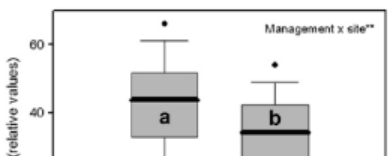
Total carbon (%)=0.764+(2.304 x 10-3 microbial biomass C – expressed as mg kg-1)+(0.936 catalase activity – expressed as mM H_2O_2 g-1 h-1)+(0.017 urease activity – expressed as mM N-NH_4^+ g-1 h-1)+(0.206 phosphomonoesterase activity – expressed as mM pNP g-1 h-1).

The biochemical equilibrium of the soil was estimated by comparing the total C content, which was measured using the dichromate oxidation method (Cr), with the total C content as determined from the equation (Ct). Theoretically, the value of the Ct/Cr ratio in soils in the biochemical equilibrium should be 100. In the study of Paz-Fereiro et al. [209], the Ct/C index varied widely between 40 and 180 and

the values mainly depended on the soil management system and revealed that the unmanaged grassland was in biochemical equilibrium throughout the study period, while no such balance was observed in the managed grassland.

The geometric mean of the assayed enzyme activities (GMea) was used as an index of soil quality in order to compare 18 pairs of organic and neighboring conventional olive orchards in southern Spain [195]. The GMea was calculated as the geometric mean of the enzymes tested as:

$$GMea=\sqrt[6]{AcP\times AlP\times Glu\times Ary\times DEhy\times PN}$$



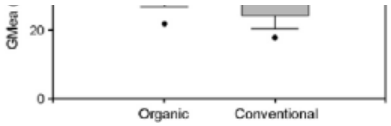


Figure 4: Box-plot representation of the geometric mean of the assayed enzyme (GMea) activities in the soils of the organic and conventional olive oil farms. Boundaries of the boxes closest to, and furthest from zero indicate the 25th and 75th percentiles, respectively. The thin and thick lines within the box mark the median and average, respectively. Bars above and below the box indicate the 90th and 10th percentiles, respectively. Outliers are represented as black dots. Average values with the same letter in each figure indicate no significantly differences between management types ($P<0.05$). Probability of the effect of interaction ''management practices x site'' on GMea is indicated and was lower than 0.01 (**). Adopted from García-Ruiz et al. [195].

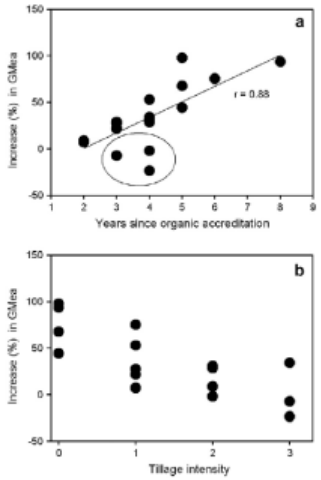


Figure 5: Relationship between the increase in the GMea in the organic relative to the comparable conventional farms and (a) years since organic accreditation of the organic farms, and (b) tillage intensity. Farms within the circle in (a) were excluded or the calculation of the coefficient of correlation because of the high tillage intensity. The coefficient of correlation was significant at $P<0.01$. Adopted from García-Ruiz et al. [195].

In conclusion, the use of complex expressions in which biochemical (enzymatic) along with various chemical properties seems to be a promising direction in developing a universal fertility index. The inclusion of different properties makes it possible to better reflect the complexity of a soil system, at least for the condition in which they have been designed [193]. The main problem with the currently available indicators is that they usually have not been tested in locations or under conditions other than those for which they were developed. As a result, they can be applied only on a regional but not on a global scale [194]. According to Burns et al. [210], the development of a soil enzyme index that can be used as a reliable measure of soil fertility is one of the key research priorities in soil enzymology today. One absolute indicator for the evaluation of soil fertility under different soil management systems and under various climatic conditions and geographic regions is difficult to develop due to the intrinsic variability of biological properties and several site-specific factors that affect soil enzyme activity. The attempt to develop a global indicator of soil fertility should be undertaken at the international level, taking into account some important site-specific factors for the study area, such as climatic parameters, different soil types and vegetation cover.

7. Conclusions and Future Challenges

The sustainability of agricultural systems has recently become an important issue all over the world. Many issues of sustainability are related to soil fertility and its changes over time. The activities of soil biological properties have been proposed as one of the important indicators of soil fertility. Among these biological properties, soil enzymes have been suggested as potential indicators of soil fertility due to the fact that they are involved in the cycling of the most important nutrients and that they correlate well with nutrient availability. Extracellular enzymes, especially, often catalyze the rate-limiting step of decomposition and nutrient cycling, thus making their expression and kinetics potential useful parameters for nutrient turnover models. Moreover, soil enzymes quickly respond to changes in soil management when compared with other biological and physicochemical properties and are simple to measure. Therefore, in most of the papers cited here, it was stated that soil enzymes have a great value as early and sensitive indicators of soil fertility that are induced by different agricultural management systems. Due to their sensitivity and capacity to provide information that integrates many environmental factors, enzymes are useful tools to assess the effect of farming practices on the capacity of the land to remain productive and on soil fertility. In fact, enzymes provide early warnings of a system's collapse and allow us to react before irreversible damage is done to the integrity and functioning of the soil ecosystem.

The bibliography cited in this review, especially those that date from last ten years, has indicated that the interest in enzyme measurements due to their possible use as soil fertility indicators is still high. Many studies have analyzed soil fertility in agricultural soils using individual enzyme activities, which was highly criticized because single enzyme activities cannot represent the rate of all of the metabolic processes unless they usually catalyze one specific reaction. That is why it has been proposed that several enzyme activities be measured, sometimes along with other biological or chemical properties and that they are integrated in an index [191,192,195,205,206,208] that better reflects all of the soil metabolic processes. In addition, the currently available indexes have not been widely used on a large scale, even in similar types of soils and under similar climatic condition and management strategies. The shortcomings of the use of soil fertility indicators in order to compare the results obtained in different researches are due to the lack of a standard methodology that is used in all laboratories, which is as a fundamental problem when interpreting the results; differences in sample collection, storage and the pre-

treatment of soil samples and finally, the high degree of variability in the data because it is affected by seasonal and edaphic factors. Additionally, there is the lack of reference values or a broad database for high-quality soils that could be used to make comparisons [193]. The advantages and disadvantages of the existing indicators suggest that a good soil fertility index should be sensitive enough to the presence of the greatest possible number of management practices, but that it should not be too sensitive to seasonal and among-sites variation, since this could mask any changes that are caused by different management strategies. Secondly, a good indicator should display consistency in the direction of the change that has been undergone in response to a given factor and should clearly reflect the gradual increase or decrease in the level of a given factor. Apart from this, it should be easily interpretable and easy and inexpensive to obtain [193].

Soil enzymes are not only used as indicators of soil status that is due to diverse agricultural practices. One of the key research priorities in soil enzymology is to develop methods of manipulating soil extracellular enzymes for ecosystem services, e.g., in agriculture to enhance the plant nutrient content or to control pathogens and pests [210]. Another option is the application of exogenous enzymes directly into soil in order to regulate the rate and direction of certain soil processes. For example, the application of exogenous cellulase and protease was used effectively to accelerate straw decomposition in soil (reference). Immobilized carbonic anhydrases are being tested for their feasibility for sequestering CO2 into bicarbonate [211], whereas purified phytase improved maize seedling growth when P was supplied in the form of phytase [212]. The problem is the loss of enzyme activity over time, which may occur when enzymes are immobilized by or entrapped within clays, organic matter or organo-mineral complexes [213] or degraded by soil proteases. This is an economic issue due to the high costs of producing the exogenous enzymes for the applications may be unprofitable unless the enzyme has a prolonged activity.

Although molecular properties have not been included in soil quality and fertility indicators, the development of genomic, transcriptomic or proteomic methodologies could be important in the evaluation of such indicators. These methods could provide information about what the role of specific microorganisms and their enzymes are in the key processes that are related to soil functionality [194]. Since the soil is complex and dynamic biological system, it is difficult to determine which microbial genotypes are responsible for the production of certain enzymes. It is necessary to understand the relationship between genetic diversity and community structure and function [214] and because all extracellular enzymes have corresponding genes, they represent an ideal model system for linking microbial identity to specific and critical ecosystem processes. That is why, advances in proteomics, metabolomics and transcriptomics are of great potential [210]. The metagenomic approach can reveal potential gene coding for an enzyme catalyzing targeted reaction, while only transcriptomics and proteomics can assess the actual levels of enzyme expression and indicate which enzyme can be used as an ecological soil indicator [12].

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