

Division - Soil Processes and Properties | Commission - Soil Biology

Contribution of enzymes to soil quality and the evolution of research in Brazil

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ABSTRACT: Extracellular soil enzymes are fundamental for the functioning of ecosystems. Several processes in the soil depend on the activity of these enzymes, including plant decomposition, soil organic matter formation/mineralization, and nutrient cycling. Moreover, extracellular enzyme activity occurs in the soil and is therefore influenced by environmental factors. Due to the high sensitivity to these factors, extracellular enzymes are used for monitoring soil quality. This review aimed to present the main contributions of soil enzymes to agriculture, emphasizing the dynamics of elements in the soil and the environmental factors that modulate enzyme activity. With this knowledge, the relationship of extracellular enzymes to soil quality is demonstrated and their use as a tool for soil monitoring. Finally, the evolution of research on soil enzymes in Brazil is presented, and the perspectives of basic and applied studies necessary to expand the knowledge and use of enzymes in soil management are pointed out. Soil enzymes play a key role in numerous soil processes, thereby making them useful indicators of productive capacity and soil quality. Research on enzymes in soil has developed significantly in the last two decades, which has made it possible for farmers to analyze and interpret enzyme activity in the soil in the laboratory.

Keywords: soil organisms, enzymatic activity, bioindicator.

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INTRODUCTION

Soil is a mediator of several chemical, physical and biological processes that are fundamental to maintain the functioning of terrestrial ecosystems (Dick and Burns, 2011). Soil colloids and minerals help filter water and adsorb environmental pollutants (e.g., inorganics, organics, radionuclides and microorganisms) (Gavrilescu, 2014). Furthermore, soil nutrients support the global production of food, fiber, oil and wood, and provide humans with over 98 % of their food (Kopittke et al., 2019). Organic matter plays a critical role in soil fertility, the global carbon cycle, and heavy metal complexation (Simpson and Simpson, 2016; Gmach et al., 2020), and soil organisms depend on enzyme-mediated catalytic activity (Dotaniya et al., 2019). For instance, soil enzymatic activity is directly related to organic matter decomposition and nutrient cycling (Tabatabai, 1994), biodegradation of toxic organic pollutants (Karigar and Rao, 2011; Badzinski et al., 2021), and plant-pathogen control (Baldoni et al., 2020).

Catalytic soil enzymes can exist in the cytoplasm or on the surface of membranes of viable cells, be excreted into the soil solution, or complexed in the soil matrix or microbial debris (Dick and Kandeler, 2005). Enzymatic catalysis carried out internally in the microbial cell corresponds to intracellular enzymes (IE), and this enzyme group generally acts on specific metabolic pathways in the cellular environment. Thus, IEs only act on small substrates absorbed by microbial cells and under controlled environmental conditions. Nonetheless, extracellular enzymes (EE) are associated with the outer part of cell walls or are released into the extracellular environment to hydrolyze high molecular weight organic substrates into oligomers or monomers (Dilly and Nannipieri, 1998; Burns et al., 2013). The action of EE occurs in the heterogeneous and dynamic environment of the soil, where extracellular enzymatic activity depends on the combination of minimally adequate factors to occur, including substrate availability, water, temperature, pH, among others (Wallenstein and Burns, 2011).

Considering that key biological soil functions depend on enzyme-mediated catalytic activity (Dotaniya et al., 2019), evaluating soil IE and EE may provide information on nutrient release in soil by organic residue degradation and microbial activity and even serve as indicators of changes in the soil environment (Kumar et al., 2013), being able to detect the quality of the soil. A classic concept of soil quality corresponds to the ability of a soil to function within the limits of the ecosystem to sustain biological productivity, maintain environmental quality and promote the health of plants and animals (Doran and Parkin, 1994).

Brazil has a large territory and different climates, resulting in a broad diversity of soils, many of which are highly fragile (Mendes et al., 2018a). Soil use and management are often inadequate, leading to degradation, and determining the activity of enzymes in the soil can be a strategic tool for the early diagnosis of soil degradation or improve and advance decision-making by farmers. In agricultural areas from Brazil, subjected to the same management practices for long periods, correlations observed between soil enzyme activity and plant productivity can be used to diagnose soil quality and guide targeted management to improve soil health and increase crop yields (van Es and Karlen, 2019; Mendes et al., 2021a).

This review aims to present the fundamental contributions of soil enzymes to agriculture, emphasizing the dynamics of elements in soil and the environmental factors that modulate their activity. With this knowledge, the relationship of extracellular enzymes with soil quality is demonstrated and their use in soil monitoring. Lastly, the evolution of research on soil enzymes in Brazil and the perspectives of basic and applied studies necessary to expand the knowledge and use of enzymes in soil management are presented.

ENZYMES AND THE DYNAMICS OF ELEMENTS IN SOIL

Carbon (C), nitrogen (N), sulfur (S) and phosphorus (P) are part of the structure of the molecules that form organic compounds, and their dynamics in the soil depend on biological activity. In organic waste degradation, the structural heterogeneity of biopolymers requires the interaction of several enzyme classes to reduce them to constituent monomers available for microbial consumption (Sinsabaugh et al., 2008). Extracellular enzymes are agents of organic compound decomposition, and key enzymatic reactions include those involved in the biogeochemical cycle of C (cellulose, hemicellulose and lignin degradation), those that hydrolyze organic N reservoirs such as proteins, chitin and peptidoglycan, those that mineralize P from nucleic acids, phospholipids, and other ester phosphates, and the ones that catalyze the hydrolysis of organic S esters (Sinsabaugh et al., 2008; Henry, 2012; Turner et al., 2016). Hence, the main groups of soil enzymes investigated due to their relationship with soil nutrition and health are cellulases (C cycle), protease and urease (N cycle), phosphatase (P cycle) and arylsulfatase (S cycle).

Main soil enzymes related to the carbon cycle

Cellulose is the most abundant natural polymer on the planet and constitutes a significant fraction of plant biomass (Lynd et al., 2002; Gessner, 2020). It is a long chain of glucose ($C_6H_{10}O_5)_n$, in which the monomers are joined by glycosidic bonds. Glucose molecules are the primary energy source of heterotrophic soil microorganisms, albeit they cannot directly access the glucose molecules present in cellulose structures. This access depends on the breakdown of this polymer through the action of three extracellular enzymes (endoglucanases, cellobiosidases and β -d-glucosidases) that compose a generic group called cellulases (Figure 1).

The first step in cellulose depolymerization occurs by the action of the enzyme endoglucanase, which belongs to the group of glucanases. The endoglucanase (endo-1,4- β -glucanase; EC 3.2.1.4) randomly cuts the β -1,4 glycosidic bonds, thus breaking the long cellulose chain, with the broken molecular chains having one reducing end and one non-reducing end (Wallenstein and Burns, 2011; Chen, 2015). Subsequently, the action of the cellobiosidase on the short cellulose chains occurs; the cellobiosidase (exo-1,4- β -glucanase; EC 3.2.1.91) comprises two components that cut, respectively, glucose and cellobiose from the reducing end of the long-chain (Wallenstein and Burns, 2011; Chen, 2015; Thapa et al., 2020).

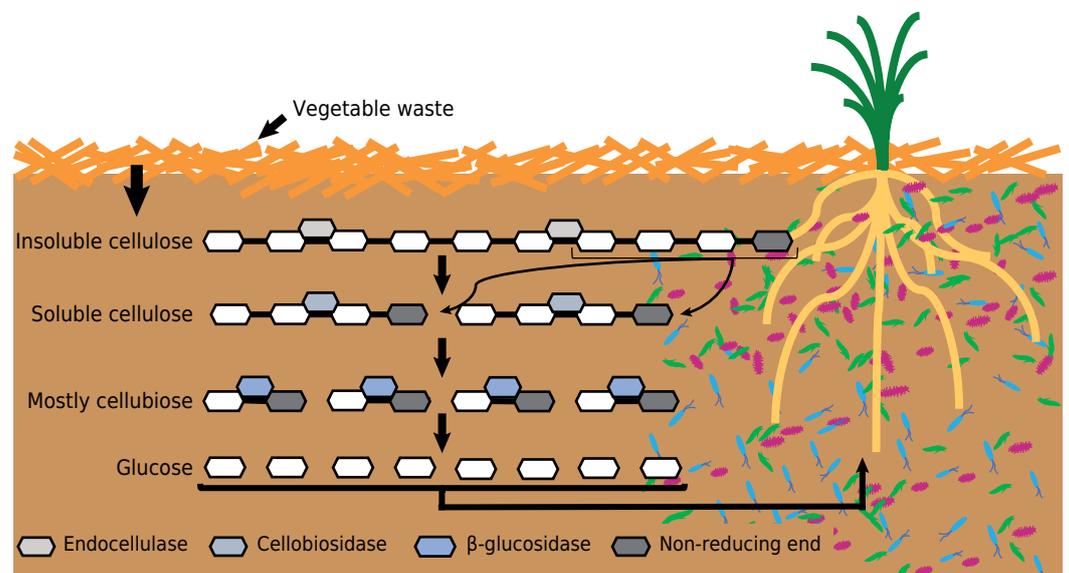


Figure 1. Soil enzymes linked to carbon in the decomposition process of plant residues. Source: Adapted from Wallenstein and Burns (2011).

After the action of the cellobiosidase, cellobiose fragments are released ($C_{12}H_{22}O_{11}$), which are disaccharides composed of two glucose molecules joined by β (1 \rightarrow 4) linkages. The β -d-glucosidase (EC 3.2.1.21) acts on cellobiose and other water-soluble cellodextrins from the reducing end, resulting in glucose molecules that can then be absorbed by microbial cells (Figure 2) (Wallenstein and Burns, 2011).

Although depolymerization is complex, organisms have specialized strategies for cellulose degradation, resulting in the vast availability of glucose (Wallenstein and Burns, 2011). The main cellulase producers are fungi, mainly basidiomycetes and ascomycetes. Martinez et al. (2008) identified the fungus *Trichoderma reesei* E.G. Simmons (*Hypocrea jecorina* Berk. & Broome) as a producer of numerous cellulose-degrading enzymes.

Cellulase enzymes do not supply nutrients to plants, although the resulting glucose is still necessary for the growth of soil microorganisms, which in turn control the availability of N, P, S and other nutrients and promote plant growth by other means (Dotaniya et al., 2019). Given that β -glycosidase is the last enzyme in the cellulose depolymerization process, its activity can be considered a suitable parameter to assess microbial activity and soil health.

Main soil enzymes related to the nitrogen cycle

Organic sources of N in the soil include humic substances, proteins, chitin, cell wall and nucleic acids (Vieira, 2017), originating from rhizodeposition, burlap, or the necromass of soil micro, meso and macroorganisms. The main source of N to soils is in the form of protein through the addition of plant and microbial residues (Greenfield et al., 2020). Proteins are polymers of amino acids held together by peptide bonds (Cox and Nelson, 2008), and in the soil, their decomposition process can be divided into two steps. The first step is proteolysis, which is carried out by protease enzymes (EC 3.4.2.21-24) that break the protein chain into smaller peptides, which subsequently release amino acids due to peptidase action (Figure 2i) (Alef and Nannipieri, 1995). In fact, the “proteases” are actually a group of different enzymes that catalyze reactions in proteins and differ from each other in several characteristics, such as molecular structure, type of reaction catalyzed, and affinity of the active site for substrates. Many consider that protease enzyme activity occurs in free proteins in solution as the ability of enzymes to act on proteins adsorbed on the surface of soil minerals, although this is still not yet well understood (Greenfield et al., 2020).

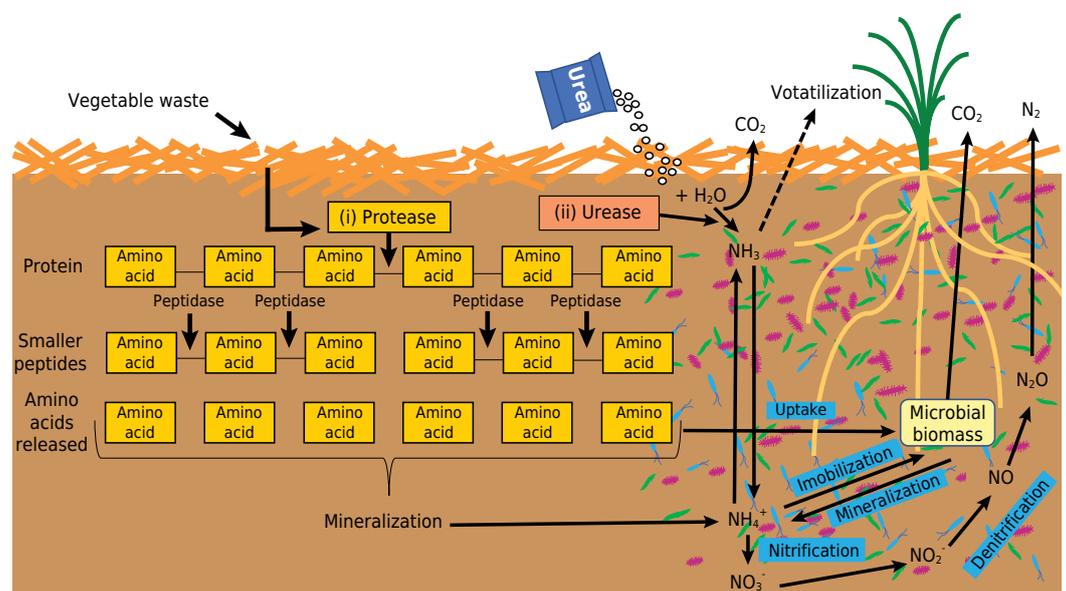


Figure 2. Nitrogen-binding soil enzymes on plant residue decomposition.

The second step takes place when the soil microbial community consumes amino acids as a source of nitrogen, carbon and energy. Inside the microbial cells, these molecules undergo deamination reactions, in which ammonium (NH_4^+) is produced and can be excreted into the soil solution. During these processes, part of the C contained in the amino acid structure is mineralized as CO_2 . In solution, part of the NH_4^+ is nitrified into nitrite (NO_2^-), and both forms can be absorbed by plant roots (Figure 2i) (Balota et al., 2013; Vieira, 2017).

Proteases also play an essential role in the degradation of other soil enzymes since they are proteins and subject to proteolytic enzyme action. For instance, many proteases exuded by plant roots are related to resistance against pathogens (Wang et al., 2020). Therefore, various microorganisms have mechanisms such as the production of protease inhibitor molecules to regulate proteolysis in the apoplastic space of roots and externally in the rhizospheric environment. Competition for resources between prokaryotic and eukaryotic microorganisms stimulates the production of antifungal proteases by bacteria. In contrast, other proteases, for example, can cleave the cell wall proteins of fungi and nematodes.

Besides protease, urease (EC 3.5.1.5) is another important enzyme in the nitrogen cycle because it acts in the hydrolysis of urea [$(\text{NH}_2)_2\text{CO}$], converting it into ammonia (NH_3) and CO_2 (Figure 2ii). Urea is the most widely industrial source of N used in agriculture worldwide. However, plants do not absorb urea, so it must be degraded to release the ammonium (NH_3) or ammonium ions (NH_4^+) (Qin and Cabral, 2002). In addition, urea can be hydrolyzed chemically, although this process is slower than biochemically. Ureas are partially extracellular and are released at root and plant death. They can also be intracellular as part of the soil biomass, a cytoplasm component, or attached to the cell membrane. This enzyme has stability in the soil against proteolytic action and other processes that cause its inactivation because they are immobilized in organo-mineral complexes (Dharmakeerthi and Thenabadu, 1996).

Nonetheless, high activity of the urease enzyme may result in N loss through NH_3 ammonia volatilization. Because of this, numerous strategies can be developed to reduce its effects, including producing urea granules coated with materials that limit dissolution and urea derivatives that are more slowly hydrolyzed by soil urease and using soil urease inhibitors applied with urea. These techniques decrease N losses by volatilization and improve urea-based fertilizer efficiency (Mota et al., 2015; Viero et al., 2015; Cancellier et al., 2016; Lourenço et al., 2016).

Nitrification and denitrification processes are mediated by enzymes and also play a fundamental role in N dynamics. These processes are important for environmental quality due to nitrate leaching and nitrous oxide emission (N_2O), a greenhouse gas. In nitrification, microbial oxidation occurs from reduced forms of N (NH_3) to less reduced forms, including NO_3^- (Hu et al., 2015), and this process has two steps. Initially, the oxidation of NH_3 to hydroxylamine occurs and is mediated by the enzyme ammonia monooxygenase or hydroxylamine reductase (EC 1.7.1.10). Subsequently, hydroxylamine is oxidized to NO_3^- by the action of the enzyme hydroxylamine oxidoreductase (EC 1.7.3.4) (Figure 2). Bacteria from different phylogenetic groupings participate in the first step, while the second step is performed mainly by bacteria of the β subclass of proteobacteria (Kandeler et al., 2011).

Denitrification starts with the reduction of NO_3^- , coming from nitrification or added by external sources (e.g., fertilizers). The NO_3^- is reduced to N dioxide (NO_2^-) through the action of the nitrate reductase enzyme. Subsequently, NO_2^- is reduced to nitric oxide (NO) by nitrite reductase enzymes. The microorganisms that carry out these transformations have one, but not both enzymes. Next, NO is then reduced to N_2O by nitric oxide reductase and, depending on environmental conditions, N_2O is converted to N_2 gas through the enzyme nitrous oxide reductase (Figure 2) (Kandeler et al., 2011).

Denitrification can be performed in bacteria, halophilic and hyperthermophilic archaea, and fungi mitochondria (Zumft, 1997).

Main soil enzymes related to the phosphorus cycle

Phosphorus is an essential element for energy transport, cell structure and nucleic acids, being indispensable for terrestrial life. In plants, adequate availability of this element is vital for plant growth and development (Acosta-Martínez and Tabatabai, 2011). In soil, P is found in organic, inorganic, and soluble forms, and organic P in soil organic matter (SOM) and organic residues is immobile and structurally unavailable for uptake by plant roots. Moreover, P mineralization is carried out by microorganisms that produce phosphatases (Kunze et al., 2011; Silva et al., 2015; Yada et al., 2015), and through the activity of these enzymes, organic P is transformed into phosphate (HPO_4^{2-} or H_2PO_4^-), which is the form that can be absorbed by plants (Figure 3i).

The name “phosphatase” was used to describe a group of five enzymes classified by the International Union of Biochemistry and Molecular Biology (IUBMB, 2021) into: 1) phosphoric monoester hydrolases (EC 3.1.3), 2) phosphoryl diester hydrolases (EC 3.1.4.), 3) triphosphoryl monoester hydrolases (EC 3.1.5), 4) enzymes acting on phosphoryl-containing anhydrides (EC 3.6.1), and 5) enzymes acting on P-N bonds (EC 3.9), such as phosphoamidase (EC 3.9.1.1).

Phosphoric monoester hydrolases are also known as phosphomonoesterases and can be acidic or alkaline, phosphoprotein phosphatases, phytases and nucleotidases. Examples of phosphomonoesters such as β -glycerophosphate, phenyl phosphate, β -naphthyl phosphate and p-nitrophenyl phosphate are attacked by phosphatases and release mineralizable forms of P (HPO_4^{2-} or H_2PO_4^- ; Figure 3iii) (Moreira and Siqueira, 2006; Nannipieri et al., 2011). Acid phosphomonoesterases are common in acidic soils, while alkaline ones are common in alkaline soils. These enzymes hydrolyze monoester bonds, including mononucleotides and sugar phosphates (Nannipieri et al., 2011), in addition to being the most studied phosphatases.

Main soil enzymes related to the sulfur cycle

Sulfur is an essential macronutrient for plant growth, and its concentration in plant tissues varies between 0.1 and 0.5 g kg^{-1} , depending on the species. This element is present in amino acids, such as methionine and cysteine (Lucheta and Lambais, 2012), thus making organic matter the largest reservoir of soil S (Lucheta and Lambais,

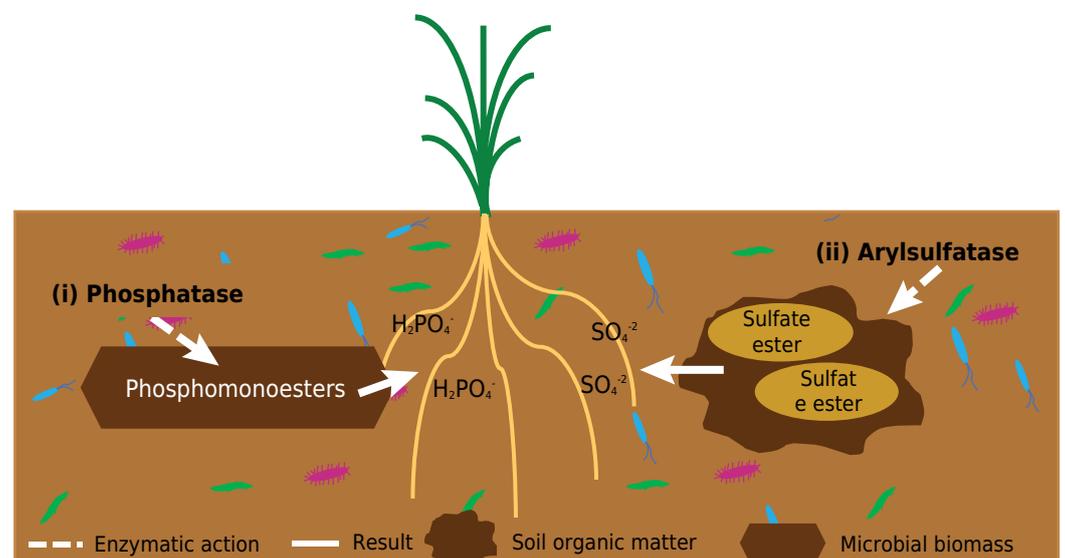


Figure 3. Enzyme phosphatase and arylsulfatase in soil.

2012; Flis and Jones, 2020). In SOM, sulfur can be highly oxidized in the form of sulfate ester, highly reduced when bound to carbon in compounds (e.g., sulfides and thiols, including amino acids such as cysteine, cystine and methionine), and oxidized in the form of sulfoxides and sulfonates, in which sulfur is bound to carbon and hydrogen (Brady and Weil, 2013).

Moreover, S uptake by roots occurs preferentially via the sulfate ion (SO_4^{-2}). However, the inorganic forms of S represent less than 10 g kg^{-1} of total soil S, and as a result, the availability of inorganic S depends directly on the sulfur mineralization of the SOM. In soil, arylsulfatase (EC 3.1.6.1) is an important enzyme that controls S mineralization from organic sources (Lisboa et al., 2012; Chen et al., 2019a). This enzyme acts on the sulfate ester by breaking the sulfur-oxygen bond and producing SO_4^{-2} (Figure 3ii) (Tabatabai, 1994). A portion of the SO_4^{-2} will be used in microbial metabolism, while another fraction will be available in the soil solution for uptake by plants. Arylsulfatase was first described by Tabatabai and Bemner (1970) and can be intracellular or extracellular and produced by plants, animals and microorganisms (Tabatabai, 1994; Rao et al., 2014).

ECOLOGY OF SOIL EXTRACELLULAR ENZYMES

The activity of extracellular soil enzymes is influenced by substrate, moisture, temperature, pH and other factors, whether chemical, physical, or biological. The substrate is the main limiting factor of enzyme activity, and this occurs for an obvious reason: in its absence, enzymes do not express their catalytic functions. The extracellular degradation of the main organic molecules (cellulose, lignin, hemicellulose, starch, pectin, among others) requires the simultaneous and/or sequential action of different enzyme classes (Wallenstein and Burns, 2011). In soil, plant cell wall polymers represent the primary substrate for the decomposing activity of microorganisms. Their decomposition occurs through the action of hydrolytic enzymes (e.g., cellulases) and oxidative enzyme activity (e.g., laccases and peroxidases). In the plant cell wall, cellulose polymers are associated with hemicellulose, lignin, pectin and various proteins, forming a compact and rigid structure where the lignin acts as a barrier around the cellulose (Zhao et al., 2012; Chen, 2014). For soil cellulases to efficiently access cellulose, oxidative enzyme activity (an energetically unfavorable reaction) is first required to depolymerize lignin (Moorhead et al., 2013; Chauhan, 2020). Hence, given the structural heterogeneity of these polymers, their degradation involves a consortium of different enzymes produced by microorganisms belonging to different groups, albeit acting synergistically (Janusz et al., 2017).

Soil moisture is a major influencing factor on the microbial community and, consequently, on enzyme catalysis (Alef and Nannipieri, 1995; Lupatini et al., 2019). Hydrolytic enzymatic reactions only occur in an aqueous medium. Contact between a substrate and an enzyme depends on transport pathways and diffusion processes within them (Guber et al., 2018). Extracellular enzymes diffuse toward the substrate and away from the parent cells through the water content of the micro- and macropores (Baveye et al., 2018; Schimel, 2018).

Furthermore, enzymes have an optimum temperature where the efficiency of the reaction peaks. Temperature variation above or below this point can reduce the speed of the reaction until its complete inactivation (Yang et al., 2019; Gómez et al., 2020). Throughout their evolution, microorganisms have developed different biochemical strategies to maintain extracellular enzyme activity against environmental temperature changes. For instance, they secrete enzymes with high thermal stability, allowing them to catalyze stable reactions over a wide temperature range, or they can produce multiple isoenzymes, each with different optimal temperatures but with a similar affinity for the substrate (Razavi et al., 2016, 2017). Protease enzymes can maintain their thermal stability and affinity for the substrate almost constant even at temperatures as variable as 0 to 40 °C (Razavi et al., 2016).

The pH determines the electrostatic behavior of the enzyme, thus having a direct effect on interfacial attraction and repulsion (Datta et al., 2017). Inside living cells, the pH is close to 7.2 and is controlled by the microorganism itself. Once released into the soil solution, extracellular enzymes are dependent on the pH of the environment (Leprince and Quiquampoix, 1996; Mónica et al., 2018; Wade et al., 2021). Soil pH values near neutrality (pH 6.0-7.0) provide higher activity of the different extracellular enzymes (Burns et al., 2013).

Enzymes interact with the surface charges of soil minerals, including metal (hydr)oxides, surfaces, and edges of argillominerals. When adsorbed on minerals, extracellular enzymes can reduce and even lose their activity due to conformational changes in their structure (Quiquampoix and Burns, 2007; Kleber et al., 2015). In addition, their diffusion towards the substrate is inhibited, leading them to become dependent on the diffusion of the substrate to its active site. These phenomena are so important that, in some cases, soil enzyme activity is more affected by organo-mineral associations than by the enzyme content present at a given site (Noll et al., 2019). However, the adsorption phenomenon can protect the enzyme from physical or chemical degradation, resulting in a pool of extracellular enzymes in the soil (Datta et al., 2017).

Due to the influence of various environmental factors, quantifying soil enzyme activity requires defined protocols with well-known assay conditions tested for a multitude of contrasting soils, such as the colorimetric determination methods described by Tabatabai (1994). Following these protocols ensures proper conditions for the enzymes in the laboratory and the ability to compare data acquired from different sites. Moreover, attention should be paid to using methods that use minimal amounts of soil, including the microplate method, as it is more susceptible to laboratory errors (Nannipieri et al., 2018).

EXTRACELLULAR ENZYMES AND THEIR RELATIONSHIP WITH SOIL QUALITY

As highlighted above, extracellular enzymes are sensitive to environmental changes, and several factors modulate their catalytic activity. These proteins are sensitive to alterations of land use, regulate SOM dynamics (Karaca et al., 2010; Acosta-Martínez et al., 2019; Adetunji et al., 2020), and relate to crop yields (Lopes et al., 2013, 2018; Mendes et al., 2019a, 2021a). Thus, enzyme activities are key soil biological indicators of soil health and quality.

Changes in land use and soil management directly impact the microbial community, enzyme activity, and nutrient status in the soil (Bissett et al., 2011; Tischer et al., 2015; Malik et al., 2018). For example, clearing a forest area to grow annual crops modifies the substrate that the microbial community will access. In native forest environments where there is no disturbance, plant residues have a higher proportion of lignin, protecting cellulose from enzymatic attack, while annual crop residues generally have lower ratios of lignin (Puttaso et al., 2011; Talbot et al., 2012). This directly impacts the degradation rates of plant cell wall polymers and the ability of the soil to sequester carbon and store it in SOM.

In agricultural environments, soil disturbance increases substrate exposure to enzymatic attack and bioavailable organic carbon protected within aggregates, resulting in loss of organic carbon because it increases extracellular enzymatic activity (Ghimire et al., 2019). Nevertheless, overall soil enzymatic activity decreases after rapid degradation of these carbon compounds due to reduced substrates availability for extracellular enzyme action. Soil disturbance also accelerates water loss through evaporation and reduces soil moisture, limiting the substrate and enzyme diffusion and consequently catalysis (Manzoni et al., 2014).

Due to greater exposure to solar radiation, elevated soil temperature may rapidly increase enzyme activity and consequently higher organic carbon degradation and mineralization

rates (Hou et al., 2016; Liu et al., 2019). This is a highly undesirable effect given our scenario of increased global average temperatures and the intensified greenhouse effect (Hassan et al., 2015; Looby and Treseder, 2018). However, temperature increases above the optimal limits for enzymes can reduce their half-life, substrate affinity and catalytic efficiency due to conformational changes and protein denaturation (Wallenstein et al., 2010; Alvarez et al., 2018).

Sustainable soil management systems (e.g., no-tillage; NT) can not only minimize soil organic carbon loss but create favorable conditions for balanced and healthy soil biological activity (Luo et al., 2020; Ramos et al., 2021). Different management systems in an Oxisol (Typic Haplorthox) were evaluated in a 24-year study. It was possible to observe the activity of the enzymes acid phosphatase, alkaline phosphatase, and arylsulfatase in NT was similar to enzymes in undisturbed forests (Balota et al., 2014). Additionally, the activity of these enzymes in NT was higher than in the conventional planting system (Balota et al., 2014). In another 22-year study, it was observed that NT in flooded Entisol and rice cultivated soil increased the C (45 %) and N (54 %) of microbial biomass and the activity of β -glucosidase (43 %), acid phosphatase (68 %), fluorescein diacetate (34 %), and urease (96 %) compared to conventional soil preparation (Carlos et al., 2021). Several studies, including short-duration ones (2 years), have shown that NT is a sustainable practice that increases microbial biomass C, N, P, S, and soil enzyme activity (β -glucosidase, arylsulfatase, phosphatase, and protease) compared to the conventional tillage system (Babujia et al., 2010; Mathew et al., 2012; Chen et al., 2019b).

In recent years, the Brazilian Agricultural Research Corporation (Embrapa) has developed the soil bioanalysis technology SoilBio, which provides a simple, effective, and practical tool for monitoring and interpreting soil health at the farm scale (Mendes et al., 2018b, 2021a,b). This technology adds the microbiological component to the physicochemical analyses commonly used by farmers by evaluating the enzymes arylsulfatase and β -glucosidase, which were selected because they are robust soil health bioindicators (Mendes et al., 2019a, 2021a). With the results of the enzyme analyses, interpretation of the values is performed, falling into ranges of high, medium, or low. Subsequently, the technology includes calculating the FertBio soil quality index, where the values of the chemical analyses are integrated with the data of the biological analyses. The tables for interpreting the results of the enzymatic analyses are specific to different soils and crops; so far, there are only tables available for the conditions of the Brazilian Cerrado biome. Currently, commercial laboratories of soil analysis are being capacitated to perform the enzyme analysis, and tables are being generated for other conditions, which will allow expanding the technology to other regions and make it available to more farmers.

EVOLUTION OF RESEARCH ON SOIL ENZYMES IN BRAZIL

In an attempt to demonstrate the growth of research on extracellular enzyme activity in the soil, a search on the Web of Science® database was performed using the terms (“enzymatic activity” * OR enzymatic * OR enzyme *) NEAR/3, and the search filter “Brazil,” and 134 scientific articles were found. To broaden the horizons of this theme, we also manually selected articles on soil enzymes by accessing the curriculum of several researchers on the Lattes platform (<https://lattes.cnpq.br/>). The data obtained from both search methods were compiled into a single database, and a total of 194 articles on enzymes in soils from Brazil were counted.

The first papers on enzyme activity in Brazilian soils were published in 1982 (Kulinska et al., 1982; Melo et al., 1982), while studies of the same nature in other countries had been published as early as the 1950s (Galstian, 1959). In the case of Brazil, the late publication of these studies compared with the rest of the world is because the most scientific research on soils began after the creation of the first graduation courses and Embrapa in the early 1970s. During the entire 1980s, only three articles were published on enzyme activity

in Brazilian soils (Figure 4a), which is quite similar to the number of publications of the same nature in the following decade. Thereafter, the number of publications significantly rose in the years 2000 and 2010, reaching 115 articles between 2010 and 2019; in other words, 59 % of all papers on enzyme activity in soils in Brazil were published in this period (2010 to 2019). However, even more remarkable growth seems yet to come because, from January 2020 to July 2021, 38 articles have already been published.

The 194 articles on enzymes in soils from Brazil were published in 86 scientific journals, 71 % in international journals, and 57 % in journals with the highest impact factor (Qualis A, according to the classification proposed by the Coordination for the Improvement of Higher Education Personnel Foundation; CAPES). The journals with the highest number of publications were *Revista Brasileira de Ciência do Solo* (14.4 %), *Pesquisa Agropecuária Brasileira* (5.2 %), and *Applied Soil Ecology* (4.1 %).

For the publication survey, the soil collection sites were considered. If the study presented soil collections in more than one state of Brazil, it was counted for both. The states with the most soil samples collected for enzymatic studies were São Paulo (18 %), Minas Gerais (15 %), Pernambuco (11 %) and Paraná (10 %) (Figure 4b). Most publications were registered in

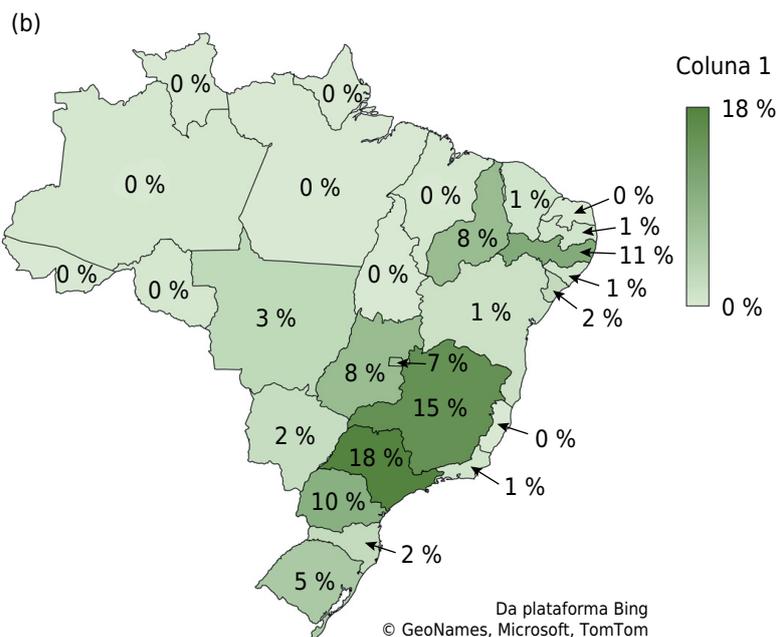
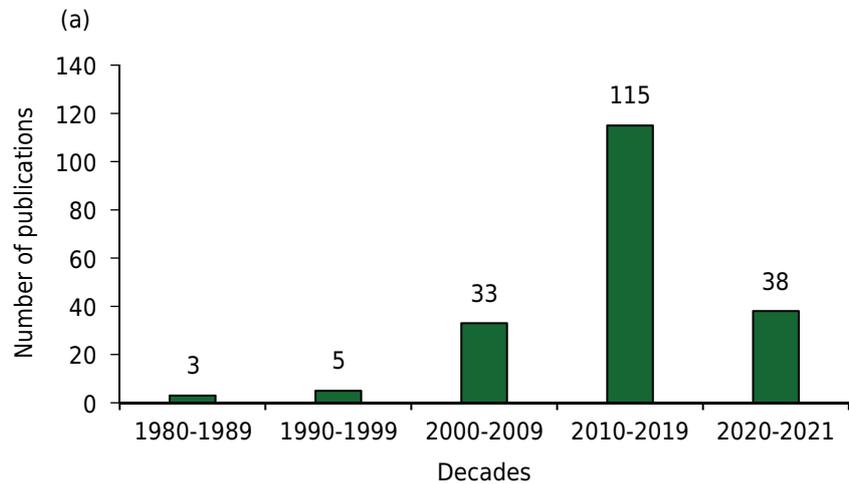


Figure 4. Current status of publications on soil enzymes in Brazil. Distribution of publications per decade (a); distribution of publications per Brazilian state (b).

the southeastern (33.66 %), northeastern (25.85 %), and mid-western (19.51 %) regions. Notably, there are few studies on soil enzymes in the northern states (1.46 %), which is where the Amazon Rainforest is located. Significant growth of research on soil enzymes in this region is expected to occur very soon due to the environmental importance and land-use changes caused by deforestation, representing a great research opportunity for Brazilian institutions. According to this survey, there is an increasing number of studies on extracellular enzyme activity in the soil, and application of this knowledge in the sustainable management of agricultural environments, with even better future prospects.

CONCLUSIONS

The agriculture practiced in many countries and recent decades has been based on monoculture, synthetic fertilizers and pesticides, and intensive water and agricultural machinery use. Over the years, this agricultural system became unsustainable due to the high economic and environmental cost of the inputs and the impact caused on agro-ecosystems. Many soils became degraded due to inefficient use and management, reducing their productive capacity. Simultaneously, the demand for food in the world has been increasing in quantity and quality.

For many years, scientific discussions and research have been carried out to find a way to produce more and better food and use less synthetic inputs and without advancing over areas still occupied by natural ecosystems. A consensus resulting from this effort is the permanent need to monitor the quality of agricultural soils to detect any damage to their chemical, physical and biological properties early on, and new practices can be adopted to avoid degradation. Once degraded, the productive capacity and ecosystem activity carried out by the soil are compromised, and its recovery is an expensive and time-consuming process.

For many decades, numerous studies have shown that soil enzyme activity can be used to monitor recent changes, for better or for worse, that occur in the soil environment resulting from its agricultural use. As demonstrated herein, the participation of soil enzymes in processes essential to environmental quality and plant productivity and their sensitivity to environmental factors make them a key indicator to help monitor biological quality. Given this scenario, enzymes related to the carbon cycle (cellulase), nitrogen (protease and urease), phosphorus (phosphatase), and sulfur (arylsulfatase) are paramount. In Brazil, the SoilBio technology is training various laboratory professionals to analyze and interpret arylsulfatase and β -glucosidase enzymes, consequently making this technology available to many farmers.

Although significant advances have been made, there is still much research to be done on soil enzymes. Most studies performed have so far focused on a few enzymes, while many have been poorly studied. The variations in soil environmental factors, natural and/or imposed by use, make it necessary to study enzyme activity under different conditions. For this reason, the interpretation tables of enzyme activity must be calibrated to different soils and crops (Mendes et al., 2019b), which is particularly difficult in a country with a wide diversity of climates, soils, and crops such as Brazil. Given these reasons, the study of soil enzymes is still challenging, albeit the results obtained thus far have proven to be promising and show that, indeed, it is possible to make technology available to farmers to evaluate the biological quality of soils.

SUPPLEMENTARY DATA

Supplementary data to this article can be found online at https://www.rbcjournal.org/wp-content/uploads/articles_xml/1806-9657-rbcs-45-e0210109/1806-9657-rbcs-45-e0210109-suppl01.pdf.

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