Nitrogen Footprints and the Role of Soil Enzymes

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Abstract

In a climate change scenario soil microbial population is affected by the impacts on soil biotic and abiotic factors, with a strong influence on soil microorganisms affecting enzyme production and activity. This influences soil organic matter turnover and nutrient cycling in soil. Nitrogen is one of the most, if not the most important, nutrient for all living organisms. Besides its vital role in maintenance of life on Earth and need to maintain nitrogen availability to produce enough food for the world population, nitrogen losses into the environment cause negative effects in all environmental compartments. To quantify the impact of each individual contribution to nitrogen pollution a concept of nitrogen-footprint was created, to measure nitrogen lost as a result of food and energy consumption. Enzymes play a role in the response of soils to nitrogen pollution and the mitigation and adaptation to climate change effects on nitrogen-footprint. Enzymes are affected by abiotic factors alterations driven by climate change but may alter their activity as a result of human actions, e.g. agricultural

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management practices affecting microbial populations. Enzymes may thus be a vehicle of both increase and reduction of nitrogen availability and therefore impact on nitrogen-footprint in a positive or negative way.

Keywords

Enzymes · Footprint · Nitrogen · Soil

1 Introduction

This chapter looks at the role that soil enzymes may play in determining Nitrogen (N) Footprints. Climate change and increased global demand for food and energy are consequences of an exponential growth in the world population. The need to produce more food requires greater agricultural production which will necessarily mean higher nutrient availability requirements. Future global food demand can be met by continuing the on-going agricultural intensification and the reliance on inorganic fertilizers or alternatively through an Input–Output optimization at the farm level to increase N use efficiency (NUE). Different production paradigms have different implications in terms of N management (and N pollution) for a nutrient that indispensable for food production [1]. Furthermore, there are other pathways of N management between where N is used in food production and eventually appears "on the table" (e.g. transportation, energy production, wastewater treatment) [2]. While meeting food demand by intensifying agriculture and using more fertilizers, N pollution will also continue to increase unless action is taken to reduce losses to the environment. Although nitrogen is indispensable for food production, N losses lead to a series of negative impacts on human and environmental health. The N-footprint concept was created in 2011 as a tool to allow individuals and institutions to understand how their personal behaviour influences N losses to the environment. The quantification of the N lost as a result of food and energy consumption, as a N-footprint, allows us to take action with the objective of reducing N-footprints at different levels, with the ultimate goal of reducing the global amount of reactive N released by human activities. The calculation of N-footprint depends upon the calculation of crop- and country-specific virtual nitrogen factors (VNF) defined as the units of reactive N released to the environment per unit of reactive N consumed in the process [3]. VNFs are specific for each crop and region and the 'Applied N' is only a part of the chain of N losses considered (see example in Fig. 1). Increasing nitrogen use efficiency (NUE) in soils to reduce Applied N losses is a major challenge. For example, estimated N losses from agricultural soils to the environment for the EU28, through gaseous emissions, leaching and runoff, are approximately 50% or greater of the N inputs to agricultural soils (including atmospheric deposition) [4]. The remaining 50% being recovered by crops (field losses associated with imported crops are not considered). Increasing our understanding of the role of enzymes and

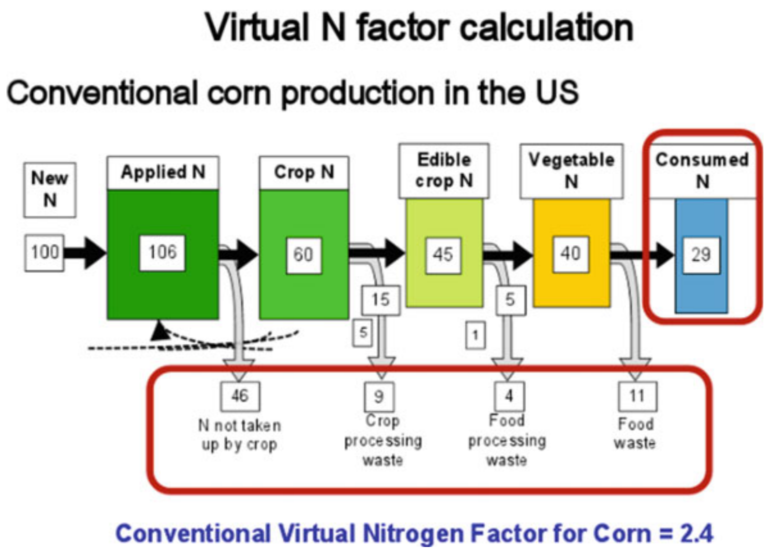

Fig. 1 Virtual nitrogen factor for maize crop in the United States (Leach et al. [3]; www.n-print.org)

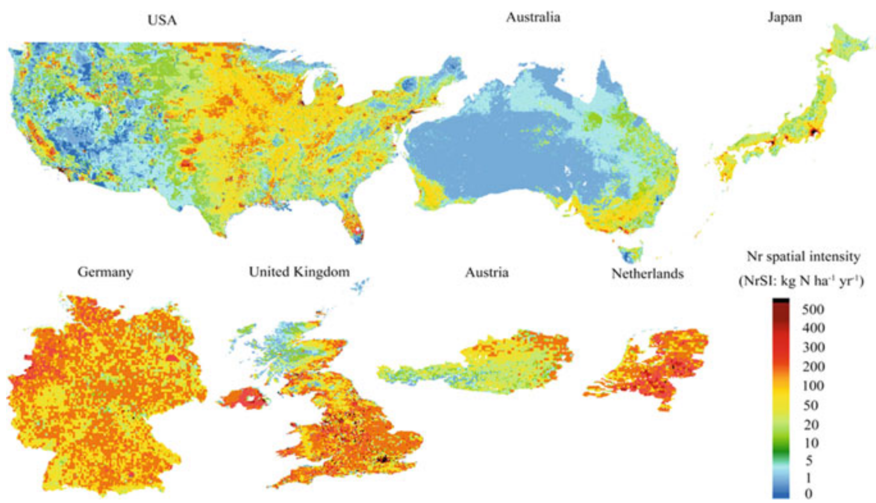

Fig. 2 Spatial variation of the anthropogenic N losses in different countries [5]

how they influence NUE and subsequent N losses to the environment is therefore essential. The spatial variation of the total anthropogenic N released to the environment following the N footprint [5] is shown in Fig. 2.

There are no current estimates given for the impacts of climate change, and enzymatic activity, on the N footprint but a simple theoretical framework can be developed based on the literature available.

2 Soil Quality, Nutrients and Crop Production

Soil quality is key to sustaining crop production in a context where the population is expected to increase up to 9.7 billion in 2050. Although soil quality is a decisive factor to ensure nutrient supply for plants' growth, current trends of soil use have led to soil quality decline, compromising soil capacity to produce enough food. Indeed, since the green revolution and the increased use of inorganic fertilizers for crop growth, it has been estimated that an exponential increase in crop production has allowed half of the world's population to be alive today [6]. The necessary increase in food production cannot be maintained for much longer due to its impactful environmental costs through the excessive mining of soil organic matter (SOM) and other nutrients from the soil, in addition to those supplied by fertilizers and by chemical pesticides to suppress diseases. Additionally, the impact of climate change in agriculture is still uncertain but already noticeable in different regions of the world through extremes in rainfall and drought. The increase in crop productivity is declining and the need for inorganic fertilizer is increasing accordingly, misleading farmers to believe that over-applying fertilizers enhance yields proportionally, thereby further decreasing the soil biological quality. The decline of soil organic matter content reduces the nutrient transformation from SOM mineralization and therefore the availability of plant nutrients in the soil solution [7]. For example, the release of N taking place during SOM breakdown and subsequent transformations are a critical part of the nitrogen (N) cycle in soil [8]. On the contrary, over-application of inorganic fertilizers leads to soil contamination, e.g. phosphorus, and water contamination or nitrate excess to the demand of plant and soil biota. Furthermore, reduction in SOM leads to poor water retention in soils enhancing the susceptibility to drought. So, for sustainable crop production today's agriculture requires appropriate management of nutrients including good soil management (light weight machinery, reduction of pesticides, crop rotation systems including grain and nitrogen fixers) and in addition the right application rate, timing, type of fertilizer and mode of application vis-a-vis plant and soil biota requirements, bearing in mind that nutrient availability is affected by climatic conditions and soil physical, chemical, and biological properties, including the effects of root exudates in the soil [9].

3 Relevance of Enzymes in Soils

Soil fertility depends upon three different but interacting components: chemical, physical and biological. Soil biological fertility regulates many functions that are beneficial to plant production: (i) releasing nutrients from soil organic matter

(SOM) (microorganisms, enzymes), (ii) fixing atmospheric N (rhizobia, bradyrhizobia), (iii) increasing phosphorus availability (mycorrhizal fungi), (iv) degrading pesticides (microorganisms, enzymes), v) controlling pathogens (microorganisms) and (vi) improving soil structure (bacteria, fungi). Soil enzymes have a major biogeochemical significance because they catalyze most of the reactions in soil chemistry processes (Fig. 3). More than 100 enzymes have been characterized in soils and are responsible for catalyzing reactions occurring in these ecosystems. Studying enzyme activity in soils is, therefore, a useful tool to assess soil functional state in environmental and ecosystem management, because any changes in soil properties will alter the activity of soil organisms and their species composition and biodiversity, including that of enzymes. In fact, enzymes play an important role in soil health as they carry out an array of crucial functions, e.g. decomposition of organic matter, production of inorganic nutrients for plant growth, nitrification, denitrification, detoxification of xenobiotics. They also have a crucial role in the biochemical cycles of essential nutrients such as C, N, P and S [10]. So, the

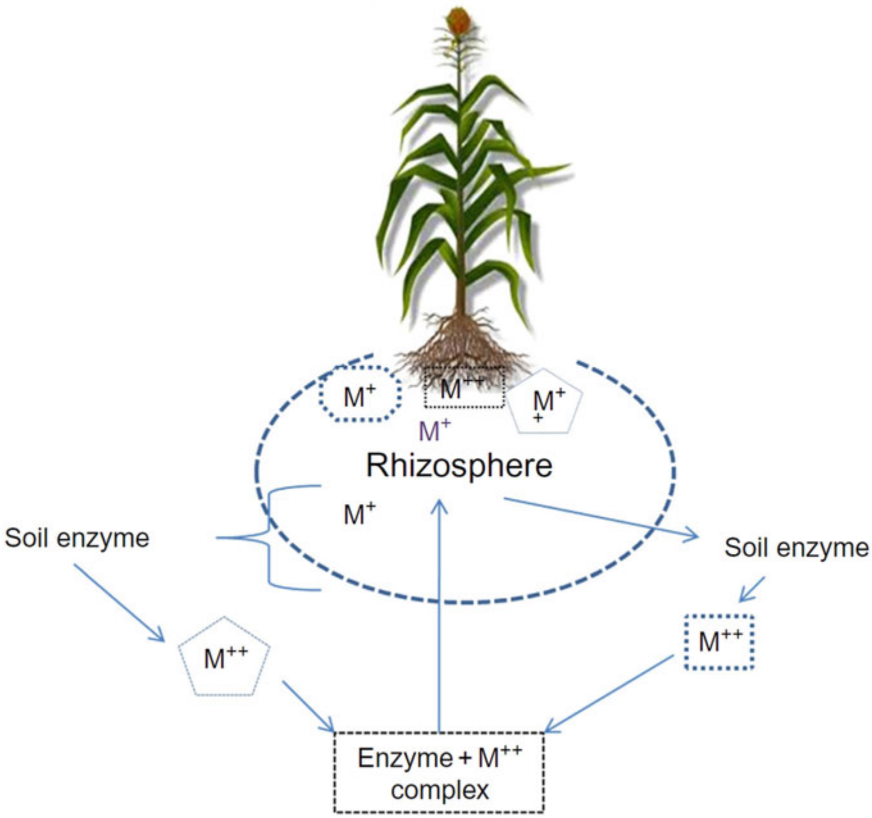

Fig. 3 Role of soil enzyme in plant nutrient dynamics [12]

management of soil enzymes can provide valuable information on microbial community functions in space and time, related to the understanding key nutrient cycles, such as nitrogen. However, only small amounts of enzymes can be directly extracted from soils, so, enzymes are mainly studied through the observation of their respective activity, which may vary with edapho-climatic conditions [11]. Seasonal variation affects microbial community responses to the environment, enzymes decrease vertically from the soil surface, vary according to microbial community distribution and also at landscape level, soil type being a major controlling factor (especially regarding soil texture and SOM content) together with soil management.

Furthermore, soil type is also a major controlling factor, particularly soil texture and SOM content. Changes in soil use and soil quality due to management affect several enzymes long before changes in soil organic matter levels can be detected [12]. This gives enzyme studies a high potential as a suitable tool for sustainable ecosystem management in the long-term. Therefore, besides the potential to anticipate soil quality depletion, enzymatic studies may show the level of degradation of highly disturbed soils and recovery in reclaimed landscapes.

Enzymes can exist on viable cells either internally or on membranes surface, but they can also be excreted into soil solution and may be found in the soil matrix and in microbial debris. Except for the case of Error! Reference source not found and a few other enzymes that exist only in viable cells, most of the other soil enzymes can be found either in viable or in complexed forms, independent of viable cells, and stabilized in the soil matrix [11]. Extracellular enzymes or exoenzymes, are secreted by cells and have the main role of hydrolyzing substrates that are too large or insoluble to be directly absorbed by microbial cells of some communities. They maybe secreted by bacteria and fungi as well and, in this case, may be used in environmental bioremediation, the ones producing hydrolases being especially useful [13]. When enzymes are found in stabilized forms on colloid surfaces and incorporated into soils, a degree of degradation of certain contaminants has been observed in soils. In fact, *enzyme activity* measurements are used as useful tools to assess certain heavy metals bioavailability in soils. Moreover, enzymes catalyze and take part in metabolism processes connected to SOM and to energy processing in soils. Therefore, the use of indicators for evaluating soil microbial diversity *and* microorganism's activity is key to understanding soil dynamics and fertility.

In summary, soil enzymes are vital not only to maintain soil fertility and health, but also to protect the environment by degrading pollutant molecules [14].

4 How Enzymes Influence Nitrogen Availability in Soils

The most important soil enzymes belong to three different classes: oxidoreductase, hydrolase, and lyase. Many of them are directly involved in the processes regulating the nitrogen cycle. Dehydrogenase (DHA) belongs to the oxidoreductase class that also includes laccases and all enzymes involved in the oxidation of different substrates (e.g. those involved in oxidative degradation of toxic organic pollutants). DHA mediates the transfer of hydrogen atoms from organic compounds, accompanied by energy generation. DHAs are present in all microorganisms and do not exist as extracellular enzymes, being specific for different substrates. The DHA activity in the soil reflects the total oxidative metabolic capacity of microbes, being considered as a good indicator of soil biological activity [15]. DHA intervenes in the oxidation of SOM by mediating proton and electron transfer from organic matter to suitable inorganic acceptors. These enzymes are affected by soil type, moisture content, and the redox state (aeration) in the soil [12].

The dominant class of extracellular enzymes found in the soils is the hydrolase class [12]. These hydrolytic enzymes are involved in the breakdown of macromolecules to obtain smaller forms utilisable by plants and microbes but are also responsible for mediating the removal of inorganic groups or ions to allow the release of the inorganic available forms [12]. This class includes amidases, amylases, cellulases, glucosidases, phosphoesterases, sulfatases and ureases.

Lyase enzymes act without hydrolysis and mediate the removal of certain chemical species breaking covalent bonds. These enzymes include ammonia lyases, decarboxylases and dehydratases. Ammonia lyases are the most important enzymes in this class as they deaminate amino acids. Enzymes representing other classes are not so common in the soil environment and less important from the ecological point of view.

Proteases are important enzymes that act in the mineralization of organic N in soil playing a vital role in maintaining the ecosystem function and in ensuring N nutrition for plant growth. Because it is an extracellular enzyme, it is linked to both organic and inorganic colloidal substances in soil. Protease degrades protein to release short peptides through hydrolyzation of the peptide bonds. If proteins are further degraded, amino acids are released and act as N sources for the soil microbes. Amino acids may be taken up by the microbes or may be further mineralized to release ammonia for plant N nutrition [16–18].

Urease is another enzyme affecting N balance in soils and can be found both in extra and in intracellular forms in the microorganisms [16]. These enzymes are released by almost all the soil microbial groups, including fungi, bacteria, algae, yeast and even some plants roots, and have as main role to hydrolyse urea into ammonium and carbon dioxide. N dynamics are influenced by urease as it increases the ammoniacal nitrogen concentration in soils. If not taken up by the plants ammonium release leads to soil pH increase after urea fertilization, and is also volatilized as ammonia, hence negatively impacting air quality and reducing the nitrogen use efficiency (NUE) of fertilizers. The use of polymer-coated urea reduces the effect of soil urease, hence reducing N losses while increasing NUE (e.g. as now widely practiced in India—Neem coating).

β-glucosidase activity, one of the most common enzymes in soils [19], plays a key role in the breakdown of low molecular weight carbohydrates of SOM, which is strongly related to the carbon cycle in soils and the products of its enzymatic activity supplies energy to soil microorganisms. Since these enzymes are proteins and therefore very sensitive to both anthropogenic and natural variable factors, monitoring their activity is a helpful tool to assess soil quality. This is particularly true in soils subjected to mixed organic and/or mineral fertilization included in different crop rotations [20].

Nitrogenase also plays an important role in the N cycle as it is a metalloenzyme responsible for catalyzing atmospheric nitrogen fixation, by ensuring the reduction of di-nitrogen (N_2) to ammonia (NH_3) which is a vital process for all forms of life on Earth.

Soil fertility is based on intense enzymatic and *microbial activity* and on metabolic diversity of *microorganisms*. SOM mineralization and transformation of nutrients are brought about by the microbial enzymes, both extracellular secreted enzymes as well as endogenous enzymes of microbial cells [12] (Fig. 4). Extracellular enzymes are secreted into the soil environment where they intervene in the decomposition or in the transformation of the organic and inorganic nutrient forms into plant available

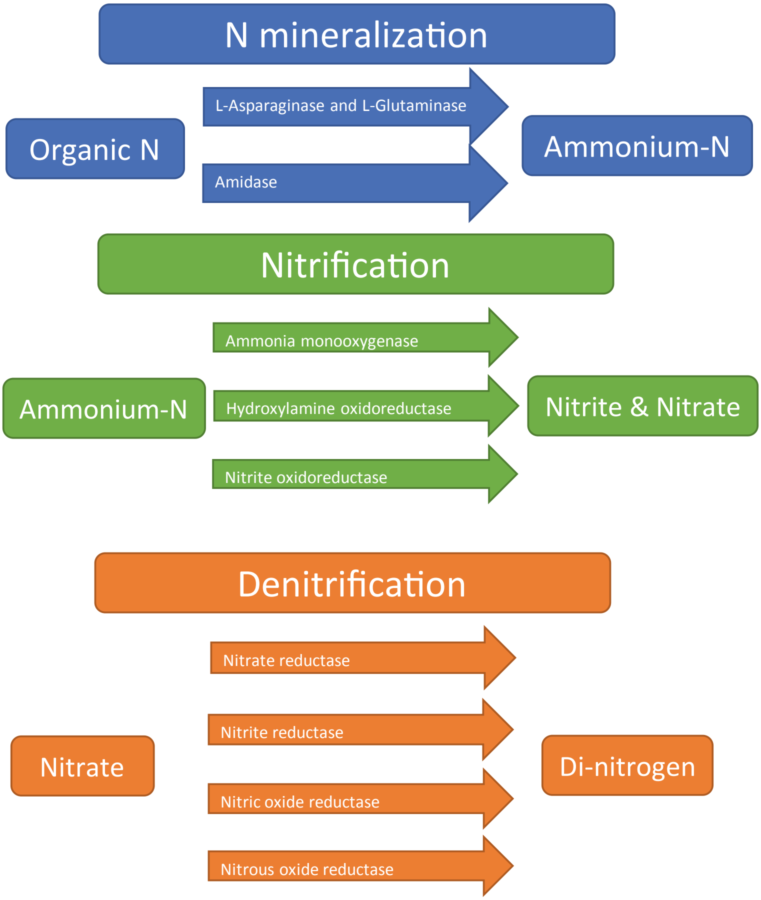

Fig. 4 Enzymes involved in the main nitrogen transformation processes in soils

forms [12]. During field application, the ammonia contained in inorganic fertilizers and animal manures is rapidly hydrolysed to the ammonium (NH_4^+) ion, ammonium compounds or nitrate (NO_3^-). This serves as a direct pathway for crop nutrient uptake, while organic amendments and SOM breakdown require depolymerization by extracellular enzymes and microbes to enhance N availability [21].

5 Soil Characteristics Influencing Enzymatic Activity

Changes in soil's physio-chemical characteristics, mostly as a result of land-use change, vegetation type and microbial status of the soil, produce a strong effect on enzymes, proving their high sensitivity to such parameters. Each soil enzyme has a specific range of pH for optimum activity at which enzymes are more stable. On the contrary, as pH deviates from optimal values, enzyme activity is reduced until becoming inactive at extremely high or low pH values where irreversible denaturation occurs. Changes in the soil concentration of H^+ ions (protons) have a strong influence on enzyme dynamics, influencing substrate degradation and acting as a co-factor in nutrients ionization and solubility properties [22]. Although this is generally true, the influence of pH in enzymatic activity is enzyme-specific and the degree of pH sensitivity is variable. For example, optimum pH for β-glucosidase is 6.0 while for urease it is 7.0 [23].

Drying and rewetting cycles in soil also affect enzyme activity to variable degrees. For example, β-glucosidase activity is reduced in dry soils showing that lower soil moisture content greatly reduces the activity of extracellular enzymes produced by microbes associated with the breakdown of SOM. The release of glucose during SOM decomposition influences the growth of soil microorganisms since glucose is the preferred carbon source for many of them [12]. β-glucosidase activity serves as one of the best predictors for evaluating the effect of crop management practices on soil health and soil quality changes. Dry conditions promote the adsorption of enzymes onto mineral surfaces where their activity is reduced as well as their access to SOM substrate. Besides reducing β-glucosidase activity, this can also reduce other enzymes rate of degradation and therefore their contribution to SOM breakdown following the next rainfall [19]. As soils dry, solutes in the water are concentrated, leading to increasingly negative osmotic potential between the inside and outside of microbes. As a response to this stress, many microbes will accumulate electrolytes and organic solutes to balance osmotic and matric potentials, which in turn may slow down the activity of enzymes within the organisms [24].

Generally, enzyme activity increases with increasing temperature, doubling the reaction rate about every 10 °C. However, beyond the enzyme-specific threshold enzymatic activity decreases drastically and becomes inactivated at high temperature. Moreover, the temperature sensitivity of the different enzymes, and thus the dynamics of nitrogen and carbon mineralization, are not the same and may be influenced by climate change in different ways [25]. As for pH, the sensitivity of enzymes to temperature varies with enzyme type and source.

6 Effect of Agricultural Management Practices (e.g. Tillage, Organic Fertilization)

Agricultural management practices and soil enzymatic activity are closely linked. On one hand, agricultural systems benefit from higher soil enzymatic activity due to an improved land management responsiveness. On the other hand, monoculture-based systems limit inter-species interactions, and thus bacteria associations, since these are influenced by root exudate components that vary in type and quantity according to different crop species [26]. Crop rotations, no-tillage, organic amendments, the use of low weight machinery and cover crops are some Conservation Agricultural (CA) practices known to favour enzyme activity [27]. A meta-analysis of 62 studies demonstrated that no till or reduced tillage promotes large microbial communities and greater enzymatic activity [28], although further study is necessary to understand the long-term (>10 years) impact on the microbial communities under CA, e.g. [28] stated till and no-till microbial activity show similar results after a 10 year period.

Tillage is performed to increase the aeration of the topsoil and provide weed control. However, conventional tillage techniques can result in soil compaction due to heavy machinery [29], which alters soil vertical structure, reducing soil organic matter, plant nutrient availability over time and microbial biomass [30]. In fact, soil compaction leads to a change in the soil atmosphere which may have negative effects on soil biological activity that, in turn, will affect soil physical properties. Therefore, plant growth may be repressed due to the negative effects on plant roots, because aeration characteristics of soil and its effects on plant growth depends mostly on the composition of air in the soil [31]. Curci et al. [32] studied the influence of tillage (shallow ploughing, deep ploughing and scarification) on enzyme activity and concluded the enzymes β-glucosidase, galactosidase, nitrate reductase and dehydrogenase were all affected negatively by tillage. These enzymes have different soil functions and different pressure responses: glucosidase activities—responsible for the hydrolysis of plant biomass—are inhibited in the presence of heavy metals (e.g. copper [33]) and when there is soil acidification; dehydrogenase activities are highly influenced by pesticides, remaining low when high doses of pesticides are traced [34]. Conversely, no-till coupled with the incorporation of crop residues increases microbial biomass as a response to an increase in SOM [32, 35, 36]. Urease is also influenced by tillage activities as it is highly influenced by SOM content. Urease catalyzes the hydrolysis of urea into carbon dioxide and ammonia and is commonly used for soil quality evaluation versus its respective management [37, 38], although enzyme activity performance is also dependent on environmental factors such as, pH, oxido-reduction potential and, in particular, temperature and moisture [39].

Crop monoculture can lead to an imbalance in the main enzymes which has a negative impact on soil function [23] subsequently causing a decline in soil quality [40]. Microbial and biochemical analyses of soil under winter wheat in a field trial with various cultivation systems (organic, conventional and monoculture) were performed during three growing seasons [41]. The activities of the tested enzymes (dehydrogenase and phosphatases) and microbial biomass C and N contents in the monoculture soil were generally significantly lower than those in the soil from the organic and conventional–short rotation systems, indicating that substantial disturbances may occur in the microbial activity of the monoculture soil. Crop rotations are an efficient measure to enhance microbial diversity in the rhizosphere, increase soil organic carbon (SOC), total nitrogen (TN) and build up resistance to disturbance and suppress root diseases [41, 42]. Cover cropping can be a useful approach to improve diversification of soil microbial communities whether through a form of rotation diversification or through its cover crop residues [30, 43]. Indeed, [44] reported an increase in soil enzymatic activity around 20% in soils with a cover crop mixture of oat/radish/vetch, with increases also in soil C and N storage. Pasture rotated with well- managed crops can also increase enzyme activity in soil because overgrazing leads to a decrease in soil microbial biomass [45].

Irrigation, aside from its primary function, helps to determine the enzymatic activity of the soil with regard to dry conditions which limit the decomposition rate and therefore microbial biomass. Protease and urease—N-cycling enzymes—seem to be the most affected by drought [46].

It is widely reported that using organic fertilizers such as manure promotes enzyme activity. In soils that are organically farmed crop residues and rhizodeposits support higher microbial biomass, leading to enhanced enzyme activities [47]. For example, it was found that enzyme activity associated with C, N and S cycling were higher under organic farming practises compared to conventional farming [47]. More specifically, [48] demonstrated that activities of soil β-1,4-glucosidase, β-1,4-*N*-acetylglucosaminidase, and leucine aminopeptidase increased with manure application. Application of chemical fertilizers such as phosphorus and N negatively affects microbial activity, particularly with long term use [48]. For example, nitrogen application may negatively affect enzyme activity, such as nitrogenase. Nitrogenase activity provides N to the soil, through catalyzing N_2 fixation from the atmosphere into two molecules of NH_3—also known as biological nitrogen fixation and [49] recorded higher activity of this enzyme with no urea-N application and inhibition of *Stenotrophomonas* sp. population (significantly important for nitrogen and sulphur cycle) with an application of 300 mg L^{-1} urea-N. The way nitrogen-based fertilizer affect nitrifying bacteria might be associated not only to the cultivar, but also to its age and to the diazotrophs present in the environment [50]. Diazotrophs are Plant- Growth-Promoting Rhizobacteria or PGPR [51, 52], although they can colonize the interior of plants (xylem vessels and intercellular spaces) where the potential for nitrogenase activity increases [52].

7 Relation of Enzymes with N and C Cycles

Soil enzymatic activity plays a major role in key processes related to the quality of SOM which, in turn, influences the efficiency of microbial nutrient assimilation since carbon is required in microbial metabolism [54]. Moreover, soil enzymatic activity can impact the availability and/or storage of C and N in SOM pools. Because a lot of the enzymatic activity occurs in the rhizosphere from associations between soil microorganisms and root exudates (plant-soil interaction) enzymes may be used as a proxy for potential plant growth and for nutrient availability [55], especially for nitrogen. While this may not directly promote crop nutrient uptake and thus, crop growth, it does so indirectly by improving the availability of soil microorganisms that play a key role in nutrient availability for plant nutrition. These degrade complex organic carbon compounds to release simple utilizable C compounds for microorganisms' survival and growth (e.g. sugars, organic acids). Moreover, the enzymes involved in nutrient (e.g. N, P and S) cycle processes, mineralize organic compounds of the respective nutrients into inorganic compounds, which can be readily used by microorganisms and plants. The influence of soil enzymatic activity in soil nutrient cycling is a relatively well-researched topic (e.g. [56–59]). By playing a vital role in initiating and maintaining nutrient biogeochemical cycles, enzymes play a vital role and ensure soil fertility for plants development [60, 61]. Enzyme activity is more intense in the rhizosphere than in the bulk soil due to the direct contact with plant roots and influence of root exudates, as well as with bacteria and mycorrhiza. The rhizosphere is a uniquely rich environment where enzymes and microorganisms mediate the biogeochemistry of minerals and better nourish the soil–plant ecosystems [62].

8 The Importance of Nitrogen Fertilization

Nitrogen (N) is the most important of nutrients within the structural and functional molecules that make up the plant structure, and is also essential for the *biosynthesis* of structural *molecules* such as the *nucleotides* and *amino acids* that are building blocks for plants, animals and other organisms. Despite this important role of nitrogen, fertilizer application can negatively affect the enzyme activity, such as for nitrogenase, that catalyzes atmospheric N fixation as mentioned before. Similarly, atmospheric deposition of reactive nitrogen reportedly reduces the activity of lignin-modifying enzymes and hence of C decomposition, which positively affects terrestrial C sequestration [63]. While ligninase activity increases in soils with low SOM quality under nutrient deficiencies, its activity is progressively reduced as nutrient deficiency is replenished [64]. By contrast, cellulase activity derived from N deposition was reported by [63] not to correlate with changes in soil C stocks.

One of the main anthropogenic factors affecting soil enzymatic activity is both organic and mineral fertilization that has a crucial influence on soil biological status and the enzymatic activity in soils. The application of organic fertilizers such as farmyard manure, has a positive effect by increasing organic C and N concentrations in soil and affects the quality and quantity of SOM. On the contrary, if manure is too rich in inorganic N (NH_4^+), it may promote immobilization and N losses and have a negative effect. Data shows that the impact of agricultural nutrient management practices on enzyme activity depends on the type of fertilizer (i.e.

inorganic and organic), (in)organic N content, application rate and mulching materials [65]. For instance, the application of N fertilizer and leaf mulching has significantly increased the activities of dehydrogenase and *β-glucosidase* [66]. Soil mulched with white clover and crown vetch also considerably increases the activity of soil urease, invertase and alkaline phosphatase [67, 68] observed a higher enzymatic activity from mulching when compared to mulched no-till treatments, which is a typical practice of conservation agriculture, particularly popular in drylands. Additionally, mulching can enhance enzyme activity when coupled with earthworms during rice and wheat cropping systems [68] and under bare soil [69], soil compaction [70] which can negatively impact nutrient availability and crop production [71]. Indeed, soil compaction can be arguably more important than N fertilization regarding soil enzymatic activity [29]. Red clover mulches and different levels of N fertilization were found to significantly impact the activity of different enzymes (e.g. acid phosphatase, protease) [72]. Furthermore, [20] showed a 10–26% increase in the enzymatic activity when applying lower N fertilization rates to catch crops (40 and 80 kg N ha^{-1} $year^{-1}$), while the opposite was found following the application of poultry organic manures [73, 74] also found higher enzymatic activity following the N fertilization of organic fertilizers compared to inorganic fertilizers. In addition, the application of N fertilizers over longer periods of time positively affects enzyme activity [75].

By contrast, N fertilizers can negatively affect enzyme activity such as nitrogenase. Nitrogenase enhances soil N availability by catalyzing atmospheric biological N fixation. N fertilizers also affect nitrifying bacteria that convert ammonia to nitrate (NO_3^-) according to the cultivar and to the diazotrophs present in the environment [50]. Conversely [77], observed a strong negative effect of L-asparaginase, a *N*-acquiring enzyme, to C-acquiring enzyme (BG) ratio, on total N concentration in an agricultural field previously amended with different fertilization plans. It is therefore worth mentioning that plant production is generally N limited, while soil microorganisms may be carbon (C) or N limited.

9 Climate Change Impact and Mitigation, and Related Effects on Nitrogen Footprint

The most recent concerns about climate change impacts on soil processes, along with a growing depletion of soil quality worldwide have stimulated experimental research towards the development of methods of fighting and adapting to these impacts. Improving carbon sequestration and promoting plant nutrition from bio-based sources have become priorities. In fact, the use of natural processes to restore soil quality while retaining C to decarbonize production, and monetization of microbially mediated soil nutrients resources (e.g. N) are undoubtedly the pathway for environmental recovery.

Changing conditions and reducing resources will lead to promoting adaptation strategies that allow the production of enzymes with a minimum of carbon and nutrient costs for the cell but still obtaining maximum benefits. In this sense, the enzymatic activity will be a result of the efficiency achieved by microorganisms, in terms of spending resources to produce enzymes versus the benefit of increasing the availability of assimilable mineral nutrients, energy sources and low molecular weight organic compounds. While in a climate change scenario, microbial cells face the need to reduce the energy they use to produce enzymes, they must, on the other hand, maintain a sufficiently high concentration of the reaction products to ensure the maintenance of cell function and maintain viability of their populations. The products necessary to guarantee the microbiological functions in the soil and the balance of nutrient cycles, are C and the nutrients (especially nitrogen and phosphorus) necessary to ensure the existence of energy (i.e. ATP) and the synthesis and secretion of enzymes (proteins). Therefore, N is a crucial element to maintain soil functions as well as microbial and plants survival.

Because of climate change, soil temperature is increasing, soil wetting and drying cycles are more frequent and carbon dioxide and other greenhouse gases are increasing in the atmosphere [25]. These abiotic phenomena will have marked effects in the microbial community composition and may increase biomass and enzyme activities, which can occur as a direct effect or as a result of plant growth, increases in litter deposition and root exudation. So, any attempt to mitigate the impacts of global warming in plant production and soil quality must take into account the microbial responses, including soil enzyme activity dynamics.

A good SOM turnover and balanced nutrient cycles greatly depend on enzymatic activity which in turn is dependent on soil conditions such as temperature and water content but are also influenced by enzyme pool size [19]. The rate of enzymes production by soil microbial populations versus the rate of degradation in the environment, determine pool size. Both production and turnover are affected by soil conditions that vary seasonally but are also affected by climate change, that produces temperature, moisture and atmosphere composition alterations. Enzyme production by microbes requires energy and nutrients, and an adequate stoichiometry of their biomass targeting specific C, N or P rich compounds [19]. Besides the mere maintenance of the enzymatic pool, temperature and moisture can affect both the global rate of enzyme production and the relative rate of production of the different enzymes present in soils. This is due to climatic effects on substrate availability for microbes, microbial efficiency and finally on enzyme efficiency. Therefore, whether climate changes affect environmental conditions locally and regardless of the duration and timing of the impacts, it is certain that this will affect enzyme pool sizes and will have an effect on N-footprint of plant production. Whenever the enzymatic activity increases as a consequence of higher soil temperature, in the presence of available substrate for microbes, enzyme production may be reduced if microbial biomass remains unchanged [77]. Different enzymes are differentially affected by temperature thus, climate change is enzyme-specific. Reference [78] observed that N-degrading enzymes have lower temperature sensitivity compared to C-degrading enzymes, which will lead to a higher production of the former enzymes and consequently reducing N availability. N pool reduction in soils will require heavier fertilization for agricultural production which in turn will increase N-footprint related to crop production. If it is true that the increase in temperature has an immediate effect reducing enzyme activity, it is also true that the continued effect of climate change has a negative impact on the production of enzymes by microorganisms compromising enzyme pools and turnover rates. When enzymes activity is affected by temperature, C degradation will decrease with consequent reduction on C availability for microorganisms that will slow down N availability for plant absorption. On the contrary, as mentioned, N-degrading enzymes will still be active, with cumulative depletion effect on N pool for crop nutrition.

Diffusion of substrates, enzymes and therefore the products of enzyme activity, are affected by soil water availability. So, climate change driven drought conditions will limit diffusion of enzymes and substrates in the soil affecting enzymes contribution to nutrient balances in soils. Indeed, Burns et al. [25] predicted that the reduction of soil moisture would potentially decrease enzyme activity in response to a lower microbial biomass and enzyme production. However, the enzyme pool under drought conditions was stable, which could be explained in two ways: either mass-specific enzyme production was higher under low water availability, or enzyme turnover decreased in dry soils, which was the most probable reason to this observation. The stability of enzymatic processes was not necessarily due to enzyme activity in situ in dry soils, as reported by the same authors. If this is the case, N availability is compromised both for protein production for microbes and for plant nutrition.

Another effect of climate change is the alteration of atmosphere CO_2 content. This may not directly affect microbial activities because CO_2 concentration in soils are naturally much higher than in the atmosphere [24]. However, plant direct responses to elevated CO_2 levels may strongly affect microbial communities due to the changes in plant's metabolism and processes. These may include increased water use efficiency, increased exudation of labile C by roots and a faster nutrient uptake due to a higher plant productivity [25]. Increased rhizodeposition tends to stimulate microbial biomass, therefore increasing potential enzyme production and microbial respiration, although [79] did not observe significant effects of high free air CO_2 presence. CO_2 enrichment is also expected to positively impact nitrogenase activity and biological N fixation by leguminous plants by (i) increasing plant mass/N demand and decreasing soil N availability which limits N fixation and (ii) increased C allocation to root nodules [80, 81]. Whether this is enough to offset the higher need for N fertilization from the reduced soil N availability, with a positive impact in the N footprint by enhancing N recycling rather than through the Haber–Bosch process, remains to be seen.

The greater the knowledge on the degradation of lignin, which constitutes the recalcitrant fraction of SOM, the more evident is the relationship between C and N in the enzymatic processes in the soil, since the distribution of potentially lignin-degrading organisms in soils respond to disturbances associated with anthropic N deposition and climate change [25]. This leads to the assumption that C sequestration and SOM are important to the maintenance of the equilibrium of C/N in soils, thus benefiting microbial activity and ensuring enough enzymes for nutrient cycling. Indeed, climate warming affects soil carbon (C) dynamics, with possible serious consequences for soil C stocks and atmospheric CO_2 concentrations but, the mechanisms underlying changes in soil C storage are not well understood, hampering long-term predictions of climate C-feedbacks [82]. A meta-analysis has shown that reductions in soil C stocks with warming are associated with increased ratios of ligninase to cellulase activity that can be used to track changes in the predominant C sources of soil microbes and can thus provide mechanistic insights into soil C loss pathways. Results suggest that warming stimulates microbial utilization of recalcitrant C pools, possibly exacerbating long-term climate-C feedbacks [82].

A long-term field manipulation experiment has provided evidence that soil aggregate size independently mediates soil microbial feedbacks to multiple climate change factors [83]. Altered microbial enzyme activities, enzyme stoichiometry, and specific enzyme activities under climate change were mainly consistent across soil aggregate size classes. An exception was that C degrading enzyme activities were greatest where C concentrations were highest, namely in the micro-aggregates. Moreover, climate change increased specific enzyme activities for C decomposition, suggesting positive feedbacks between microbial activities related to SOM decomposition and climate change. The distribution of aggregates within soils is affected by both physical and biological processes, and therefore not only affects microbial function but is also affected by it. Previous studies have found that soil aggregate size exerted strong impacts on soil C dynamics and microbial activity. For example, a study of microbial community profiles and activities among aggregates of winter fallow and cover-cropped soil has shown that microorganisms and their activities can be heterogeneously distributed among soil aggregates, and their distribution may change in response to management practices that affect aggregate [84]. Lack of community differentiation may be due to the frequent mixing of soil during cultivation and tillage events, whereby microbial communities become evenly distributed among soil aggregates.

Gong et al. [85] show the response of soil enzyme activity to warming and nitrogen addition in a meadow steppe. Soil enzyme activity, soil microclimate and soil nutrients were measured to investigate the response of soil enzyme activity to N addition and experimental warming. Warming enhanced phosphatase activity (35.8%) but inhibited the cellulase activity (30%). Nitrogen addition significantly enhanced the activities of urease (34.5%) and phosphatase (33.5%) but had no effect on cellulase activity. Significant interactive effects of warming and N addition on soil enzyme activity were observed. In addition, warming reduced soil C (7.2%) and available P (20.5%), whereas N addition increased soil total N (17.3%) and available N (19.8%) but reduced soil C (7.3%), total P (14.9%) and available P (23.5%). Cellulase and phosphatase activity was highly correlated with soil temperature and water content, whereas urease activity was determined primarily by soil N availability. It is difficult to generalize the effects of warming on enzyme pools, as they are also affected and affect other abiotic factors such as the above mentioned frequent and intermittent drying of soils, that will originate matric and osmotic stresses with related impact on enzyme composition and activity.

The results show that climate change not only significantly affects soil enzyme activity, but also affects the mineralization of soil nutrients. These findings suggest that global change may alter grassland ecosystem C, N and P cycling by influencing soil enzyme activity.

Despite the current knowledge on enzymes activity, there is still considerable gaps requiring more information to understand the *ecology* and function of extra-cellular enzymes in soils because of the diversity and complexity of the soil physical and chemical environment and microbial communities. In fact, microbial and enzymatic responses to the effects of climate change are complex because they not only depend on several climatic factors and the relations among them, but are also cumulatively affected by enzymes activity and microbial turnover which in turn are dependent on the formerly mentioned climatic factors. Due to this complexity and missing information, the use of enzyme-based technology requires careful consideration for interpretation and application. This is particularly true when enzymes are used to evaluate soil quality because soil *enzyme activities* should be used in correlation with other key soil measurements. Since enzymes can be independent of soil type, further research on calibrating and interpreting soil enzyme technologies is needed.

10 Conclusion

The amounts of N applied to the soil from different sources are only a part of the chain of N losses considered by the N Footprinting methodology, but they do tend to be one of the larger ones and increasing NUE in soils is a major challenge to reduce these losses. Increasing the understanding of the role of enzymes and how they are affected by the factors listed above will help improve N Footprints. But there is a lot of uncertainty and responses will be site and crop specific. Climate change results in global warming, uncertain and erratic precipitation patterns and atmosphere composition alteration and, because the activity of enzymes in their natural environments is affected by abiotic factors and biotic processes (e.g. enzyme production and turnover) they are likely to be affected by climate change driven phenomena. These, in turn, have important consequences for ecosystem functions including those happening in the soils, such as the maintenance SOM and added organic material decomposition, nutrient cycling and plant—microbe interactions. These effects will ultimately have an impact on crop productivity, net C balance in soils, N use efficiency and N-footprint.

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