



Review

Integrating Ecological Principles for Addressing Plant Production Security and Move beyond the Dichotomy ‘Good or Bad’ for Nitrogen Inputs Choice

Maria Amélia Martins-Loução , Teresa Dias and Cristina Cruz

Centre for Ecology, Evolution and Environmental Changes (cE3c), Change-Global Change and Sustainability Institute, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal; mtdias@fc.ul.pt (T.D.); cmhoughton@fc.ul.pt (C.C.)

* Correspondence: maloucao@fc.ul.pt

Abstract: Mankind’s strong dependence on nitrogen (N) began when we started farming and, ever since, we have depended on nitrogen in the soil for plant production. More than a century has passed since the discovery of N as an element until the advent of synthetic fertilizers. Today, after a century of Haber–Bosch innovation, many other endeavors and challenges can be launched to understand how the effects of N in the environment can be perceived as ‘good’ or ‘bad’. All this knowledge evolution was truly dependent on the scientific advances, both technological and methodological, and particularly on the approaches at the micro and macro level. As with nearly everything in our lives (e.g., events, people, food, decisions, world history), we tend to use the dichotomy ‘good or bad’ to categorize, and scientific advances are no exception. The integration of scientific and technological advances allows us to move beyond this simple dichotomy ‘good or bad’ and to make choices. Here, we review the main marks in understanding plant nutrition throughout time, with special emphasis on N, from the Greeks to the most recent trends in the 21st century. Since improving plant N use efficiency is a main avenue to meet several Sustainable Developmental Goals (e.g., SDG2 zero hunger, SDG12 responsible production and consumption, SDG15 life on land), the European Green Deal, and The Farm to Fork strategy, we propose that the ecological principles must be integrated in agro-ecosystem management. During the last 40 years, our research group has contributed to: (i) the clarification of the so-called dichotomy of choices when it comes to the environmental effects of N; and (ii) fetching natural solutions for N manmade problems. This was based on the knowledge that life is a continuous symbiotic interplay between mutualism and parasitism depending on environmental conditions and that there is a need for feeding people, assuring food quality and diminishing environmental impacts. We argue that, as a society, we have the scientific and technological means to learn from nature and to apply the ecological rules in agro-ecosystems. However, this is a choice we must make as individuals and as a society.

Keywords: biofertilizers; agrosystems; ecosystems; nitrogen; nitrogen use efficiency; plant nutrition; soil biodiversity



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1. Introduction

The availability of more food is fully linked to the transformation of human societies, shaping the size of human population [1]. The switch from hunting to farming, with the use and spread of domesticated grains [2], was a social change that triggered a positive feedback where the increased availability of food created the need for more reliable and constant crop yields. This positive feedback was maintained until the present day and will be stronger due to the current human population growth and consumption patterns [3,4]. Achieving food security and sustainability is very much dependent on our understanding of plant nutrition. Agriculture management transformations are associated with the growing knowledge of plant nutrition and the use of synthetic fertilizers (especially N-containing

fertilizers) through a series of chronological events. Curiosity, common sense, scientific advancement, and societal concern have been the drivers of mankind's dependence on fertilizers, especially nitrogen (N), through plant domestication. Curiosity was triggered by the need for food production that led to understand plant needs and, consequently, to acquire information about the best plants to cultivate and their use. Common sense was the basis of early sustainable agricultural practices: people learned how to exploit without degrading soil resources, while maintaining economic viability. Science provided knowledge about N as a chemical element that may be present in distinct forms with distinct chemical characteristics and biological consequences for crop productivity. More recently, societal concern grew with the recognition of N as a regulator of the ecosystem functioning [5] and the associated negative effects on human health and ecosystems. Within the European Union alone, the impacts of N losses have been estimated at €70–320 billion per year [6].

During the last 40 years, our research group has contributed to the clarification of the so-called dichotomy of choices when it comes to the environmental effects of N. Many works have been derived and evolved since the first recognition that carob—a legume tree—did not follow the 'normal' nitrate preference as an N source. It was a whole group creation and ramification till today that supports this work revision about this dichotomy of choices between N sources. In this analysis, we end up with an ecological approach as a way to help the present challenge of following the need of feeding people while assuring food quality and diminishing environmental impacts under all the global changes we face today.

To better understand the knowledge evolution on plant nutrition, this revision begins with the history of plant nutrition and its progresses through time towards an efficient acquisition and use of N from vegetative to reproductive organs, with increased plant yield under reduced N inputs. A higher plant nitrogen use efficiency (NUE) is one of the key factors to enhance crop output and decrease cost cultivation while maintaining environmental quality. Early in history, humanity understood that crop productivity relies on N fertilization; however, only 40 years ago humanity became aware of the unintended negative environmental consequences of its inadequate use [7]. A dramatic example of the consequences that long-term excessive use of fertilizers can have are the large dead zones formed in the Gulf of Mexico around the Mississippi Delta [8]. Many other examples worldwide reinforce the need to improve the distinct processes that contribute to plant NUE, including N uptake, translocation, assimilation, and remobilization, which are governed by multiple interacting genetic and environmental factors comprising N availability.

It has been well known for a long time that plant NUE has a strong genetic component that leads to the selection and breeding of crop varieties [9]. However, only recently did we start to understand that soil biodiversity, and in particular that of the microbiota, is a strong component of plant NUE and therefore cannot be overlooked. The recognition of the strong negative environmental impacts of agriculture together with the constraints posed by the ongoing climate change drove the search for more sustainable food production systems, with the consequent need for increased research on plant nutrition and innovation of fertilizer production and application. The increase of temperature may produce positive impacts on agriculture in northern countries, while in southern countries water shortage may cause lower harvested yields. This means that climate change may differently affect the NUE of crops which are critical for addressing the present challenges of food security and environmental degradation. Here, we discuss the fundamental advances in the technological advances in fertilizer use, plant–microbe interactions, and plant nutrition towards better agricultural practices with less negative impacts on the environment and human health. The revision concludes with a brief review of the one health concept and its role within the present challenges of producing high quality crops while minimizing environmental impacts and avoiding biodiversity loss. Taking advantage of the ecological principles, where we know that there are always subtle evolutive and adaptive changes,

we propose a new view and interpretation for plant nutrition data towards better human health, socio-economic viability and resilience, and ecological conservation.

2. Plant Nutrition: The History of Humans on Earth

Understanding plant nutrition is fundamental for humankind and instrumental for agriculture, and it represents a great collective effort of science, industry, technicians, and citizens. However, the history of discoveries (Figure 1) concerning the way plants feed is full of misconceptions and incorrect theories.

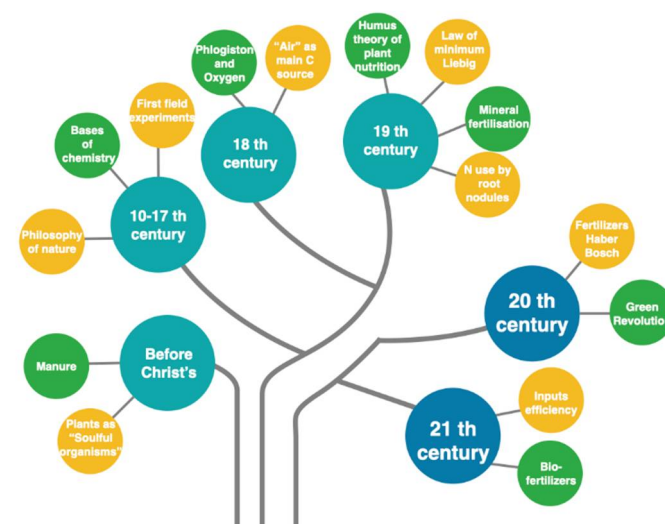


Figure 1. Evolution of some historical marks on plant nutrition.

The ancient Greeks were interested in the way plants feed and how farmers could facilitate their growth. Manure, compost, straw, animal residues, river and pond silt, green manure, ashes, bones, marl, lime, and gypsum were recommended to be used for soil fertilization [10,11]. Aristotle (384–322 BC) taught that plants take up food from soil through roots in the organic form [12]. However, Marcus Porcius Cato (234–149 BC) was the first to give advice on sowing and soil fertilization with the goal of obtaining maximum profit from land cultivation with low expenditure. In ancient Rome, the army (500,000–750,000 soldiers) required about 7500 kg of cereals a day, implying that food production covered large areas and had to be supported by knowledge. This was the beginning of farming 'specialization', and with it came large fields of cereals, grapevines, and olives [12].

In the Middle Ages, attention was drawn to the relationship between plant yield, growth, and development on different soils. According to Fink [13], between the 12th and 15th centuries, 1 kg of seeds provided, on average, 3–4 kg of cereal grain (compared with the present 50–60 kg).

The science of plant nutrition started to develop around the XVII century based on the carefully planned quantitative research of Jean Baptiste van Helmont (1579–1644) that led him to the conclusion that plants feed on water only [14]; a conclusion repeated by many knowledgeable men in England, France, Germany, and Russia. All, with their high reputation, contributed to the credibility of this incorrect theory of plant nutrition for almost two centuries; an interpretation that was corrected by John Woodward (1655–1728).

In 1733–1804 Joseph Priestley discovered oxygen and showed that plants, contrary to animals, purify air since they emit oxygen under certain circumstances. Jan Ingen-Housz (1730–1799) was the first to state that plants emit oxygen only under sunlight since under dark conditions they emit carbon dioxide. This observation was an important step to show how plants feed on CO₂ [12]. Nicolas-Theodore de Saussure (1767–1845) was the first to apply Lavoisier's principles of modern chemistry to conclude that '...the atmosphere is

the main source of carbon for a plant, and soil is only a supplier of ash components and water' [15].

However, the 19th century was a breakthrough in the understanding of plant nutrition. Two hypotheses gain power: 'The humus theory for plant nutrition' by Albrecht Daniel von Thaer (1752–1828) and the 'Theory of mineral plant nutrition' by Justus von Liebig (1803–1873). The humus theory for plant nutrition was the dominant view of plant nutrition for decades. Justus von Liebig (1803–1873) was the first to explain, through his experimental works, the basics of plant mineral nutrition, that not humus but mineral salts are taken up with water by roots from soil. For stable plant yields, soil should be supplied with mineral fertilizers to replenish the deficiency of nutrients caused by their removal from the field. However, in his theory, Liebig marginalized the issue of nitrogen fertilization, assuming that plants acquire nitrogen (like carbon dioxide) through a closed circulation cycle in nature. In 1842, a German scientist Carl Sprengel (1787–1859) formulated the 'Theory of Minimum', which now is known as 'Liebig's Law of the Minimum'. This theory states that plant growth is limited by the essential nutrients at the lowest concentration; this revolutionized contemporary views about fertilization dominated by the humus theory both among scientists and farmers. This law is still valid and is the basis for current systems of mineral fertilization diagnostics. However, Liebig's conviction that only ash components decide on the value of manure, and that nitrogen did not play a significant role, was strongly criticized by Boussingault (1802–1887), who believed that the presence of N in natural fertilizers (formerly known as organic fertilizers) is the main yield-forming factor. Boussingault was the first scientist to pay attention to the circulation of elements—important for plant nutrition—which provided the basis for the cycle evidence in nature. Experiments conducted at Rothamstead (England) also confirmed N as a basic fertilizer component [12]. After many years of research, Boussingault's views on the role of N in plant nutrition were coupled with the role of ash components, promoted by Liebig, formulating a coherent concept that would form the basics of mineral plant nutrition to date.

In 1886, Hermann Hellriegel (1831–1895) and Hermann Wilfarth (1853–1904) showed that Fabaceae plants can use nitrogen by means of root nodules with the participation of microorganisms living in them [16,17]. Just as Liebig's discoveries constituted the beginning of modern agricultural chemistry, John Lawes's patent (1842) for the manufacture of superphosphate as well as setting up the first factory to produce it (Liverpool 1848) should be regarded as the beginning of the mineral fertilizer production technology [18].

Currently, it is commonly assumed that, apart from using mineral fertilizers to maintain and increase soil fertility, natural and organic fertilizers are also necessary, since they act as the substrate for humus formation [19]. The global importance of the principles of mineral fertilization formulated by Liebig and Boussingault for agriculture is well explained in a sentence from Liebig, 'Rome has thrown the entire fertility of Sicily into the gutter', meaning that each ship that brought Sicilian grain to Rome also brought minerals, which, instead of being returned to the soil with excrements, disappeared in municipal waste. The result was the impoverishment of the old Roman fertile soils of Sicily [20]. In past civilizations, minerals taken up from the soil could not be restored, which was a great barrier for the development of past civilizations.

In the 20th century, the scientific discoveries regarding mineral plant nutrition, as well as the import of guano from South America and soda niter from Chile and a vision of famine as a result of the exploitation of Chilean saltpeter deposits, were the drivers behind chemical innovations and include a dynamic growth of the fertilizer industry in the 20th century. The industrial revolution was made available by Fritz Haber's brilliant invention of ammonia synthesis in 1909. It was the first time that a lab experiment was able to mimic the bacterial ability of converting molecular N into ammonia. Later, in 1913 Carl Bosch reached the greatest achievements of implementing that catalytic process at the industrial scale. Thus, obtained ammonia became the basic fertilizer intermediate product in the growing fertilizer industry. Most of the modern fertilizer technologies were introduced in the first three decades of the 20th century (ammonia, nitric acid, triple superphosphate). The

amazing effectiveness of the ‘chemicalization’ of agricultural production and the utilization of the achievements of the Green Revolution in the 1980s gave the technological capacity to feed the earth’s population [21]. The use of fertilizers allowed for a great increase in yields from a unit of area, and thus for a reduction in food prices. Therefore, it significantly contributed to the improvement of living standards of modern societies. However, that did not solve the problem of feeding the population [20].

In the middle of the 20th century, when it seemed that mineral fertilizers were the ‘cure’ that would ensure food security for mankind, people realized that the increase in fertilizer doses not only makes the unit yield increase smaller and smaller, but after exceeding the upper productivity limit, a reduction in yield takes place. Further studies showed that excessive or unbalanced fertilization contributes to the deterioration of the biological value and quality of yields. Following von Liebig’s Law of the Minimum, production grows linearly until the fertilizer reaches a critical dose till the time at which another factor (whether a mineral or not) becomes limiting to growth. It was Mitscherlich, (1912) who, after years of experimentation, stated the Law of Diminishing Returns to express the decreasing marginal productivity outputs as levels of the limiting factor are raised. These observations led Voisin (1903–1964) to the formulation of two new fertilizer laws in the 1960s known as the Law of the Maximum and the Law of the Priority of Biological Quality. His concern was for the risk of considering only yield and ignoring soil conservation and the ‘biological quality’ of the collected product. He tried to establish the importance of the relationship between crop production and fertilizer levels to achieve productive crops as crucial knowledge for the monitoring of fertility programs, the development of corrective strategies for the soil, as well as for the economic use of resources. According to the Liebig and Mitscherlich laws, returns from fertilization are proportional to the difference between maximum and current productions in such a way that the returns tend to zero as production approaches its maximum value [22]. Thus, the Law of the Maximum states that ‘an excessive amount of nutrient availability in soil limits the effectiveness of other substances and in consequence leads to decreased yields’. These laws, next to Liebig’s Law of the Minimum, are the basis of the present-day systems of fertilization diagnostics in agriculture.

In modern times, agriculture is a symbiosis between the practical application of scientific research on chemistry and chemical technology, biology, physiology of mineral nutrition, and other related sciences. The driving force in agriculture in the 20th century was the widely understood chemical progress (the dominant part of which were mineral fertilizers) and the generation of an explosion of knowledge and technology towards the development of genetically engineering crops [23], with its boom and sophistication in the 21st century. Today, in an era of metabolomics and transcriptomics, the challenge is to pull the knowledge from classical crop breeding to crop engineering and from laboratories to replicated field trials. In relation to plant production, this progress is represented by, above all, the creation of new cultivars better adjusted to the requirements of the consumer and of the agri-food industry. The role of mineral fertilization is still very important but is focused on fertilizer technologies that are adjusted to the trends of how to use plants and to their requirements. By creating new genotypes, biological progress sets (to some degree) frameworks that specify the measures which need to be taken in fertilization to keep pace with the ever-changing new conditions. The effect of these changes is a fundamental increase in the specificity of fertilizers. It is estimated that the fertilizer market includes almost a thousand different types of fertilizers [24].

However, chemical innovations in plant nutrition include various strategies used separately or in combination. For example, genetic engineering is used to improve the uptake of macro- and micronutrients by plants. One of the greatest successes in this area was the expression of ferritin, a protein that stores iron in a bioavailable form. Recombinant soybean ferritin was added to many grains, and pea ferritin was expressed in rice. Research is being conducted to gain a more complete understanding of physiological processes of plants, biochemical changes, and to work out methods for designing new compounds for

plant feeding and protection. Another research focuses on increasing the effectiveness of nutrient uptake from the soil and on the use of biofertilizers for biological nitrogen fixation.

3. Plant Nitrogen Use Efficiency

Since N is required for plant photosynthesis (e.g., chlorophyll, enzymes) [25,26], crop productivity relies heavily on N fertilization. From 1972 to 2022, the world population has increased by more than 100%, raising the agricultural demand to feed people. N fertilizer consumption by the Haber–Bosch process increased from 82 Tg N yr to 110 Tg N yr (34%) [27]. Cultivation-induced biological nitrogen fixation (C-BNF) has also increased in several agricultural systems, with crop, pasture, and fodder legumes being the most important. The C-BNF estimate for 1970 was 11.5 Tg N and, because of the increase in soybean and meat production over the past five decades, increased to more than 40 Tg N in 2020 [28]. The large fertilizer consumption enlarged cereal and meat production by 20% and 26%, respectively, but increased reactive nitrogen (Nr) creation by 120% [29]. N availability to plants depends on the amount present in the soil and on the rate at which N cycling occurs through the soil–plant system. However, when soil N availability increases (e.g., upon fertilizer application and peak in N mineralization), N is easily lost from the root soil profiles of eco- and agro-ecosystems by runoff, leaching, denitrification, volatilization, and crop harvesting.

On a global scale, creation and use are identical as there are no internal transfers. This makes Nr creation a good indicator of global human reactive N production. The loss of Nr in the environment increased from approximately 15 Tg N in 1860 to 226 Tg N in 2020 [28]. In this century, the increase in consumption of fertilizer N by the Haber–Bosch process was due to two drivers: East and South Asia development [27]. Although, there is a substantial variation by region, process, and time, the Haber–Bosch process is the most important source of new Nr creation in all regions. The exception is Latin America, where the majority of Nr creation is C-BNF, mainly due to the large-scale soy production [28].

N losses have to be compensated for by soil amendments, particularly N mineral fertilizers that are of critical importance to maintain modern crop productivity. Since the amount of fertilizer required depends on many environmental factors, it is not surprising that we still cannot reliably predict plant N requirements. Many advances have been made, particularly at the plant molecular clock level, providing a mechanistic explanation for changes in plant speciation [26], which, combined with fertilizer recommendations, could greatly improve prediction accuracy of seasonal growth conditions and N requirements. The adjustment of the N fertilizer supply to the level of soil-available N requires a reliable and practical scientific knowledge of N use efficiency (NUE) by plants, a process integrating N uptake, reduction and assimilation, and root to shoot transport according to the plant growth rates. Knowledge of these metabolic factors is of great importance to halt the increase in the production costs and the different environmental impacts of reactive N (Nr, all N compounds except N₂) [30–32] created by the modern use and misuse of nitrogen fertilizers. Despite some scientific knowledge improvement, NUE has not increased since 1980 [33]. Therefore, present priorities are to improve crop NUE and to design agronomic, biotechnological, and breeding strategies for better fertilizer use.

These and other considerations have determined that N uptake and its assimilation into amino acids and from them into other organic N compounds has been thoroughly investigated during the last decades [25,34–40]. The study of inorganic N uptake and assimilation in plants requires the integration of physiological and molecular research and approaches [41] for the full understanding of the processes involved and their regulation. Evidence for genetic variation in both acquisition efficiency and internal-use efficiency was given for plants under N-sufficient and N-limited conditions [42]. More recent reports have also discussed how nitrate transporter and assimilation genes can be used as molecular tools to improve NUE in crops [43]. A deeper understanding of the processes in wheat cultivars showed limited genetic variations, suggesting a requirement for broader germplasm within older varieties, landraces, and even wild relatives [44]. In pearl millet, on the contrary,

extensive genetic variation across population parents showed contrasting genetic stocks [45]. A deep knowledge and understanding of species genetic variations is essential to select efficient genotypes with improved NUE (to then be included in breeding programs) and therefore achieve climate resilience.

The estimation of energetic costs (measured as respiratory or relative growth rates) associated with N uptake in different plant species [46–48] has disclosed the spatial and temporal heterogeneous distribution of N sources in the soil [49,50], explaining differences in NUE. The low NUE may increase as the external concentrations of N, particularly nitrate, are reduced. Under natural Mediterranean conditions, a long-term N-manipulation study (since 2007) shows that N availability (and ammonium in particular) is a driving force for biodiversity changes above- [51,52] and belowground [53–55], suggesting that the biotic compartments (living plants, plant litter, soil biota) have a crucial role in preventing N losses [50]. Whether low NUE is actually a feature of plant species' response according to the availability of different N sources under natural environments [51,52] is still rather difficult to generalize, particularly for agro-ecosystems.

Besides heterogeneity in soil N availability, N uptake and assimilation along plant roots are also heterogeneous [56], which is associated with changes in root system mycorrhization [57] or not [58]. Knowledge on N transport mechanisms and their biochemical regulation are advancing fast in different wild (e.g., *Ceratonia siliqua* L., *Cistus albidus* L., *Olea europaea* L.) and cultivated species (e.g., *Arabidopsis thaliana* L., *Lycopersicon esculentum* L., *Lactuca sativa* L., *Lupinus albus* L., *Pisum sativum* L. and *Spinacia oleracea* L.) [39,41,56,59,60]. Although many previous studies refer to activities taking place in specific organs (e.g., roots versus leaves) [36,61], currently roots and shoots are regarded as functionally interdependent and controlled by carbon and nitrogen metabolic pathways [62,63]. The 'whole plant approach' allows to disentangle such relationships that depend on an efficient root shoot communication through signaling processes mediated by phytohormones (auxin and cytokinin), carbohydrates [43,64], and nitric oxide (NO) [65]. N is not only a nutrient but also a component of simple inorganic signaling compounds such as NO and nitrate (NO_3^-) [66]. The presence of beneficial rhizosphere plant-microbe interactions is often involved in phytohormone- and NO-mediated responses that improve N uptake, assimilation, and NUE [67].

Genetic differences determine to a significant extent the ability of plants to take up and use inorganic N for growth [60,63,68]. Although previous N uptake studies had concluded that nitrate is the preferred N source for most herbaceous crops [69–71], several studies provided evidence that many species, including conifers and other woody plant species (e.g., *Ceratonia siliqua* or carob tree, *Dimorphandra wilsonii*) and herbaceous crops, grow well and occasionally better with ammonium [34,72,73]. The long-standing idea of ammonium toxicity, which dates to Charles Darwin, is still a matter of debate [74]. Nevertheless, ammonia is continuously produced in living organisms by a number of biochemical processes (e.g., photorespiration), suggesting that enzymes [40,75] and transporters are responsible for maintaining the delicate balance of ammonium fluxes in plant tissues and, thus, plant tolerance of ammonium. Taking advantage of the existence of N stable isotopes, modern and sensitive methods have been developed to understand and interpret the role of ammonium in plant nutrition [76,77] and thus provide new insights into the controversy surrounding ammonium transport systems and mechanisms. Much of the ammonium sensitiveness of plants was explained by the main occurrence of NH_3 diffusion across biological membranes [78], independently of the known strong evidence that a high affinity NH_4^+ transport system is over a broad range of organisms [79]. Apparently, the transport of ammonium across cellular membranes is conserved throughout all domains of life and the NH_4^+ deprotonation process may be a mechanistic feature conferring selectivity against K^+ and for NH_3 transport after NH_4^+ recruitment [77].

During the 20th century, research has been directed to modeling N utilization by crops, taking into account crop demand, root development, and crop uptake of soil mineral N [80]. However, the application of N fertilizers to soils is still very inefficient, as 50% of the

applied N-fertilizer is not taken up by the plants [81], and will therefore have an impact in the receptor ecosystems (both terrestrial and aquatic) [82]. This highlights the importance of N-transformations occurring in soils in relation to specific environmental conditions. Although too much ammonium is toxic to most crops, the last five decades showed that the majority of plant species grow more in the presence of a mixture of ammonium and nitrate, according to natural and artificial conditions [34,63,83,84]. A plant's preference for nitrate or ammonium may be associated with the external and/or internal factors. Ammonium tolerance can be improved with the presence of nitrate, even when present in very low concentrations and with the addition of potassium fertilizers. Therefore, having access to ammonium and nitrate may be advantageous to plants as they can make use of different morphological and physiological adaptations [72] and/or promote beneficial interactions with microorganisms, facilitating a cross-talk signaling that promotes plant growth and development [85,86].

Reducing N fertilizer pollution while maintaining high crop yields can be done by exploiting new tools that select and breed crops able to take advantage of the ammonium/nitrate balance and by recruiting beneficial soil microbes, as we can observe under natural and semi-natural environments.

4. Nature-Based Solutions for Better NUE

Since most natural and semi-natural ecosystems are N-limited, the increased N availability can change plant community and, consequently, ecosystem functioning [87]. Thus, managing N availability is one of the major environmental challenges of the 21st century. This is an economic, social, and ecological issue that requires a more efficient conversion of inorganic N inputs into organic N compounds in agricultural systems (with less N losses and more N available for longer periods).

The strong interactions between agrosystems and ecosystems is becoming more evident, especially in patchy landscapes where different land uses co-exist (e.g., conservation areas and agricultural farms). The steady intensification of crop production has acquired many of the characteristics of an industry with harmful byproducts and waste beyond the levels that ecosystems can normally cope with. Adaptation measures can include novel fertilizers and improved agronomic practices that increase soil carbon, organic matter, and soil water retention. Agricultural efficiency could be improved, but all evidences suggest that the threat for food demand to tackle climate change in agriculture justifies the introduction of various innovations in chemical products [88] and the growing appreciation of soil microorganisms as biofertilizers and as important elements to preserve soil fertility. Thus, creative and innovative strategies must be built upon sound scientific basic research through a holistic ecological approach following the model of nature-based solutions.

The ongoing modernization of the production of fertilizers and agrochemicals meets the requirements of the IPPC (Integrated Pollution Prevention Control) directive and BAT (Best Available Technique) standards. Innovating in plant nutrition requires comprehensive and multidisciplinary technological research focusing on the chemical and biological products and their production processes and also on the beneficial properties of such products. As an example, fertilizers coated with different polymers for slow and controlled release of nutrients during plant vegetative stages are a new trend [89] as they can improve soil structure and soil water holding capacity [90] and function as carriers of microelements. The fertigation system is another chemical innovation used in modern plant production. Recovering nutrients that can be found in domestic water waste is another innovation approach, especially when considering urbanization: agrosystems are constantly exporting nutrients in the form of food products that enter metropolitan areas, whereas minerals and organic substances found in domestic waste find their way (in the form of sewage) to surface waters or are accumulated on landfills, creating huge environmental problems and the necessity to incur high costs of their utilization that can replenish the soils in agrosystems [91]. New innovative methods of processing this waste into organic and organic-mineral fertilizers have been recently elaborated. This approach also applies to

agricultural and forestry waste [92] and to mushroom-growing cellars or agricultural biogas plants [93].

In response to the global issue of misuse and overuse of pesticides and mineral fertilizers, feeding the increased global population is further challenged by limited availability of agricultural land. Thus, the requirement today is to have a production system that has a higher productivity in a small area and time for cultivation. Moreover, plant microbial biotechnology can potentially help in developing sustainable agriculture practices, since microbes play a vital role in determining the fertility and soil structure and in reducing problems associated with the use of chemicals fertilizers [94]. The development of alternate, sustainable, and cost-effective biologically available nutrient resources can be achieved with microbial inoculants acting as biofertilizers. The term ‘biofertilizer’ describes soil live microorganisms that increase plant growth and development by acting through various mechanisms: nutrient uptake, minimizing nutrient loss, excretion of phytohormones, controlling plant pathogens [95], protecting plants from different biotic and abiotic stress, and pollutant detoxification [96]. Increasingly, biofertilizers have gained relevance in agricultural industries and are viewed as key components of sustainable agriculture. As a result, the global biofertilizer market reached a value of USD 2.3 billion in 2020 and it is projected to increase to almost double in 2026 (<https://www.marketsandmarkets.com/Market-Reports/compound-biofertilizers-customized-fertilizers-market-856.html> (accessed on 26 October 2021)). Unlike mineral fertilizers, which are associated with soil degradation and low efficiency in the use of natural resources, biofertilizers have been pointed out as a promising tool in the transition to a more sustainable agriculture, because: (i) in general, biofertilizers do not constitute an environmental pollution risk; (ii) an effective biofertilizer application can significantly reduce the mineral fertilizer application, thus reducing the negative effects associated with the use of this type of fertilizers; and (iii) as biofertilizers enhance soil microbial activity and promote beneficial symbiotic relationships and ecological interactions, the use of biofertilizers contributes to a more diverse and resilient soil, increasing the soil health as well as its long-term sustainability [94].

This agricultural innovation (i.e., biofertilizers) corresponds to fetching natural solutions, mainly for manmade problems, since high diverse ecosystems rarely present a serious disease breakout. Since microorganisms are more likely to survive and reproduce under stress, plant–microorganism interactions can establish symbiotic microbiomes that benefit plants directly and/or indirectly by modifying the (a) biotic environment. Variation in plant–microbiome composition across sites and time [86] and functional redundancy in the microbial community will allow host selection on microbiome function rather than taxa per se [97]. Considering microbiome recruitment as a symbiotic model on plant–soil ecology and adaptation [98] would allow the application of this knowledge in biofertilizer design and production [4,99].

Although the use of microorganisms in agriculture is not new [100], the complexity of functions and interactions in plant-associated microbiomes as well as their performance and persistence in the environment is yet to be harnessed [101]. Microbial inoculants can be considered safe, low-cost, and convenient additives that are a nature-based option for promoting plant growth and quality and disease control [102,103]. Researchers play an important role in the transfer of technology to farmers, but studies to understand the needs and preferences of target consumers are also needed. Therefore, research into the widespread use of biofertilizers is one of the mainstream tasks in scientific work towards the sustainability of agriculture, minimizing environmental hazards and soil nutrient losses (Figure 2). In view of the above stated facts, the long-term use of biofertilizers arises to be productive and accessible to marginal and small farmers because it would be the viable option to increase productivity per unit area while reducing environmental impacts and external N inputs. To help the function of whole systems, agricultural areas are not cleared out, facilitating the attraction of different pollinators or pest deterrents; this does not mean that there are no constraints. On the contrary, there are still technological, financial, and environmental constraints before it can be widespread. However, the understanding of

the whole ecological web and connectivity is the pillar and a new paradigm that even the European Union is interested in following according to its Green Deal and The Farm to Fork strategy.

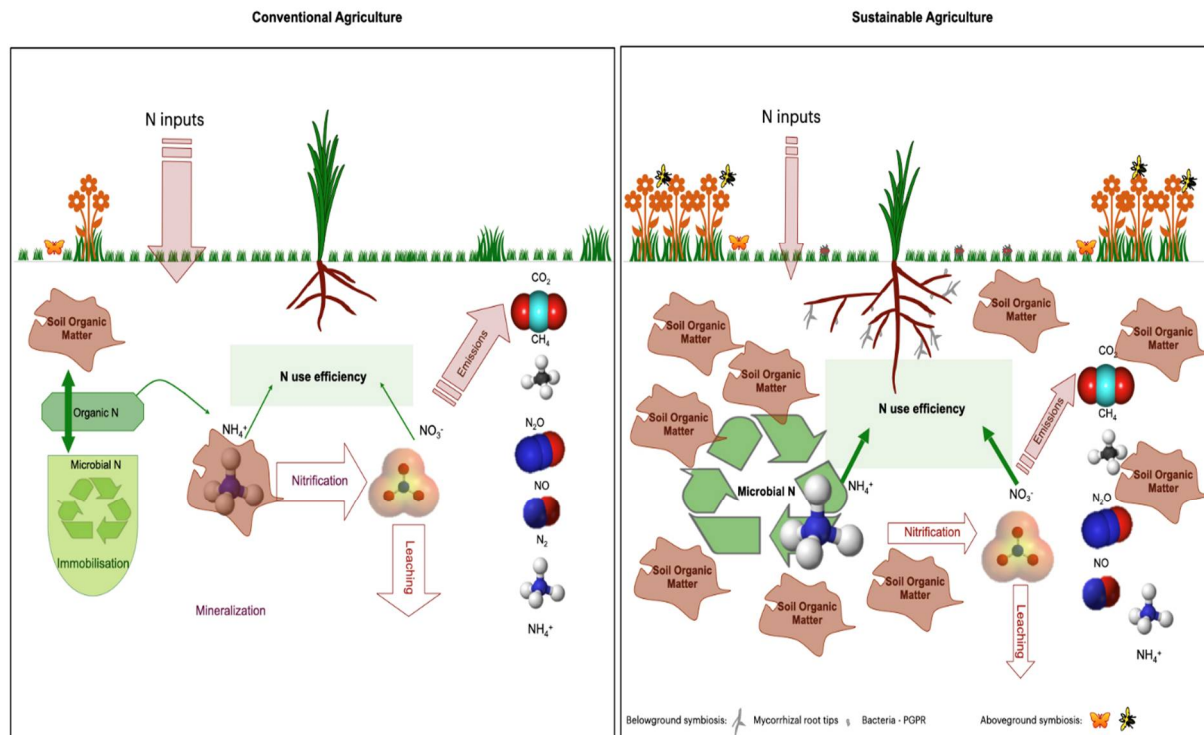


Figure 2. The effect of agrosystem management (conventional and sustainable agriculture) on plant adaptations and production. N availability (concentrations and forms) in the soil determines biomass partitioning between roots and shoots. Plants grown under conventional agriculture (on the **(left)**) grow faster, present low root/shoot ratios, have a higher turnover rate, and generate low decomposability litter (i.e., most part of N is taken out during harvest, litter has high C/N). These plants depend on high external inputs and show fewer and less efficient symbiosis below- and aboveground. In these systems, the amount of nitrate and ammonium in the soil varies depending on the type of added fertilizer and climate, and soil organic matter and microbial diversity are low. The N that is not taken up by the plants is highly susceptible to be lost from the system through runoff, nitrate leaching, and gas emissions as CO₂, methane (CH₄), nitric oxide (NO), nitrous oxide (N₂O), or ammonia. Plants grown under sustainable agriculture (on the **(right)**) receive much less external N inputs, show a high root/shoot ratio, and production is much more dependent on plant–microbial interactions—bacteria and fungi—forming an entire plant microbiome. The plant microbiome promotes microbial N turnover, higher N use efficiency, and fewer N losses. Since fields are not fully cleared out, other plant species that attract pollinators can establish and therefore ensure the functioning of an aboveground ecological network. The presence of this system promotes the accumulation of soil organic matter and the maintenance of high soil biodiversity even after harvest.

Biofertilizers are designed to target one or more desired effects: biocontrol of pathogens, N fixation, improving plant nutrition, weathering of soil minerals, and nutritional or hormonal effects. However, for developing biofertilizers we need to cultivate the microbes (e.g., bacteria) *in vitro*. Several studies have identified a group of microorganisms normally found within plants through the use of throughput sequencing-based studies [101]. Plant Growth Promoting Rhizobacteria (PGPR), which are nonpathogenic microorganisms present in the rhizosphere, possess abilities to promote plant growth by improving mineral and water absorption, producing plant growth stimulating compounds, and suppressing the growth of pathogens [95]. The promotion of plant growth by PGPR, especially bacteria, can be traced back for centuries. The first patented microbial product was registered nearly

100 years ago and used *Rhizobium* sp. as an active ingredient. In addition, inoculation with non-symbiotic rhizosphere bacteria such as *Azotobacter* began in the 1930s and 1940s and was revived in the late 1970s [100]. The use of microorganisms in disease control was developed later; the first product to be used as a biocontrol agent was registered by the US Environmental Protection Agency (EPA) in 1979 [104]. Various mechanisms are involved in the synergistic interactions between plants and microorganisms [105]. Direct mechanisms involve nutrient acquisition, such as N, phosphorus and iron [85], hormone or nitric oxide-level modulation [106,107], and stress release [108]. Thus, identifying those mechanisms is a major challenge due to the diversity and difficulty in measuring plant growth and performance under different biotic or abiotic conditions [73,109].

Understanding the ecological processes that govern the establishment, stability, adaptation, and evolution of symbiotic relationships is crucial for a successful application of biofertilizers and biostimulants. This knowledge is pivotal for developing sustainable agricultural systems that guarantee the provision of nutrients (mainly N and P) to the plants while reducing mineral fertilizer inputs, which will reinforce food security (i.e., the state of having reliable access to sufficient, safe, affordable, and nutritious food at all times) and consequently follow a One Health target.

5. One Health Context within the Ecosystem

Although plant health is currently part of the definition of One Health, plants have typically not been well integrated into discussions of One Health approaches [110]. However, plant health is vital to sustain human and animal health and is a critical component of the complex interactions between the environment, humans, and animals. Maintaining plant health has important consequences for human and animal health as an important driver of food security and safety, as a source of livelihoods in plant-based agriculture, as a source of pharmaceuticals, and as part of healthy environments [111].

Plants provide over 80% of the food consumed by humans and are the primary source of nutrition for livestock. Food security—the state of having reliable access to sufficient, safe, affordable, and nutritious food at all times—is key to healthy and productive societies. Food security is also a crucial aspect of One Health and is a pillar of the United Nations Sustainable Development Goals (SDGs). The UN definition of food security identified four key pillars: (1) availability, (2) access (both economic and socio-cultural), (3) utilization, including food preparation and safety, and (4) the stability of these three pillars [88]. Food security thus reflects a complex value chain of production, food processing and distribution, and food access, beginning with plant health in the field.

Science, society, and policy agree that the use and misuse of all kinds of N-containing fertilizers and pesticides to promote plant growth and pest control are becoming obsolete and should therefore be replaced by new agricultural systems able to respond to climate change and to safeguard the capacity of food systems to ensure global food security. With this productive strategy we could save enough N to offset most of the new demand for the nutrient application, beginning with more efficient use of the nutrient in cropping and ending with much less wasteful animal feeding and food consumption, while safeguarding the whole environment.

To employ the One Health concept, which predicted the integration of the interface with ecosystems in the One Medicine concept, we need an approach that is holistic and transdisciplinary and incorporates ecological expertise in applying the ecosystem approach. It is now more knowledgeable that the sustainability of agro-ecosystems depends on their ability to deliver multiple ecosystem services, rather than food and feed production alone. In an anthropocentric point of view, ‘healthy’ soils, plants, and animals in an agro-ecosystem is seen in the services they provide to humankind. From an ecological point of view, values and qualities of all organisms within an ecosystem, independent of their utility to human societies, are recognized. Being this is a revision within an agronomic perspective, the One Health concept is here focused on plant productivity, though with an ecological and holistic perspective. This implies that to follow a One Health concept we need to focus on a smaller

impact on the agricultural soil environment and a smaller dependence on nutrient inputs. Thus, we need to identify the thresholds beyond which irreversible changes might occur, to understand whether they follow the unraveling trajectories of native ecosystems and their symbiotic interactions; particularly, when we are facing climate change and biotic invasions of disease vectors that may ruin crop quality. Therefore, the challenge is to identify those thresholds for soil structure, since its degradation causes the disappearance of key microbe species and a larger dependence on external inputs for plant production.

We need to adopt a holistic approach and understand that, in nature, there are different symbiotic interactions that confer subtle adaptations along different gradients of stressful conditions. This means that soil ecosystems are a common home to the plants (which take support, nutrients, and water) and a larger number of microorganisms (commensal, pathogenic, and beneficial). Plant–microbe interactions often involve the exchange of nutrients [57] maintained by complex signaling pathways [107]. Whether soil biodiversity changes trigger important changes in ecosystem functions and are responsible for environmental dynamics affecting coevolved interactions is still a matter of study [112]. Traditional monitoring strategies are often expensive and associated with a delay in problem recognition and accessing data. New ecological approaches are needed to differentiate between the potential interactions between climate and land-use effects [113,114]. Some of these include the use of structural equation models [112], others take advantage of the available technology for time–series monitoring and the so-called ‘space-for-time substitution’ method to analyze ecosystem dynamics in a more comprehensive manner [115].

6. The Gospel of Choice

During the last century, human activities have greatly increased the availability of N interfering with N cycling between living organisms and their environment (soil, water, and atmosphere) [116]. The greatest human-driven increase in global N supplies is a consequence of the need to produce more and better harvests to improve the protein nutrition of men and animals, which in 1900 was a worldwide concern. The solution brought by the Haber–Bosch process was so successful that, without it, approximately half of the current human population would not exist [117]. As a consequence, N fertilization has increased in this time about tenfold, resulting in the increase of the global pools of various forms of N in the air and in water resources as well as affecting soil inorganic pools [50], strongly influencing decomposition and microorganisms diversity [32,55], and affecting all ecosystem compartments [118]. Thus, in one century, humans have profoundly altered the N cycle focusing on the feeding of an increase in human population. The global interference in the N cycle is so strong that it is one of the four planetary boundaries that have been crossed, threatening our planetary safety [119,120]. Despite decades of research trying to better understand crop NUE, the negative consequences of too much available N in the biosphere and available technical controls remain elusive.

Given the magnitude of N as a global change driver on its own, and in close interdependence with others (e.g., biosphere integrity, climate change, land-system change) [120], the current challenge is to advance science, technology, and practices to assure the world’s future food security while reducing agriculture’s environment footprint. Under this circumstance, we need to rethink agricultural systems. The current generalized idea that mineral N inputs are ‘bad’, similarly to what occurred in the 1990s with nitrate versus ammonium N-inputs, is a false problem. It is time to understand that soil N availability (too much or too little) varies in space and in time, and so does its impacts. In addition, synthetic fertilizer application is highly unevenly distributed on a global scale and we should not jeopardize its use as just a bad thing. Therefore, we suggest an ecological causality approach in discussing the better choice of N applications (Figure 3). This approach involves user options, sustainable development principles, and the development of eco-technologies to follow the causal links between soil biodiversity, plant–microbe interactions, and plant productivity. The challenge is to look at the soil as an ecosystem.

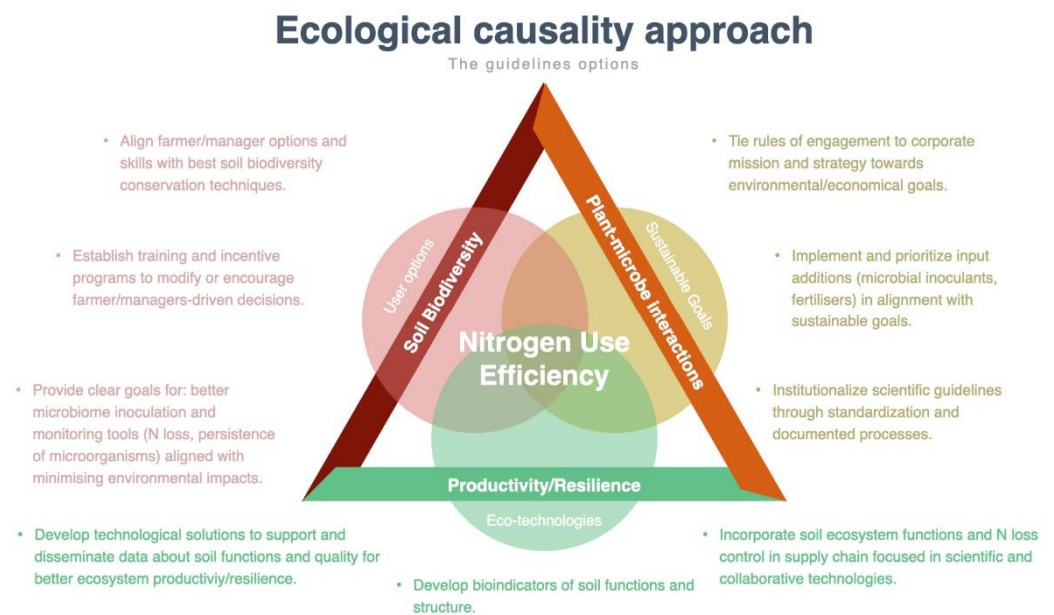


Figure 3. Using the ecological causality approach (based on analyzing long-time research data in different agro- and ecosystems and different conditions), our research team quantifies various causal links among key ecosystem components, including soil biodiversity, plant–microbial interactions, and ecosystem functioning and services provision. This analysis emphasizes the importance of soil biodiversity in promoting efficient plant–microbial interaction to harness a more sustainable balance between ecosystem functioning and plant productivity. The present diversity–nutrient–biomass triangular approach intends to reveal how the strengths of these ecological networks influence N use efficiency in natural and manmade ecosystems. For each part, different applied proposals are envisaged to reach the goal: improve N use efficiency, minimize N environmental impacts, and safeguard farmers’ socioeconomic viability. This ecological causality approach advocates that the use of sustainable technologies (e.g., organic amendments, biofertilizers, no tillage, crop rotations) is in line with the Farm to Fork strategy—one of the central pillars of the European Green Deal—as the European Commission aims to see a reduction in nutrient losses by at least 50%, and a reduction in fertilizer use by at least 20% by 2030, while ensuring no deterioration in soil fertility (https://ec.europa.eu/info/food-farming-fisheries/sustainability/environmental-sustainability/low-input-farming/nutrients_en (accessed on 26 October 2021)).

Soil is the most biologically diverse ecosystem in the biosphere. Soil microorganisms strongly influence biogeochemical cycles, forming and decomposing organic matter and plants, such as other organisms, and hosting and selecting a diverse set of microbes. The assembly of these microbial communities is shaped by different symbiotic plant–microbe and microbe–microbe interactions, dictated by a dynamic and complex balance of inhibition and facilitation of growth. These dynamic biotic interactions depend on different biotic and abiotic factors, and ultimately contribute to ecosystem adaptation and resilience. The understanding and knowledge of those interactions needs the training of stakeholders, the effective collaboration of scientists, and the support of policies. The trophic complexity present under natural and semi-natural conditions explains why wild plants rarely present a serious disease breakout and show resilience under stochastic disturbances. This means that scientists need to develop reliable amounts of microbial inoculants and to establish clear and objective guidelines for stakeholders to follow.

We, as a society, have the scientific and technological means to learn from nature and apply the ecological rules in agro-ecosystems. However, this is a choice we all have to make as individuals and as a society (Figure 3). To follow the UN definition of food security, we need to focus primarily on plant health in the field and, thus, on the availability of nutrient resources to attain economical sufficient productivity. Accordingly, an economic

productive exploitation must be based on crop NUE. Current development of our knowledge on different aspects of N use by different crops [121], woody plants, as well as wild species [122] enables us, as a research group, to: (i) offer better guidelines for an efficient use of N fertilizers [54,90]; (ii) advise on the best fitted genotypes for food production and nutritious food under sustainable agriculture [92] within rationally co-existing eco- and agrosystems [102,123]; and (iii) monitor the negative impacts of N pollution in natural ecosystems [118].

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References

- Richards, J.F. Land transformation. In *The Earth as Transformed by Human Action: Global and Regional Changes in the Biosphere over the Past 300 Years*; Turner, B.L., Clark, W.C., Kates, R.W., Richards, J.F., Mathews, J.T., Meyer, W.B., Eds.; CUP Archive: Cambridge, UK, 1990; pp. 163–178.
- Spengler, R.; Frachetti, M.; Doumani, P.; Rouse, L.; Cerasetti, B.; Bullion, E.; Mar'yashev, A. Early agriculture and crop transmission among Bronze Age mobile pastoralists of Central Eurasia. *Proc. R. Soc. B Biol. Sci.* **2014**, *281*, 20133382. [[CrossRef](#)] [[PubMed](#)]
- Dias, T.; Dukes, A.; Antunes, P.M. Accounting for soil biotic effects on soil health and crop productivity in the design of crop rotations. *J. Sci. Food Agric.* **2015**, *95*, 447–454. [[CrossRef](#)]
- Dias, T.; Pimentel, V.; Cogo, A.J.D.; Costa, R.; Bertolazi, A.A.; Miranda, C.; de Souza, S.B.; Melo, J.; Carolino, M.; Varma, A.; et al. The Free-Living Stage Growth Conditions of the Endophytic Fungus *Serendipita indica* May Regulate Its Potential as Plant Growth Promoting Microbe. *Front. Microbiol.* **2020**, *11*, 562238. [[CrossRef](#)]
- Galloway, J.; Aber, J.; Erisman, J. The Nitrogen Cascade. *Bioscience* **2003**, *53*, 341–356. [[CrossRef](#)]
- Sutton, M.A.; Howard, C.M.; Erisman, J.W.; Billen, G.; Bleeker, A.; Grennfelt, P.; van Grinsven, H.; Grizzetti, B. *The European Nitrogen Assessment*; Cambridge University Press: Cambridge, UK, 2011.
- Smil, V. Nitrogen and Food. *Ambio* **2002**, *31*, 126–131. [[CrossRef](#)] [[PubMed](#)]
- Malakoff, D. Death by suffocation in the Gulf of Mexico. *Science* **1998**, *281*, 190–192. [[CrossRef](#)]
- Han, M.; Okamoto, M.; Beatty, P.H.; Rothstein, S.J.; Good, A.G. The Genetics of Nitrogen Use Efficiency in Crop Plants. *Annu. Rev. Genet.* **2015**, *49*, 269–289. [[CrossRef](#)]
- Clarkson, D.T.; Hanson, J.B. The mineral nutrition of higher plants. *Annu. Rev. Plant Physiol.* **1980**, *31*, 239–298. [[CrossRef](#)]
- Fageria, N.K. *The Use of Nutrients in Crop Plants*; CRC Press: Boca Raton, FL, USA, 2016.
- Antonkiewicz, J.; Łabętowicz, J. Chemical innovation in plant nutrition in a historical continuum from ancient Greece and Rome until modern times. *Chem.-Didact.-Ecol.-Metrol.* **2016**, *21*, 29–43. [[CrossRef](#)]
- Finck, A. Fertilizers and Fertilization. In *Introduction and Practical Guide to Crop Fertilization*; Verlag Chemie: Weinheim, Germany, 1982.
- Starck, Z. Fizjologia roślin: Jak było wczoraj, jak jest dziś, a co przyniesie jutro? *Kosmos* **2014**, *63*, 569–589.
- Guntzer, F.; Keller, C.; Meunier, J.-D. Benefits of plant silicon for crops: A review. *Agron. Sustain. Dev.* **2012**, *32*, 201–213. [[CrossRef](#)]
- Hoagland, D.R.; Arnon, D.I. The water-culture method for growing plants without soil. *Circular. Calif. Agric. Exp. Stn.* **1950**, *347*, 32.
- Burris, R.H. Biological nitrogen fixation: A scientific perspective. *Plant Soil* **1988**, *108*, 7–14. [[CrossRef](#)]
- Marciniak, B. Misja nauk chemicznych w zrównoważonym rozwoju. *Nauka* **2012**, *1*, 123–132.
- Gondek, K. Effect of fertilization with farmyard manure, municipal sewage sludge and compost from biodegradable waste on yield and mineral composition of spring wheat grain. *J. Elem.* **2012**, *17*, 231–245. [[CrossRef](#)]
- Voisin, A. Fertilizer Application. In *Soil, Plant, Animal*; Crosby Lockwood: London, UK, 1965.
- Kozhakhyanov, T.E.; Lukashenko, S.N.; Larionova, N.V. Accumulation of artificial radionuclides in agricultural plants in the area used for surface nuclear tests. *J. Environ. Radioact.* **2014**, *137*, 217–226. [[CrossRef](#)] [[PubMed](#)]
- Ferreira, I.E.P.; Zocchi, S.S.; Baron, D. Reconciling the Mitscherlich's law of diminishing returns with Liebig's law of the minimum. Some results on crop modeling. *Math. Biosci.* **2017**, *293*, 29–37. [[CrossRef](#)]
- Huang, J.; Pray, C.; Rozelle, S. Enhancing the crops to feed the poor. *Nature* **2002**, *418*, 678–684. [[CrossRef](#)]

24. Filipek-Mazur, B.; Lepiarczyk, A.; Tabak, M. Nitrogen and sulphur fertilization on yielding and zinc content in seeds of winter rape 'Baldur' cultivar. *Ecol. Chem. Eng. A* **2013**, *20*, 1351–1358.
25. Cruz, C.; Lips, S.H.; Martins-Loução, M.A. The effect of nitrogen source on photosynthesis of carob at high CO₂ concentrations. *Physiol. Plant* **1993**, *89*, 552–556. [[CrossRef](#)]
26. Kelly, S. The Amount of Nitrogen Used for Photosynthesis Modulates Molecular Evolution in Plants. *Mol. Biol. Evol.* **2018**, *35*, 1616–1625. [[CrossRef](#)] [[PubMed](#)]
27. Heffer, P.; Prud'homme, M. Global nitrogen fertilizer demand and supply: Trend, current level and outlook. In Proceedings of the Solutions to Improve Nitrogen Use Efficiency for the World, Melbourne, Australia, 4–8 December 2016.
28. Galloway, J.N.; Bleeker, A.; Erisman, J.W. The Human Creation and Use of Reactive Nitrogen: A Global and Regional Perspective. *Annu. Rev. Environ. Resour.* **2021**, *46*, 255–288. [[CrossRef](#)]
29. Galloway, J.N.; Townsend, A.R.; Erisman, J.W.; Bekunda, M.; Cai, Z.; Freney, J.R.; Martinelli, L.A.; Seitzinger, S.P.; Sutton, M.A. Transformation of the Nitrogen Cycle: Recent Trends, Questions, and Potential Solutions. *Science* **2008**, *320*, 889–892. [[CrossRef](#)]
30. Pinho, P.; Dias, T.; Cruz, C.; Tang, Y.S.; Sutton, M.A.; Martins-Loucao, M.A.; Maguas, C.; Branquinho, C. Using lichen functional diversity to assess the effects of atmospheric ammonia in Mediterranean woodlands. *J. Appl. Ecol.* **2011**, *48*, 1107–1116. [[CrossRef](#)]
31. Pinho, P.; Theobald, M.R.; Dias, T.; Tang, Y.S.; Cruz, C.; Martins-Loução, M.A.; Máguas, C.; Sutton, M.; Branquinho, C. Critical loads of nitrogen deposition and critical levels of atmospheric ammonia for semi-natural Mediterranean evergreen woodlands. *Biogeosciences* **2012**, *9*, 1205–1215. [[CrossRef](#)]
32. Dias, T.; Oakley, S.; Alarcón-Gutiérrez, E.; Ziarelli, F.; Trindade, H.; Martins-Loução, M.A.; Sheppard, L.; Ostle, N.; Cruz, C. N-driven changes in a plant community affect leaf-litter traits and may delay organic matter decomposition in a Mediterranean maquis. *Soil Biol. Biochem.* **2013**, *58*, 163–171. [[CrossRef](#)]
33. Subbarao, G.V.; Searchinger, T.D. Opinion: A "more ammonium solution" to mitigate nitrogen pollution and boost crop yields. *Proc. Natl. Acad. Sci. USA* **2021**, *118*, e2107576118. [[CrossRef](#)]
34. Cruz, C.; Lips, S.H.; Martins-Loução, M.A. Growth and nutrition of carob plants as affected by nitrogen sources. *J. Plant Nutr.* **1993**, *16*, 1–15. [[CrossRef](#)]
35. Cruz, C.; Lips, S.H.; Martins-Loução, M.A. Interactions between nitrate and ammonium during uptake by carob seedlings and the effect of the form of earlier nitrogen nutrition. *Physiol. Plant* **1993**, *89*, 544–551. [[CrossRef](#)]
36. Cruz, C.; Lips, S.H.; Martins-Loução, M.A. Nitrogen assimilation and transport in carob plants. *Physiol. Plant* **1993**, *89*, 524–531. [[CrossRef](#)]
37. Cruz, C.; Lips, S.H.; Martins-Loução, M.A. The effect of root temperature on carob growth. Nitrate versus ammonium nutrition. *J. Plant Nutr.* **1993**, *16*, 1517–1530. [[CrossRef](#)]
38. Cruz, C.; Lips, S.H.; Martins-Loução, M.A. Uptake of ammonium and nitrate by carob (*Ceratonia siliqua*) as affected by root temperature and inhibitors. *Physiol. Plant* **1993**, *89*, 532–543. [[CrossRef](#)]
39. Cruz, C.; Lips, H.; Martins-Loução, M.A. Nitrogen use efficiency by a slow-growing species as affected by CO₂ levels, root temperature, N source and availability. *J. Plant Physiol.* **2003**, *160*, 1421–1428. [[CrossRef](#)] [[PubMed](#)]
40. Cruz, C.; Bio, A.F.M.; Domínguez-Valdivia, M.D.; Aparicio-Tejo, P.M.; Lamsfus, C.; Martins-Loução, M.A. How does glutamine synthetase activity determine plant tolerance to ammonium? *Planta* **2006**, *223*, 1068–1080. [[CrossRef](#)]
41. von Wirén, N.; Gazzarrini, S.; Gojon, A.; Frommer, W.B. The molecular physiology of ammonium uptake and retrieval. *Curr. Opin. Plant Biol.* **2000**, *3*, 254–261. [[CrossRef](#)]
42. Ladha, J.K.; Kirk, G.J.D.; Bennett, J.; Peng, S.; Reddy, C.K.; Reddy, P.M.; Singh, U. Opportunities for increased nitrogen-use efficiency from improved lowland rice germplasm. *Field Crop Res.* **1998**, *56*, 41–71. [[CrossRef](#)]
43. Iqbal, A.; Qiang, D.; Alamzeb, M.; Xiangru, W.; Huiping, G.; Hengheng, Z.; Nianchang, P.; Xiling, Z.; Meizhen, S. Untangling the molecular mechanisms and functions of nitrate to improve nitrogen use efficiency. *J. Sci. Food Agric.* **2020**, *100*, 904–914. [[CrossRef](#)]
44. Hawkesford, M.J. Genetic variation in traits for nitrogen use efficiency in wheat. *J. Exp. Bot.* **2017**, *68*, 2627–2632. [[CrossRef](#)]
45. Pujarula, V.; Pusuluri, M.; Bollam, S.; Das, R.R.; Ratnala, R.; Adapala, G.; Thuraga, V.; Rathore, A.; Srivastava, R.K.; Gupta, R. Genetic Variation for Nitrogen Use Efficiency Traits in Global Diversity Panel and Parents of Mapping Populations in Pearl Millet. *Front. Plant Sci.* **2021**, *12*, 625915. [[CrossRef](#)]
46. Poorter, H.; Lambers, H. Is interspecific variation in relative growth rate positively correlated with biomass allocation to the leaves? *Am. Nat.* **1991**, *138*, 1264–1268. [[CrossRef](#)]
47. Atkin, O.K.; Botman, B.; Lambers, H. The causes of inherently slow growth in alpine plants: An analysis based on the underlying carbon economies of alpine and lowland *Poa* species. *Funct. Ecol.* **1996**, *10*, 698–707. [[CrossRef](#)]
48. Mata, C.; van Vemde, N.; Clarkson, D.T.; Martins-Loucao, M.A.; Lambers, H. Influx, efflux and net uptake of nitrate in *Quercus suber* seedlings. *Plant Soil* **2000**, *221*, 25–32. [[CrossRef](#)]
49. Cruz, C.; Bio, A.M.F.; Jullioti, A.; Tavares, A.; Dias, T.; Martins-Loução, M.A. Heterogeneity of soil surface ammonium concentration and other characteristics, related to plant specific variability in a Mediterranean-type ecosystem. *Environ. Pollut.* **2008**, *154*, 414–423. [[CrossRef](#)] [[PubMed](#)]
50. Dias, T.; Martins-Loução, M.A.; Sheppard, L.; Cruz, C. The strength of the biotic compartment in retaining nitrogen additions prevents nitrogen losses from a Mediterranean maquis. *Biogeosciences* **2012**, *9*, 193–201. [[CrossRef](#)]

51. Dias, T.; Malveiro, S.; Martins-Loução, M.; Shepard, L.; Cruz, C. Linking N-driven biodiversity changes with soil N availability in a Mediterranean ecosystem. *Plant Soil* **2011**, *341*, 125–136. [\[CrossRef\]](#)
52. Dias, T.; Clemente, A.; Martins-Loução, M.; Sheppard, L.; Bobbink, R.; Cruz, C. Ammonium as a Driving Force of Plant Diversity and Ecosystem Functioning: Observations Based on 5 Years' Manipulation of N Dose and Form in a Mediterranean Ecosystem. *PLoS ONE* **2014**, *9*, e92517. [\[CrossRef\]](#)
53. Dias, T.; Crous, C.J.; Liberati, D.; Munzi, S.; Gouveia, C.; Ulm, F.; Afonso, A.C.; Ochoa-Hueso, R.; Manrique, E.; Sheppard, L.; et al. Alleviating Nitrogen limitation in Mediterranean maquis vegetation leads to ecological degradation. *Land Degrad. Dev.* **2017**, *28*, 2482–2492. [\[CrossRef\]](#)
54. Ulm, F.; Gouveia, C.; Dias, T.; Cruz, C. N fertilization in a Mediterranean ecosystem alters N and P turnover in soil, roots and the ectomycorrhizal community. *Soil Biol. Biochem.* **2017**, *113*, 60–70. [\[CrossRef\]](#)
55. Dias, T.; Crous, C.J.; Ochoa-Hueso, R.; Manrique, E.; Martins-Loução, M.A.; Cruz, C. Nitrogen inputs may improve soil biocrusts multifunctionality in dryland ecosystems. *Soil Biol. Biochem.* **2020**, *149*, 107947. [\[CrossRef\]](#)
56. Cruz, C.; Lips, S.H.; Martins-Loucao, M.A. Uptake regions of inorganic nitrogen in roots of carob seedlings. *Physiol. Plant* **1995**, *95*, 167–175. [\[CrossRef\]](#)
57. Cruz, C.; Green, J.J.; Watson, C.A.; Wilson, F.; Martins-Loução, M.A. Functional aspects of root architecture and mycorrhizal inoculation with respect to nutrient uptake capacity. *Mycorrhiza* **2004**, *14*, 177–184. [\[CrossRef\]](#) [\[PubMed\]](#)
58. Cruz, C.; Lips, S.H.; Martins-Loucao, M.A. Changes in the morphology of roots and leaves of carob seedlings induced by nitrogen source and atmospheric carbon dioxide. *Ann. Bot.* **1997**, *80*, 817–823. [\[CrossRef\]](#)
59. Domínguez-Valdivia, M.D.; Aparicio-Tejo, P.M.; Lamsfus, C.; Cruz, C.; Martins-Loução, M.A.; Moran, J.F. Nitrogen nutrition and antioxidant metabolism in ammonium-tolerant and -sensitive plants. *Physiol. Plant* **2008**, *132*, 359–369. [\[CrossRef\]](#) [\[PubMed\]](#)
60. Dias, T.; Neto, D.; Martins-Loucao, M.A.; Sheppard, L.; Cruz, C. Patterns of nitrate reductase activity vary according to the plant functional group in a Mediterranean maquis. *Plant Soil* **2011**, *347*, 363–376. [\[CrossRef\]](#)
61. Dias, T.; Martins-Loução, M.; Sheppard, L.; Cruz, C. Plant tolerance of ammonium varies between co-existing Mediterranean species. *Plant Soil* **2015**, *395*, 243–252. [\[CrossRef\]](#)
62. Marschner, H.; Kirkby, E.A.; Cakmak, I. Effect of mineral nutritional status on shoot-root partitioning of photoassimilates and cycling of mineral nutrients. *J. Exp. Bot.* **1996**, *47*, 1255–1263. [\[CrossRef\]](#)
63. Martins-Loução, M.A.; Cruz, C. The Role of Nitrogen Source in Carbon Balance. In *Modes of Nitrogen Nutrition in Higher Plants*; Srivastava, H.S., Singh, R.P., Eds.; Oxford & IBH Publishing Co. Pvt: New Delhi, India, 1999; pp. 231–282.
64. Wang, L.; Ruan, Y.-L. Shoot–root carbon allocation, sugar signalling and their coupling with nitrogen uptake and assimilation. *Funct. Plant Biol.* **2016**, *43*, 105–113. [\[CrossRef\]](#)
65. Buet, A.; Galatro, A.; Ramos-Artuso, F.; Simontacchi, M. Nitric oxide and plant mineral nutrition: Current knowledge. *J. Exp. Bot.* **2019**, *70*, 4461–4476. [\[CrossRef\]](#)
66. Forde, B. The role of long-distance signalling in plant responses to nitrate and other nutrients. *J. Exp. Bot.* **2002**, *53*, 39–43.
67. Kang, A.; Zhang, N.; Xun, W.; Dong, X.; Xiao, M.; Liu, Z.; Xu, Z.; Feng, H.; Zou, J.; Shen, Q.; et al. Nitrogen fertilization modulates beneficial rhizosphere interactions through signaling effect of nitric oxide. *Plant Physiol.* **2022**, *188*, 1129–1140. [\[CrossRef\]](#)
68. Kronzucker, H.J.; Glass, A.D.M.; Siddiqi, M.Y. Nitrate induction in spruce: An approach using compartmental analysis. *Planta* **1995**, *196*, 683–690. [\[CrossRef\]](#)
69. Lips, S.H.; Ben Zioni, A.; Vaadia, Y. K⁺ recirculation in plants and its importance for adequate nitrate reduction. In *Recent Advances in Plant Nutrition*; Samish, R.M., Gordon, Breach, Eds.; Gordon and Breach Science Publishers Inc.: New York, NY, USA, 1970; Volume 1, pp. 207–215.
70. Ben-Zioni, A.; Vaadia, Y.; Lips, S.H. Nitrate uptake by roots as regulated by nitrate reduction products of the shoot. *Physiol. Plant* **1971**, *24*, 288–290. [\[CrossRef\]](#)
71. Lips, S.H.; Soares, M.I.M.; Kaiser, J.J.; Lewis, O.A.M. K⁺ modulation of nitrogen uptake and assimilation in plants. In *Inorganic Nitrogen Metabolism*; Syrett, P.J., Ulrich, W.R., Aparicio, P.J., Castillo, F., Eds.; Springer: Berlin, Germany, 1987; pp. 233–239.
72. Martins-Loução, M.A.; Cruz, C.; Correia, P.M. New approaches to enhanced ammonium assimilation in plants. In *Nitrogen in a Sustainable Ecosystem: From the Cell to the Plant*; Martins-Loução, M., Lips, S.H., Eds.; Backhuys: Leiden, The Netherlands, 2000; pp. 349–360.
73. Fonseca, M.B.; Dias, T.; Carolino, M.M.; França, M.G.C.; Cruz, C. Belowground microbes mitigate plant-plant competition. *Plant Sci.* **2017**, *262*, 175–181. [\[CrossRef\]](#) [\[PubMed\]](#)
74. Esteban, R.; Ariz, I.; Cruz, C.; Moran, J.F. Review: Mechanisms of ammonium toxicity and the quest for tolerance. *Plant Sci.* **2016**, *248*, 92–101. [\[CrossRef\]](#)
75. Cruz, C.; Domínguez-Valdivia, M.D.; Aparicio-Tejo, P.M.; Lamsfus, C.; Bio, A.; Martins-Loução, M.A.; Moran, J.F. Intra-specific variation in pea responses to ammonium nutrition leads to different degrees of tolerance. *Environ. Exp. Bot.* **2011**, *70*, 233–243. [\[CrossRef\]](#)
76. Ariz, I.; Cruz, C.; Moran, J.F.; González-Moro, M.B.; García-Olaverri, C.; González-Murua, C.; Martins-Loução, M.A.; Aparicio-Tejo, P.M. Depletion of the heaviest stable N isotope is associated with NH₄⁺/NH₃ toxicity in NH₄⁺-fed plants. *BMC Plant Biol.* **2011**, *11*, 83. [\[CrossRef\]](#)

77. Ariz, I.; Boeckstaens, M.; Gouveia, C.; Martins Ana, P.; Sanz-Luque, E.; Fernández, E.; Soveral, G.; von Wirén, N.; Marini Anna, M.; Aparicio-Tejo Pedro, M.; et al. Nitrogen isotope signature evidences ammonium deprotonation as a common transport mechanism for the AMT-Mep-Rh protein superfamily. *Sci. Adv.* **2018**, *4*, eaar3599. [\[CrossRef\]](#)
78. Antonenko, Y.N.; Pohl, P.; Denisov, G.A. Permeation of ammonia across bilayer lipid membranes studied by ammonium ion selective microelectrodes. *Biophys. J.* **1997**, *72*, 2187–2195. [\[CrossRef\]](#)
79. Ninnemann, O.; Jauniaux, J.; Frommer, W. Identification of a high affinity NH₄⁺ transporter from plants. *EMBO J.* **1994**, *13*, 3464–3471. [\[CrossRef\]](#)
80. Martins-Loução, M.A.; Lips, S. *Nitrogen from the Cell to the Plant: Recent Progress and Perspectives*; Martins-Loução, M.A., Lips, S., Eds.; Backhuys: Leiden, The Netherlands, 2000; pp. 3–6.
81. Ladha, J.K.; Pathak, H.; Krupnik, T.J.; Six, J.; van Kessel, C. Efficiency of Fertilizer Nitrogen in Cereal Production: Retrospects and Prospects. In *Advances in Agronomy*; Academic Press: Cambridge, MA, USA, 2005; Volume 87, pp. 85–156.
82. Baron, J.S.; Barber, M.; Adams, M.; Agboola, J.I.; Allen, E.B.; Bealey, W.J.; Bobbink, R.; Bobrovsky, M.V.; Bowman, W.D.; Branquinho, C. The effects of atmospheric nitrogen deposition on terrestrial and freshwater biodiversity. In *Nitrogen Deposition, Critical Loads and Biodiversity*; Springer: Berlin/Heidelberg, Germany, 2014; pp. 465–480.
83. Bijlsma, R.; Lambers, H.; Kooijman, S. A dynamic whole-plant model of integrated metabolism of nitrogen and carbon. 1. Comparative ecological implications of ammonium-nitrate interactions. *Plant Soil* **2000**, *220*, 49–69. [\[CrossRef\]](#)
84. Cruz, C.; Dias, T.; Matos, S.; Tavares, A.; Neto, D.; Martins-Loucao, M.A. Nitrogen availability and plant cover: The importance of nitrogen pools. In *Ecosystems and Sustainable Development Iv, Vols 1 and 2*; Tiezzi, E., Brebbia, C.A., Uso, J.L., Eds.; Advances in Ecological Sciences; WIT Press: Boston, MA, USA, 2003; Volume 18–19, pp. 123–135.
85. Cruz, C.; Fegghi, Z.; Martins-Loução, M.; Varma, A. Plant Nitrogen Use Efficiency May Be Improved Through Symbiosis with *Piriformospora indica*. In *Piriformospora Indica*; Varma, A., Kost, G., Oelmüller, R., Eds.; Soil Biology; Springer: Berlin/Heidelberg, Germany, 2013; Volume 33, pp. 285–293.
86. Dias, T.; Stürmer, S.; Chaves, S.; Fidalgo, C.; Tenreiro, R.; Correia, P.; Carvalho, L.; Martins-Loução, M.; Shepard, L.; Cruz, C. Species of Arbuscular Mycorrhizal Fungal Spores can Indicate Increased Nitrogen Availability in Mediterranean-type Ecosystems. In *Nitrogen Deposition, Critical Loads and Biodiversity*; Sutton, M.A., Mason, K.E., Sheppard, L.J., Sverdrup, H., Haeuber, R., Hicks, W.K., Eds.; Springer: Berlin, Germany; Dordrecht, The Netherlands, 2014; pp. 259–266.
87. Cruz, C.; Dias, T.; Pinho, P.; Branquinho, C.; Máguas, C.; Pinto, M.J.; Martins-Loução, M.A. Policies for plant diversity conservation on a global scale: A SWOT analysis. *Kew Bull.* **2010**, *65*, 525–528. [\[CrossRef\]](#)
88. FAO. *Climate Change and Food Security: Risks and Responses*; FAO: Rome, Italy, 2015; p. 86.
89. Lawrencina, D.; Wong, S.K.; Low, D.Y.; Goh, B.H.; Goh, J.K.; Ruktanonchai, U.R.; Soottitantawat, A.; Lee, L.H.; Tang, S.Y. Controlled Release Fertilizers: A Review on Coating Materials and Mechanism of Release. *Plants* **2021**, *10*, 238. [\[CrossRef\]](#) [\[PubMed\]](#)
90. Besharat, S.; Barão, L.; Cruz, C. New strategies to overcome water limitation in cultivated maize: Results from sub-surface irrigation and silicon fertilization. *J. Environ. Manag.* **2020**, *263*, 110398. [\[CrossRef\]](#) [\[PubMed\]](#)
91. Antonkiewicz, J.; Kołodziej, B.; Bielińska, E.J. The use of reed canary grass and giant miscanthus in the phytoremediation of municipal sewage sludge. *Environ. Sci. Pollut. Res.* **2016**, *23*, 9505–9517. [\[CrossRef\]](#)
92. Ulm, F.; Avelar, D.; Hobson, P.; Penha-Lopes, G.; Dias, T.; Máguas, C.; Cruz, C. Sustainable urban agriculture using compost and an open-pollinated maize variety. *J. Clean. Prod.* **2019**, *212*, 622–629. [\[CrossRef\]](#)
93. Gondek, K.; Mierzwa-Hersztek, M. Effect of low-temperature biochar derived from pig manure and poultry litter on mobile and organic matter-bound forms of Cu, Cd, Pb and Zn in sandy soil. *Soil Use Manag.* **2016**, *32*, 357–367. [\[CrossRef\]](#)
94. Bhardwaj, D.; Ansari, M.W.; Sahoo, R.K.; Tuteja, N. Biofertilizers function as key player in sustainable agriculture by improving soil fertility, plant tolerance and crop productivity. *Microb. Cell Factories* **2014**, *13*, 66. [\[CrossRef\]](#)
95. Jaber, L.R.; Enkerli, J. Effect of seed treatment duration on growth and colonization of *Vicia faba* by endophytic *Beauveria bassiana* and *Metarhizium brunneum*. *Biol. Control* **2016**, *103*, 187–195. [\[CrossRef\]](#)
96. Maçik, M.; Gryta, A.; Fraç, M. Chapter Two-Biofertilizers in agriculture: An overview on concepts, strategies and effects on soil microorganisms. In *Advances in Agronomy*; Sparks, D.L., Ed.; Academic Press: Cambridge, MA, USA, 2020; Volume 162, pp. 31–87.
97. Hawkes, C.V.; Bull, J.J.; Lau, J.A. Symbiosis and stress: How plant microbiomes affect host evolution. *Philos. Trans. R. Soc. B Biol. Sci.* **2020**, *375*, 20190590. [\[CrossRef\]](#)
98. Munzi, S.; Cruz, C.; Corrêa, A. When the exception becomes the rule: An integrative approach to symbiosis. *Sci. Total Environ.* **2019**, *672*, 855–861. [\[CrossRef\]](#)
99. Ramos, A.C.; Melo, J.; de Souza, S.B.; Bertolazi, A.A.; Silva, R.A.; Rodrigues, W.P.; Campostrini, E.; Olivares, F.L.; Eutrópio, F.J.; Cruz, C.; et al. Inoculation with the endophytic bacterium *Herbaspirillum seropedicae* promotes growth, nutrient uptake and photosynthetic efficiency in rice. *Planta* **2020**, *252*, 87. [\[CrossRef\]](#) [\[PubMed\]](#)
100. Antoun, H.; Prévost, D. Ecology of Plant Growth Promoting Rhizobacteria. In *PGPR: Biocontrol and Biofertilization*; Siddiqui, Z.A., Ed.; Springer: Dordrecht, The Netherlands, 2006; pp. 1–38.
101. Trivedi, P.; Batista, B.D.; Bazany, K.E.; Singh, B.K. Plant-microbiome interactions under a changing world: Responses, consequences, and perspective. *New Phytol.* **2022**, *34*, 1951–1959. [\[CrossRef\]](#) [\[PubMed\]](#)
102. Dias, T.; Correia, P.; Carvalho, L.; Melo, J.; de Varennes, A.; Cruz, C. Arbuscular mycorrhizal fungal species differ in their capacity to overrule the soil's legacy from maize monocropping. *Appl. Soil Ecol.* **2018**, *125*, 177–183. [\[CrossRef\]](#)

103. Basílio, F.; Dias, T.; Santana, M.; Melo, J.; Carvalho, L.; Correia, P.; Cruz, C. Multiple modes of action are needed to unlock soil phosphorus fractions unavailable for plants: The example of bacteria- and fungi-based biofertilizers. *Appl. Soil Ecol.* 2022, *under review*.
104. Fravel, D.R. Commercialization and Implementation of Biocontrol. *Annu. Rev. Phytopathol.* **2005**, *43*, 337–359. [[CrossRef](#)]
105. Ramakuwela, T.; Hatting, J.; Bock, C.; Vega, F.E.; Wells, L.; Mbata, G.N.; Shapiro-Ilan, D. Establishment of *Beauveria bassiana* as a fungal endophyte in pecan (*Carya illinoensis*) seedlings and its virulence against pecan insect pests. *Biol. Control* **2020**, *140*, 104102. [[CrossRef](#)]
106. Melo, J.; Carolino, M.; Carvalho, L.; Correia, P.; Tenreiro, R.; Chaves, S.; Meleiro, A.I.; de Souza, S.B.; Dias, T.; Cruz, C.; et al. Crop management as a driving force of plant growth promoting rhizobacteria physiology. *SpringerPlus* **2016**, *5*, 1574. [[CrossRef](#)]
107. Santana, M.M.; Gonzalez, J.M.; Cruz, C. Nitric Oxide Accumulation: The Evolutionary Trigger for Phytopathogenesis. *Front. Microbiol.* **2017**, *8*, 1947. [[CrossRef](#)]
108. Canton, G.C.; Bertolazi, A.A.; Cogo, A.J.; Eutrópico, F.J.; Melo, J.; de Souza, S.B.; AKrohling, C.; Campostrini, E.; da Silva, A.G.; Façanha, A.R.; et al. Biochemical and ecophysiological responses to manganese stress by ectomycorrhizal fungus *Pisolithus tinctorius* and in association with *Eucalyptus grandis*. *Mycorrhiza* **2016**, *26*, 475–487. [[CrossRef](#)]
109. Lefort, M.-C.; McKinnon, A.C.; Nelson, T.L.; Glare, T.R. Natural occurrence of the entomopathogenic fungi *Beauveria bassiana* as a vertically transmitted endophyte of *Pinus radiata* and its effect on above- and below-ground insect pests. *N. Z. Plant Prot.* **2016**, *69*, 68–77. [[CrossRef](#)]
110. Destoumieux-Garzón, D.; Mavingui, P.; Boetsch, G.; Boissier, J.; Darriet, F.; Duboz, P.; Fritsch, C.; Giraudoux, P.; Le Roux, F.; Morand, S.; et al. The One Health Concept: 10 Years Old and a Long Road Ahead. *Front. Vet. Sci.* **2018**, *5*, 14. [[CrossRef](#)]
111. Savary, S.; Willocquet, L.; Pethybridge, S.J.; Esker, P.; McRoberts, N.; Nelson, A. The global burden of pathogens and pests on major food crops. *Nat. Ecol. Evol.* **2019**, *3*, 430–439. [[CrossRef](#)] [[PubMed](#)]
112. Guerra, C.A.-O.; Delgado-Baquerizo, M.; Duarte, E.; Marigliano, O.; Görgen, C.; Maestre, F.T.; Eisenhauer, N.A.-O. Global projections of the soil microbiome in the Anthropocene. *Glob. Ecol. Biogeogr.* **2021**, *30*, 987–999. [[CrossRef](#)] [[PubMed](#)]
113. Fox, A.; Widmer, F.; Barreiro, A.; Jongen, M.; Musyoki, M.; Vieira, A.; Zimmermann, J.; Cruz, C.; Dimitrova-Mårtensson, L.M.; Rasche, F.; et al. Small-scale agricultural grassland management can affect soil fungal community structure as much as continental scale geographic patterns. *FEMS Microbiol. Ecol.* **2021**, *97*, fiab148. [[CrossRef](#)] [[PubMed](#)]
114. Barreiro, A.; Fox, A.; Jongen, M.; Melo, J.; Musyoki, M.; Vieira, A.; Zimmermann, J.; Carlsson, G.; Cruz, C.; Lüscher, A.; et al. Soil bacteria respond to regional edapho-climatic conditions while soil fungi respond to management intensity in grasslands along a European transect. *Appl. Soil Ecol.* **2022**, *170*, 104264. [[CrossRef](#)]
115. Hirota, M.; Oliveira, R. Crossing thresholds on the way to ecosystem shifts. *Science* **2020**, *367*, 739–740. [[CrossRef](#)] [[PubMed](#)]
116. Galloway, J.N.; Winiwarter, W.; Leip, A.; Leach, A.M.; Bleeker, A.; Erisman, J.W. Nitrogen footprints: Past, present and future. *Environ. Res. Lett.* **2014**, *9*, 115003. [[CrossRef](#)]
117. Erisman, J.W.; Sutton, M.A.; Galloway, J.; Klimont, Z.; Winiwarter, W. How a century of ammonia synthesis changed the world. *Nat. Geosci.* **2008**, *1*, 636–639. [[CrossRef](#)]
118. Ochoa-Hueso, R.; Munzi, S.; Alonso, R.; Arróniz-Crespo, M.; Avila, A.; Bermejo, V.; Bobbink, R.; Branquinho, C.; Concostrina-Zubiri, L.; Cruz, C.; et al. Ecological impacts of atmospheric pollution and interactions with climate change in terrestrial ecosystems of the Mediterranean Basin: Current research and future directions. *Environ. Pollut.* **2017**, *227*, 194–206. [[CrossRef](#)]
119. Rockström, J.; Steffen, W.; Noone, K.; Persson, Å.; Chapin, F.S.I.; Lambin, E.; Lenton, T.M.; Scheffer, M.; Folke, C.; Schellnhuber, H.J.; et al. Planetary Boundaries: Exploring the Safe Operating Space for Humanity. *Ecol. Soc.* **2009**, *14*, 32–63. [[CrossRef](#)]
120. Steffen, W.; Richardson, K.; Rockström, J.; Cornell, S.E.; Fetzer, I.; Bennett, E.M.; Biggs, R.; Carpenter, S.R.; de Vries, W.; de Wit, C.A.; et al. Planetary boundaries: Guiding human development on a changing planet. *Science* **2015**, *347*, 1259855. [[CrossRef](#)]
121. Cruz, C.; Martins-Loução, M.A.; Varma, A. The influence of plant co-culture of tomato plants with *Piriformospora indica* on biomass accumulation and stress tolerance. *Acta Hort.* **2010**, *868*, 123–127. [[CrossRef](#)]
122. Hessini, K.; Kronzucker, H.J.; Abdely, C.; Cruz, C. Drought stress obliterates the preference for ammonium as an N source in the C4 plant *Spartina alterniflora*. *J. Plant Physiol.* **2017**, *213*, 98–107. [[CrossRef](#)] [[PubMed](#)]
123. Mahmoudi, N.; Caeiro, M.F.; Mahdhi, M.; Tenreiro, R.; Ulm, F.; Mars, M.; Cruz, C.; Dias, T. Arbuscular mycorrhizal traits are good indicators of soil multifunctionality in drylands. *Geoderma* **2021**, *397*, 115099. [[CrossRef](#)]