Effectiveness and Fate Nitrogen in Plants Physiology, Molecular Approaches for Maximization and Improving Nitrogen Use Efficiency. A Review

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ABSTRACT
Nitrogen is the limiting nutrient element after carbon, hydrogen, and oxygen for photosynthetic process, phyto-hormonal, proteomic changes, and growth development of plants to complete its lifecycle. However, excessive and inefficient use of N fertilizer results in enhanced crop production costs and atmospheric pollution. Atmospheric nitrogen (71%) in the molecular form is not available for the plants. For world’s sustainable food production and atmospheric benefits, there is an urgent need to upgrade nitrogen use efficiency in agricultural farming systems. Nitrogen use efficiency is the product of nitrogen uptake efficiency and nitrogen utilization efficiency; varies from 30.2 to 53.2%. Nitrogen losses are too high, due to excess amount, low plant population, poor application methods, etc., which can go up to 70% of total available nitrogen. Adopting improved agronomic approaches, losses can be minimized up to 15–30%, such as optimal dosage of nitrogen, application of N by using canopy sensors, maintaining plant population, drip fertigation and legume based intercropping. Recent developments and future prospects of improving nitrogen use efficiency (NUE) in crops using various complementary approaches. Including conventional breeding and molecular genetics, in addition to alternative farming techniques based on no-till continuous cover cropping cultures and/or organic nitrogen (N) nutrition. Whatever the mode of N fertilization, an increased knowledge of the mechanisms controlling plant N economy is essential for improving (NUE) and for reducing excessive input of fertilizers, while maintaining an acceptable yield and sufficient profit margin for the farmers. Using plants grown under agronomic conditions, with different tillage conditions, in pure or associated cultures, at low and high N mineral fertilizer input, or using organic fertilization, it is now possible to develop further whole plant agronomic and physiological studies. These can be combined with gene, protein and metabolite profiling to build up a comprehensive picture depicting the different steps of N uptake, assimilation and recycling to produce either biomass in vegetative organs or proteins in storage organs. Providing a critical overview as to how our understanding of the agro-eco physiological and molecular controls of N assimilation in crops, under varying environmental conditions, has been improved. Long-term sustainability may require a gradual transition from synthetic N inputs to legume-based crop rotation, including continuous cover cropping systems, where these may be possible in certain areas of the world, depending on climatic conditions. Current knowledge and prospects for future agronomic development and application for breeding crops adapted to lower mineral fertilizer input and to alternative farming techniques are explored, whilst taking into account the constraints of both the current world economic situation and the environment. Nitrate reductase, nitrite reductase, glutamine synthetase, glutamine oxoglutarate aminotransferase, and asparagine synthetase enzyme have a great role in nitrogen metabolism. However, further studies on carbon–nitrogen metabolism and molecular changes at atomic levels are required by using “whole genome sequencing technology” to improve nitrogen use efficiency (NUE).

Keywords: Nitrate Functions, Nitrogen Fertilizer Replacement Value, Nitrogen Use Efficiency, Rhizosphere Management and Processes, Root Uptake and Root Development.
1. Introduction

Application of mineral fertilizers particularly nitrogen (N), is the main method for maintaining or restoring soil nutrients and maximization crop yields. Nitrogen used in commercial fertilizers is particularly soluble for easy uptake and assimilation by plants. Due to the simplicity of its storage and handling, nitrogen can easily be applied to plants when needed. Mineral fertilizers are the main source of nutrients applied to soils, even if the contribution of animal manure remains important, especially when there is densely populated livestock nearby. Nitrogen fertilizers used extensively to increase crop yield particularly after World War II. Using of synthetic nitrogen fertilizers has eliminated a major elemental constraint with respect to enriching the soil stock of organic C and N originally managed by organic manure amendments, leguminous cultures and fallow periods. Formation of ammonia and thus synthetic N fertilizers by the Haber–Bosch process was one of the most important inventions of the 20th century. Haber process, also called the Haber–Bosch is an artificial process, which is the main industrial procedure. Such process converts atmospheric (N₂) to (NH₃) by a reaction with (H₂) using a metal catalyst under high temperatures and pressures, ammonia had been difficult to produce on an industrial scale, Fig (1). An energy diagram can be created based on the enthalpy of the reaction of the individual steps. The energy diagram can be used to compare homogeneous and heterogeneous reactions: Due to the high of the dissociation of nitrogen, the homogeneous gas phase reaction is not realizable. The catalyst avoids this problem as the energy gain resulting from the binding of nitrogen atoms to the catalyst surface overcompensates for the necessary dissociation energy so that the reaction is finally exothermic. Nevertheless, the dissociative adsorption of nitrogen remains the rate-determining step: not because of the activation energy, but mainly because of the unfavorable of the rate constant. Although is endothermic, this energy can easily be applied by the reaction temperature (about 700 K).

![Energy diagram](image)

**Fig. 1:** Illustrates an energy diagram on the enthalpy of the reaction of the individual steps. Although the Haber process is mainly used to produce today, during it provided with a source of ammonia for the production of compensating for the ' trade blockade on, thus allowing the production of food for nearly half of the world population (Erisman et al., 2008; Galloway et al., 2008). Consequently, a dramatic escalation has occurred in global consumption of synthetic N, from 11.6 million tonnes (Tg) in 1961 to 104 Tg in 2006 (Hoang and Alauddin, 2010; Mulvaney et al., 2009).

1.1. Nitrogen use efficiency (NUE)

Over 40 years, the amount of mineral N fertilizers applied to agricultural crops increased by 7.4 fold, whereas the overall yield increase was only 2.4 fold (Tilman et al., 2002). This means that N use efficiency, (NUE) which may be defined as the yield obtained per unit of available N in the soil (supplied by the soil + N fertilizer) has declined sharply. This obviously implies that NUE is higher at reduced levels of crop production when the use of N fertilization is much lower. Nitrogen use efficiency (NUE) is the product of absorption efficiency (amount of absorbed N/quantity of available N) and the utilization efficiency (yield/absorbed N). For a large number of crops, there is a genetic variability for
both N absorption efficiency and for N utilization efficiency Hirel et al., (2007), Malcolm and Andrew (2020) reported that plant growth and yield require nitrogen, and furthermore are dependent upon multiple physiological processes Fig. (2).

![Processes contributing to and determining NUE in wheat. Measures of nitrogen use efficiency shown in grey boxes; primary traits in green boxes; physiological process in yellow boxes. After Hawkesford, (2011).](image)

Such perspective, is useful to use the productivity index and the component traits of this index Barraclough et al., (2010) to consider impacts on G x E x M. NUE may be considered the top level trait and for wheat is the yield of grain produced per unit of N available to the crop; it is expressed as kg yield per kg of available N; it is also the product of the two second level traits, N uptake efficiency (NUpE) and N utilization efficiency (NUTE). NUpE, or sometimes biomass NUpE (BioNUpE) is the ratio of N taken up by the crop compared to what is available from the soil and applied fertilizer, and is expressed as kg N (in the crop) per kg N (available). Nitrogen in the roots is ignored, but the N in the aerial biomass for wheat is that in the grain and straw combined. NUpE or grain NUpE is the amount of grain produced per unit of N taken up, and is kg (grain) per kg N. NUE is mathematically the product of NUpE x NUTE. An interaction between N management and genetic variation of these second level traits was indicated in a two-site and four-year trial of 16 wheat genotypes, in which genetic variability in NUTE rather than NUpE was reported to be of greater significance at low N inputs (Gaju et al., 2011).

Other useful measures of efficiency of N use include the nitrogen harvest index (NHI), which is the fraction of total N taken up by the crop that is partitioned to the grain, and is a refinement of harvest index (HI) which describes portioning of dry matter alone. However, NHI is independent of yield and uptake efficiency, and a low yielding crop may have a high NHI, but leave substantial unrecovered N in the soil. High grain protein concentration (GPC) is required for end uses such as flour for bread making; however, it is difficult to increase GPC without decreasing yield due to the negative relationship between the two, as high yield usually reflects high carbohydrate content that in turn dilutes N concentration. The desired trait, to increase GPC without reducing yield, can be defined as grain protein deviation (GPD), the deviation from the negative linear relationship between yield and N concentration, and reflects an ability to acquire more N in the grain for a given yield (Bogard et al., 2010; Mosleth et al., 2015). There is some uncertainty of the physiological basis of GPD but it may be related to phenology and post anthesis N uptake (Bogard et al., 2010; Bogard et al., 2011). Both GPC and GPD may be overcome agronomically with higher N-inputs, particularly later in the season when yield has been largely determined, however this inevitably leads to low NUE. Future research may develop techniques for making bread from low protein wheat’s, which would be a major breakthrough.
for increasing NUE whilst maintaining end-use suitability, although the reduced protein content may be detrimental for a healthy diet. Each of these (NUE) parameters are complex traits involving many underpinning physiological and biochemical reactions and pathways.

Genetic studies indicate the multigenic and heritable nature of the major traits and the underpinning processes. However, unravelling the traits and breeding for improved NUE is complex. Genetic variation in many traits is apparent in modern germplasm and largely in historic material, landraces and wild relatives, and could be the basis for germplasm selection. Yield is commonly the major commercial target for selection, usually at a constant N input, hence selection for NUE and NUtE is consequentially also selected for. Differentiation between NUpE and NUtE is not made consciously, however higher yielding; high protein genotypes (and hence high GPD types) will have high NUpE also. Efforts have been made to consider management protocols (M) by including selection at different N-inputs Ortiz-Monasterio et al., (1997); however, a common assumption is that ranking of variety performance is independent of N-availability. Growers will also be looking to maximize profitability, which may be different from maximizing NUE, particular if they are aiming for a quality market. Arguably, the greatest genetic improvement (G) has been the introduction of short straw (dwarf) varieties, and therefore, HI and NHI have increased. The immediate effect is that biomass allocation to the grain is favored, maximizing grain yield at the expense of straw biomass. Another direct consequence of utilizing dwarf varieties is that the reduced stature facilitates resistance to lodging, a problem particularly encountered at high levels of N fertilization, particularly when combined with conditions of high wind and rainfall. The ability to exploit higher N-rates has led to a management strategy (M) of increased N-inputs, which promotes greater yields but at lower efficiency. Higher N fertilization can increase disease and weed pressure, requiring additional agrochemical inputs. Moreover, the occurrence of interactions between the genotype and the level of N led to the conclusion that the best performing crop varieties at high N fertilization input are not necessarily the best ones when the supply of N is lower (Gallais, et al., 2005). This is mainly because breeding for most crops has been conducted over the last 50 years in the presence of high mineral fertilization inputs, thus missing the opportunity to exploit genetic differences under a low level of mineral or organic N fertilization conditions (Ceccarelli, 1995). In most intensive agricultural production systems, over 50% and up to 75% of the N applied to the field is not used by the plant and is lost by leaching into the soil (Raun, and Johnson, 1999; Asghari, and Cavagnaro, 2011).

The global challenge of meeting increased food demand and protecting environmental quality will be won or lost in cropping systems that produce maize, rice, and wheat. Achieving synchrony between nitrogen supply and crop demand without excess or deficiency is the key to optimizing tradeoffs amongst yield, profit, and environmental protection in both large-scale systems in developed countries and small-scale systems in developing countries. Setting the research agenda and developing effective policies to meet this challenge requires quantitative understanding of current levels of nitrogen -use efficiency and losses in these systems, the biophysical controls on these factors, and the economic returns from adoption of improved management practices. Although advances in basic biology, ecology, and biogeochemistry can provide answers, the magnitude of the scientific challenge should not be underestimated because it becomes increasingly difficult to control the fate of nitrogen in cropping systems that must sustain yield increases on the world’s limited supply of productive farmland. Improving nitrogen use efficiency and profit, the goal of reducing Nr while sustaining adequate rates of gain in cereal production to meet expected food demand will require increases in both NUE and REN, which in turn will require innovative crop- and soil-management practices. The economic ‘benefit to cost ratio’ has a large influence on farmer adoption of new technologies. While some management practices might increase NUE by reducing nitrogen losses or increasing the proportion of nitrogen inputs that are retained in soil organic and inorganic nitrogen pools, adoption by farmers is not likely without the promise of adequate economic return. Hence, management options for improving NUE of cereal production systems must also consider REN (N-fertilizer recovery efficiency) and PEN (N-fertilizer uptake efficiency) because these parameters determine the economic impact on grain yield in relation to applied N inputs and crop N accumulation. Recent literature on improving NUE in crop-production systems has emphasized the need for greater synchrony between crop nitrogen demand and supply from all sources throughout the growing season (Cassman, et al., 1993; Appel 1994; Robertson, 1997). The approach recognizes the need to efficiently utilize both indigenous and applied N and is
justified by the fact that losses mechanisms from all N-loss increase in proportion to the amount of available N at any given time in soil profile.

Fig. 3: The main species and interconversions in the nitrogen cycle. N2 is reduced to NH3 through biological or industrial N2 fixation (red arrows), providing N-containing fertilizers for plants (green arrows). However, excess NH3 is processed by microorganisms in the soil by nitrification (pale-blue arrows) and denitrification (blue arrows), which transform the nitrogen-containing fertilizer into environmental pollutants.

Uptake efficiency from a single N-fertilizer application typically decreases in proportion to the amount of N-fertilizer applied (Beman, et al., 2005). The same principle applies to available N derived from organic N sources such as legume green manures, cover crops and animal manures. Several researches, Stivers, and Shennan (1991), Kessavalou, and Walters, (1999), reported that nitrate leaching from manures could be equal or greater than nitrogen losses from inorganic N fertilizer. Particularly when the available N supply from either source exceeds crop demand by similar amounts for comparable periods. Increasing yield production can also contribute to greater NUE from both indigenous and applied N sources. Fast growing, plants have root systems that more effectively exploit available soil resources (Herridge et al., 2008). Crop health, insect and weed management, moisture and temperature regimes, supplies of nutrients other than nitrogen, and use of the best adapted cultivar or hybrid all contribute to more efficient uptake of available nitrogen and greater conversion of plant nitrogen to grain yield. Assuming a well-managed crop, Recovery efficiency of applied N fertilizer (REN) and profit from applied nitrogen are therefore optimized with the least possible nitrogen losses when the plant-available nitrogen pool is maintained at the minimum size required to meet crop-nitrogen requirements at each stage of growth. Fig (3).

1.2. Plant–microbe nutritional interactions

Considering the environmental damage associated with current fertilization practices, a current research priority is to optimize plant–microbe nutritional interactions for more sustainable agricultural systems. However, the specific mechanisms governing the assembly of the plant microbiome and its modulation according to plant nutritional status are extremely complex and difficult to predict. Richard Jacoby, et al., (2017), reported that natural environment, plants are part of a rich ecosystem including numerous and diverse microorganisms in the soil. It has been recognized that some of these microbes, such as mycorrhizal fungi or nitrogen fixing symbiotic bacteria, play an important part in plant performance through improving mineral nutrition. However, the full range of microbes associated with
plants and their potential to replace synthetic agricultural inputs has only recently started to be uncovered.

In the last few years, a great progress has been made in the knowledge on composition of rhizospheric micro biomes and their dynamics. There is clear evidence that plants shape microbiome structures, most probably by root exudates, and that bacteria have developed various adaptations to thrive in the rhizospheric niche. The mechanisms of these interactions and the processes driving the alterations in micro biomes are Fig. (4).

Fig. 4: Illustrates the interactions between plants, microbiota, and soil. Both plants and Microorganisms obtain their nutrients from soil and change soil properties by organic litter deposition and metabolic activities, respectively. Microorganisms have a range of direct effects on plants through, e.g., manipulation of hormone signaling and protection against pathogens. Plants communicate with the microorganisms through metabolites exuded by the roots. The major knowledge gaps for understanding the mechanisms of plant–microbe Interactions in the rhizosphere are shown in bold.

Some microorganisms are able to improve soil fertility by metabolizing the N. It is however a lengthy process which involves a major risk because mineral N, especially nitrate (NO3–) and urea CO(NH2)2 are very soluble and can run off into the surface water or flow into the groundwater. Water contaminated by nitrate is not potable and at high concentrations can be a serious risk for human health (Al-Redhaiman, 2000; Umar, and Iqbal, 2007). Moreover, the water industry must bear additional costs to remove nitrates from groundwater sources (Harris, and Skinner 1992; Cameron, and Schipper, 2010). The detrimental impacts of nitrate loss from the soil have toxicological implications for animals and humans Camarguo, and Alonso, (2006), environment leading to the eutrophication of freshwater London, (2005), and marine ecosystems (Beman et al., 2005). This phenomenon is manifested by a proliferation of green algae, reduced infiltration of light, oxygen depletion in surface water, disappearance of benthic invertebrates and the production of toxins harmful to fish, livestock and humans.

1.3. Nitrous oxide emissions from soils and their risk

Soils are also at risk from eutrophication, as excessive amounts of nutrients can cause oxygen depletion in the soil and thus prevent the proper functioning of natural microorganisms. This, in turn, affects soil fertility. Moreover, Mulvaney, et al., (2009) reported synthetic N fertilizers could promote microbial carbon utilization depleting both soil and sub-soil organic N content. Eutrophic soils are the source for the emission of N2O (nitrous oxide), which can react with the stratospheric ozone Sutton, et al., (2011) thus increasing the greenhouse effect and the emission of toxic ammonia (NH3) into the atmosphere that can contribute to acidification (Ramos 1996; David et al., 2009). Nitrous oxide (N2O) is a long-lived trace gas in the atmosphere, with an average mixing ratio of 322.5 ppbv in the year 2009. Atmospheric N2O concentrations have increased by 19 per cent since pre-industrial times, with an average increase of 0.77-ppbv yr–1 for the period 2000–2009 WMO (2010). There are two reasons why the so-called laughing gas has been attracting a considerable interest of scientists. First, it is a potent greenhouse gas (GHG), with a 100-year global warming potential 298 times that of carbon
dioxide (CO$_2$; molecule for molecule) contributing 6.24 % to the overall global radiative forcing (WMO 2010; Forster 2007). Second, it is the single most important depleting substance of stratospheric ozone (Ravinshakara et al., 2009). The dominant sources of N$_2$O are closely related to microbial production processes in soils, sediments and water bodies. Agricultural emissions owing to N fertilizer use and manure management (4.3–5.8 Tg N$_2$O–N yr$^{-1}$) and emissions from natural soils (6–7 Tg N$_2$O–N yr$^{-1}$) represent 56–70% of all global N$_2$O sources (Syakila and Kroeze, 2011). Field measurements of N$_2$O exchange between soils and the atmosphere across a wide variety of terrestrial ecosystems as well as laboratory incubation studies under controlled conditions both with soils and with pure cultures of microorganisms provide an extensive set of measured emission fluxes. Fig. (5)

![Fig. 5: A schematic representation of C–N interactions in the terrestrial ecosystem. Note that biological nitrogen fixation and denitrification are process performed by microorganisms that also need C as substrate and that the schematic is more representative of agroecosystems](image)

However, up-scaling N$_2$O budgets to national and regional scales remain an unresolved challenge with current national estimates still highly uncertain. This is mainly due to the very dynamic and variable character of N$_2$O soil losses caused by a multitude of interacting controls (Butterbach-Bahl and Dannenmann, 2011). As a result, soil N$_2$O emissions are characterized by ‘hot spots’ and ‘hot moments’, i.e. by an enormous spatio-temporal variability (Groffman 2006; Wolf 2010). Availability of reactive nitrogen (Nr: here defined as organic bound N and inorganic N compounds except N$_2$) is the major driver of N$_2$O soil emissions, fertilizer use is a key factor controlling soil N$_2$O fluxes (Syakila and, Kroeze 2011; Fowler 2009). However, elevated N$_2$O soil fluxes are not only restricted to sites were N fertilizers are applied (the so-called direct emissions), but owing to volatilization, leaching and erosion processes, nitrogen is cascading from application sites to downwind and downstream ecosystems. This might result in natural ecosystem N enrichments, thereby creating new hot spots of N$_2$O emissions (i.e. indirect emissions (Galloway et al., 2003; Sutton (2007). For a better understanding of N$_2$O soil emissions, it is, on the one hand, necessary to understand nitrogen cycling from ecosystem to regional and global scales and on the other hand, to improve our understanding of key processes involved in N$_2$O formation, consumption and emission. The challenge is to integrate the two. Here, we summarize the current understanding of processes involved in N$_2$O emissions, outlining advances and remaining challenges to characterize and quantify relevant soil processes and soil surface fluxes of N$_2$O and describe the state of development of models used to simulate N$_2$O soil fluxes from site to regional scale. (Klaus Butterbach-Bahl, et al., 2013). Stated that, although it is well established that soils are the dominating source for atmospheric nitrous oxide (N$_2$O). Recent work shows that a better understanding of the composition and diversity of the microbial community across a variety of soils in different climates and under different land use, as well as plant - microbe interactions in the rhizosphere, may provide a key to better understand the variability of N$_2$O fluxes at the soil – atmosphere interface.
Regulation of the reduction of $\text{N}_2\text{O}$ to dinitrogen ($\text{N}_2$) has increased our understanding of $\text{N}_2\text{O}$ exchange. This improved process understanding, building on the increased use of isotope tracing techniques and metagenomics, needs to go along with improvements in measurement techniques for $\text{N}_2\text{O}$ and $\text{N}_2$ emission in order to obtain robust field and laboratory datasets for different ecosystem types. Advances in both fields are currently used to improve process descriptions in biogeochemical models, which may eventually be used not only to test our current process understanding from the microsite to the field level, but also used as tools for up-scaling emissions to landscapes and regions and to explore feedbacks of soil $\text{N}_2\text{O}$ emissions to changes in environmental conditions, land management and land use. Fig (6)

The process of gaseous ammonia loss from plant foliage can range from 2 to 15 kg N/ha/year released, depending on the crop examined or the location (Sommer et al., 2004; Wang, et al., 2011). Additionally, when the plant does not take up urea fertilizers applied to the soil, up to 40% can also be lost in the form of ammonia (Fowler and Brydon, 1989; San Francisco et al., 2011). Mineral N fertilizers produced by the Haber–Bosh process are very costly in energy production Erisman; et al., (2008), Olson, (1977) and represent nowadays up to 50% of the operational cost for the farmer depending on the cultivated crop (Reganold, et al., 1990).

![Diagram of biotic and abiotic processes of nitrous oxide (N$_2$O). Processes potentially leading to N$_2$O formation and consumption, involved N compounds, their reaction pathways as well as their oxidation states are shown. According to current knowledge, anaerobic ammonia oxidation does not contribute to N$_2$O formation or consumption. By contrast, N$_2$O may at least serve as a substrate for biological dinitrogen fixation. Processes predominantly requiring anaerobic (or micro-aerobic) Grey illuminated segments underline conditions. Norg/R-](image-url)
NH2, monomeric organically bound N forms; NH4+, ammonium; NH3, ammonia; H2OH, hydroxylamine; NO₂-, nitrite; NO₃-, nitrate; NO, nitric oxide; N₂O, nitrous oxide; N₂, molecular dinitogen. DNRA, Dissimilatory Nitrate Reduction to Ammonium.

Thus, NUE and energy input are seen as important indicators for the environmental impact of the production of conventional crops but also of energy crops, since they have a large capacity to produce biomass with the minimal amount of N fertilizer Lewandowski et al., (2006) Comparatively, the net energy cost of N₂ fixation in leguminous species is lower than that necessary for an equivalent production of synthetic N fertilizers Andrews et al., (2009) and Fustec et al., (2010) Fig (7). Therefore, it will be advantageous to the farmer to include more legumes both in crop rotations and in cover crops, whether the main cultivated crop is grown for grain or biomass.

Fig 7: Main reactions involved in nitrogen assimilation in higher plants. NO₃⁻ = nitrate; NO₂⁻ = nitrite; NH₄⁺ = ammonium, N₂ = atmospheric dinitrogen. The main enzymes involved in nitrate reduction and ammonia assimilation are indicated in italics: NR = nitrate reductase; NiR = nitrite reductase e; Nase = nitrogenase; GS = glutamine synthetase; GOGAT = glutamate synthase. The ultimate source of inorganic N available to the plant is ammonium, which is incorporated into organic molecules in the form of glutamine and glutamate through the combined action of the two enzymes GS and GOGAT. Carbon originating from photosynthesis through the tricarboxylic acid cycle (TCA cycle) provides the α-ketoglutarate needed for the reaction catalyzed by the enzyme GOGAT. Amino acids are further used for the synthesis of proteins, nucleotides and all N-containing molecules.

2-Biological Nitrogen Fixations

There is growing interest in increasing the contribution of biological nitrogen fixation to the growth of crop plants in agriculture. Symbiotic nitrogen fixation is largely limited to legumes in agricultural systems, but there are a number of microorganisms, including some diazotrophs, that inhabit the rhizosphere of other crop plants, some of which have been shown to enhance plant growth Fig. (8) & (9).

The present an overview of the diversity and specificities of associations between diazotrophs and their host plants and the biology and biochemistry of these nitrogen-fixing symbiotic associations. Understanding the mechanisms, between plant and microbe, involving the formation and functions of these symbioses to solve the nitrogen fixation problem will position us to engineer these processes into no fixing food crops, such as cereals and agriculturally important. Initial challenges include identifying a suitable microbial partner, initiating intracellular accommodation, that controlling plant microbiome, and keeping cheaters under control, discussing perspectives and limitations for engineering a nitrogen-fixing ability in plants based on knowledge of symbiotic nitrogen fixation in legumes and nonlegumes.
**Fig. 8:** Illustrates Root niches for colonization by diazotrophic bacteria. Rhizospheric bacteria (orange cells) colonize the rhizosphere soil area without invading internal plant tissues. Associative bacteria (blue cells) are in close interaction with the plant surface and sometimes can be found within plant tissues. Endophytic bacteria (dark red cells) colonize any region within the epidermis of the plant root, and they can reside in apoplastic intercellular spaces and the xylem vessel apoplast. In general, the endophytes invade the internal plant tissues through sites of injury in the epidermis, root tips, and root cracks formed at the sites of lateral root emergence. Some endophytic bacteria can spread to distant plant organs (stem, leaves, seeds, and fruits).

**Fig. 9** Kosakonia sacchari PBC6.1 contains well-characterized nitrogen regulatory pathways. The regulatory network controlling nitrogen fixation and assimilation in PBC6.1 is shown, including the key nodes via AmtB, glutamine synthetase (GS; encoded by the glnA gene), GlnE depicted as the two-domainATase-AR enzyme, GlnD, and PII proteins depicted as small trimeric proteins, NiFL and NiFA.
2.1. Symbiotic Nitrogen Fixation

Diversity of nitrogen fixing plant-microbe associations. Nitrogen-fixing bacteria are found in several phyla Boyd and Peters (2013) and representatives from most (if not all) of these phyla are known to engage in nitrogen-fixing symbiosis with plants (Hardoim et al., 2015). Reciprocally, plants have developed multiple solutions to associate with and accommodate diazotrophs in order to acquire atmospheric nitrogen. Proximity between a bacterial symbiont and plant host is a key element for nutrient exchanges between them and falls into three broad categories, based on the degree of intimacy and interdependency of the plant and microbe: loose associations with free-living nitrogen fixers, intercellular endophytic associations, and endosymbiosis. Interactions between plants and associative nitrogen-fixing bacteria, which are considered a subset of plant growth-promoting rhizobacteria (PGPR) Fig. (10) are the simplest form of nitrogen-fixing symbiosis.

These associative bacteria respond to root exudates via chemotaxis to, and colonization of, the rhizosphere of many plants but typically do not invade plant tissues Santi et al., (2013), Compant et al., (2010) Fig. (11).

Nitrogen-fixing, plant growth-promoting rhizobacteria (PGPR), have been identified among the bacilli and especially among the proteobacteria (Schmid and Hartmann, 2007). Their proximity to the root enables them to impact plant resource acquisition (nitrogen, phosphorus, and essential minerals), yield, and growth ,Ahemad and Kibret (2014). Some of the best-studied species of associative PGPR belong to the genus Azospirillum, which are able to improve the fitness of several crops, including wheat, maize, and rice (Steenhoudt and Vanderleyden, 2000). Azolla ferns, which have been used as companion plants in rice agriculture for centuries, accommodate the heterocystous cyanobacterium...
Nostoc azollae (formerly Anabaena azollae) within specialized leaf cavities Peters and Meeks (1989) Fig. (12).

Many species of diazotrophic bacteria have evolved beyond surface colonization to spread and multiply within plant tissues without causing damage and eliciting significant defense reactions. These bacteria, such as *Azoarcus*, *Herbaspirillum*, and *Glucanacetobacter* Fig. (13) are classified as endophytes due to their tight association with plant tissues (Pedraza, 2008). Bacterial endophytes are ubiquitous and have been isolated from surface-sterilized tissue from almost all plants examined to date (Nair and Padmavathy, 2014).

![Fig. 11: Represents plant and soil microbiome association influencing soil microbiome.](image1)

![Fig. 12: Represents Location of the leaf cavity in Azolla filiculoides and Anabaena Azollae (cyanobacterium).](image2)
Fig. 13: Illustrates plant colonization by Herbaspirillum seropedicae. The association of H. seropedicae (red spots) with host plants under laboratory conditions starts with chemotaxis of the bacteria to the plant root and attachment onto root surfaces, preferentially in the root hair zone (1). The majority of the bacteria remain on the root surfaces, but some Herbaspirillum penetrate through discontinuities of the epidermis, such as the elongation zone (2a).

Their association can be obligate or facultative, and they exhibit complex interactions with their hosts that range from mutualism to parasitism. They typically enter plant tissues through natural openings (stomata) or through cracks at the site of lateral root emergence, for instance (Eskin et al., 2014). Research on bacterial endophytes has mainly focused on quantifying the amount of nitrogen fixed and on identification of the diazotrophs; consequently, very little is known about the molecular mechanisms involved in forming and maintaining the cooperation. Cyanobacteria are also frequently found within plant tissues. Nostoc is endophytic with two genera of liverworts (Blasia and Cavicularia) and all hornworts. Colonization can take place in dome-shaped auricles on the thallus of liverworts or in slime cavities of the thallus or mucilage-filled canals that run parallel to the thallus of hornworts (Adams and Duggan, 2008). Nostoc is also able to endophytically colonize coralloid roots of cycads. The mechanism of recruitment is unknown, but the cyanobacteria are found embedded in mucilage in a specific cortical layer of the coralloid root between elongated specialized cells Costa and Lindblad (2002) Fig. (14). The most elaborate form of nitrogen-fixing plant microbe association is endosymbiosis. Bacterial endosymbionts are generally acquired from the environment and are accommodated inside plant cells within plant-derived membranes. Some plants interact with nitrogen-fixing cyanobacteria. In the symbiosis between plants of the genus Gunnera and cyanobacteria of the genus Nostoc, seedlings recruit the endosymbiont by secretion of carbohydrate-rich mucilage. Nostoc subsequently enters through specialized glands and then is accommodated within cells of the inner cortex. The host's plasma membrane, which acts as the interface for nutrient exchange Bergman and Osborne (2002), surround filaments of Nostoc. Most well studied plant endosymbioses are those between actinorhizal plants and Frankia bacteria and between legumes and rhizobia, which we will discuss in more depth below.
Fig. 14: Represents Young shoots (⋆) and root cluster (●) of Monotropa uniflora Fig. 2 Root tip of M. uniflora covered with a fungal mantle. Numerous cystidia (●) are evident. Scale bar =100 μm Fig. 3 Coralloid root mass of Pterospora andromedea showing numerous root tips (●). Scale bar =5 mm Fig. 4 Branched root of P. andromedea. A fungal mantle (⋆) covers the main root apex and lateral roots. Scale bar =100 μm

2.2. Signaling, infection, and specificity.

2.2.1. Auxin Transport

Several studies link NF signaling to auxin transport. Spot inoculation of compatible rhizobia (but not a non-host strain) or micro targeting of specific LCOs can modify auxin gradients, as measured by the GH3: GUS reporter gene in white clover Mathesius et al., 1998). Likewise, flavonoid application also inhibits auxin transport. NF application or S. meliloti infection inhibits auxin transport from shoot to root at 24 h, as well as regulating expression of some MtPIN auxin efflux transporter genes, in an MtCRE1 dependent manner (van Noorden et al., 2006; Plet et al., 2011; Ng et al., 2015). Moreover, MtCRE1 dependent pathways also control the accumulation of flavonoids in M. truncatula roots upon infection and flavonoid application can rescue the Mtcre1 nodulation phenotype. These data suggest that NF induced, CK signaling triggers flavonoid induction and the subsequent inhibition of polar auxin transport. The resulting accumulation of auxin initiates cortical cell division and nodule organogenesis Fig. (15).

2.2.2. Strigolactone

Strigolactones (SGLs) show a dose-dependent effect on nodulation in M. truncatula (Gomez-Roldan et al., 2008; De Cuyper et al., 2015). Interestingly, several studies Larraínzar et al., (2015); van Zeijl et al., (2015a); Herrbach et al., (2017) showed a direct NF regulation of the expression of the SGL biosynthesis gene DWARF27 (D27). The promoter of the D27 gene is active in the root epidermis after a 3 h NF treatment and upon early steps of nodule organogenesis (van Zeijl et al., 2015a). Moreover, we observed that a combined auxin CNF treatment reduced the NF induction of D27 expression, suggesting that auxin can antagonize NF effects (Herrbach et al., 2017).
Fig. 15: Recapitulative spatio-temporal scheme of the interactions between NF signaling and downstream hormonal pathways. Rhizobia produce NFs that are perceived in root hairs (RHs) through LysM-RLK receptors such as NFP and LYK3. NF perception leads to calcium spiking and activation of DMI3 that acts upstream of the CK receptor MtCRE1. The signaling cascade involving DMI3 and CRE1 is likely involved in both epidermal and cortical signaling (black boxes). NF treatment triggers early CK biosynthesis gene (IPT/CYP735A/LOG) expression and CK accumulation in M. truncatula roots downstream of DMI3 but independently of CRE1 signaling (van Zeijl et al., 2015b). Although tissue specificity of this CK production was not determined, evidence from L. japonicus and M. truncatula suggests that epidermal CK accumulation is a negative regulator of RH infection (1) and NF signaling (2) (Held et al., 2014; Jardinaud et al., 2016). In contrast, cortical CK is a positive regulator of nodule organogenesis (3) (Gonzalez-Rizzo et al., 2006; Murray et al., 2007; Reid et al., 2017). Bioactive CKs are perceived by CRE1 and induce expression of NIN and ERN1 Ariel et al., (2012), which are positive regulators of both infection and nodule organogenesis (Andriankaja et al., 2007; Marsh et al., 2007). This induction might be partly through regulation of DELLA activities. GA is a negative regulator of DELLA protein stability. Bioactive GA pools are likely present in both epidermis and cortex early after NF treatment (Fonouni-Farde et al., 2016; Jardinaud et al., 2016; Herrbach et al., 2017). DELLAs play a positive regulatory role on symbiotic gene expression such as ERN1 and they negatively regulate CK degradation (Fonouni-Farde et al., 2016; 2017; Jin et al., 2016). In contrast, CK positively regulate GA inactivation enzymes and down-regulate expression of the GA20ox activation enzyme Fonouni-Farde et al., (2018), suggesting a negative feedback of CK on GA active pools. NF signaling induces ethylene production, both independently of the LHK1/CRE1 CK pathway (4) Reid et al., (2018) and downstream of CK perception (5) (van Zeijl et al., 2015b). Ethylene reduces CK production in M. truncatula roots; possibly through negative feedback on NF signaling (6) (van Zeijl et al., 2015b). Ethylene negatively regulates NF-induced calcium spiking, RH infection, and nodule organogenesis (Heidstra et al., 1997; Pennetsa et al., 2008). Regulation of auxin biosynthetic and conjugation enzyme (GH3) genes occurs in NF treated RHs and upon S. meliloti infection in an NF-dependent manner (Breakspear et al., 2014; Larraínzar et al., 2015; Jardinaud et al., 2016; Herrbach et al., 2017). Reciprocal positive and negative feedback regulatory loops between some auxin and NF regulated genes (7) was shown by comparing the combined effect of auxin and NFs to either treatment alone (Herrbach et al., 2017). Downstream of CK perception, control of auxin transport in the cortex seems regulated by differential expression of PIN genes Plet et al., (2011) or accumulation of flavonoid compounds. MtLAX2 that is induced upon S. meliloti infection in vasculature and early nodule primordia, and which is required for nodule organogenesis Ross et al., (2017), also mediates auxin accumulation. In parallel, epidermal auxin signaling controls infection, thread (IT) formation, at least partly through ARF16a (Breakspear et al., 2014). Exogenous application of high concentrations (mM range) of ABA or JA inhibits NF-induced calcium spiking Sun et al., (2006); Ding et al., (2008) but this inhibition is so far not supported by any transcriptomic data. Plain bars represent negative and plain arrows positive regulations. Dashed lines are hypothetical relationships, and solid lines have evidence from the literature. Different hormones are highlighted by different colors. CK, cytokinins; GA, gibberellins; ABA, abscisic acid; JA, jasmonic acid; NF, Nod factors. Bacteria entrapped in curled root hair and IT are shown in blue.
2.2.3. Jasmonic Acid (JA) and brassinosteroid (BR)

Jasmonic acid (JA) and BR regulate plant stress responses and plant growth, but their role in RL symbiosis is not well understood. Conflicting reports indicate that host responses to these hormones vary depending on the legume species, hormone dose, or type of treatment studied. Some evidence suggests NF regulation of JA biosynthetic enzymes. Larraínzar et al., (2015) observed NF dependent induction of two JA biosynthesis genes at 3–6 hpi and Breakspear et al., (2014) observed down regulation of a few JA biosynthetic genes in RHs after a 24 h NF treatment. In contrast, NF-dependent upregulation of several JA biosynthesis genes was observed in soybean at 48 hpi, while there seemed to be a reduction in expression of BR biosynthetic genes (Hayashi et al., 2012).

3. Nitrogen assimilation and amino acid synthesis

Reduction of nitrate to ammonium is catalyzed by nitrate and nitrite reductases in the cytosol and plastids, respectively (Miflin, (1974); Srivastava, 1980). Nitrite is transported into plastids by Nitrite Transporter NITR 2; 1 (Maeda et al., 2014). Glutamine Synthetases (GSs), glutamine oxoglutarate aminotransferase (GOGATs) and Asparagine Synthetases (ASs) are responsible for ammonium assimilation into amino acids (Masclaux-Daubresse et al., 2010). The chloroplastic GS2 mainly contributes to primary N assimilation and re-assimilation of photo respiratory ammonium in the mesophyll (Cren & Hirel, 1999). Cytosolic GS1 enzymes play a role in primary N assimilation in roots, especially under high nitrate conditions (Lothier et al., 2011; Guan et al., 2016). In mature leaves, GS1 isoforms are predominantly found in the companion cells promoting phloem loading for N translocation to sinks (Lothier et al., 2011). GS1 activity is also induced in mesophyll cells during leaf senescence for re-assimilation of ammonium originating from amino acid catabolism (Bruijere et al., 2000; Masclaux et al., 2000). Ferredoxin-dependent GOGAT isoforms are located in mesophyll chloroplasts, and NADH-GOGAT is present in plastids of leaf and root companion cells, further contributing to N assimilation and partitioning (Suzuki and Knaff, 2005). In addition to the GS/GOGAT pathway, there are several lines of evidence that the cytosolic AS participates in ammonium assimilation (Carvalho et al., 2003). Chloroplasts (and the cytosol) are the sites of de novo biosynthesis of a large number of protein organic amino acids, and membrane proteins are essential to facilitate their movement across the plastid envelope and towards the phloem (Weber and Tegeder, 2006). To date, only two chloroplast amino acid transporters have been characterized, the Arabidopsis glutamate/malate exchanger dicarboxylate transporter (DiT2) Renne et al., (2003) and the Petunia hybrid a Cationic Amino Acid Transporter (Php CAT) which functions in export of aromatic amino acids (Widhalm et al., 2015).

3.1. Nitrogen fixation and export of organic nitrogen from nodules

Nitrogen fixation occurs in root nodules of legumes plants and is carried out by bacteroids Fig. (16), (17). The resulting ammonia is transferred via the ammonia channel Nod 26 (Nodulin 26) across the symbiosome membrane to the nodule host cells and reduced to glutamine and asparagine (Fortin et al., 1987). The amides are the dominant N transport forms in temperate legumes such as pea (Pisum sativum) and alfalfa (Medicago sativa) (Miao et al., 1991; Carvalho et al., 2003). In nodules of tropical legumes, allantoin and allantoic acid are produced from glutamine (Atkins & Smith, 2000). These ureides are exported out of the nodules, and transported in the xylem to the shoot Herridge et al., (1978); Streeter, (1979). Following synthesis and depending on the legume species, ureides or amides move apoplastically towards the nodule vasculature, which is surrounded by an endodermis blocking further apoplastic flow (Pelissier et al., 2004). Import of the organic N into cortical and endodermal cells is essential for their continued movement towards the nodule xylem or phloem for delivery to the shoot and roots, respectively.

While the molecular mechanism for amide uptake into the nodule symplasm is unknown, recent studies in soybean have demonstrated that import of allantoin and allantoic acid requires the function of Ureide Permeases (UPS1-1 and UPS1-2) (Pelissier et al., 2004; Collier & Tegeder, 2012; Carter & Tegeder, 2016).
Fig. 16: Nodule morphologies across the Leguminosae (tribes indicated in parentheses). (a) Spherical determinate desmodioid-type nodules with lenticels (arrows) on the South African legume Dipogon lignosus (Phaseoleae) (photograph courtesy of Wendy Liu). (b) Determinate nodule on the Australian native Hardenbergia comptoniana (Phaseoleae). (c) Dalbergioid nodules on the upper root/lower stem of the neotropical species Aeschynomene americana (Dalbergieae); these nodules are always associated with lateral roots (arrows) (photograph courtesy of H. S. Gehlot). (d) Photosynthetic stem nodules on the West African semi-aquatic species Sesbania rostrata (Sesbanieae). (e) Indeterminate nodules on the roots of the Australian native Chorizema cordatum (Mirbelieae). (f) Indeterminate nodule of the Australian native Chorizema rhombbeum (Mirbelieae). Note the pink colouration (*), which is caused by leghaemoglobin. (g) Indeterminate nodules on the roots of the Australian native Templetonia retusa (Brongniarteae); the rhizobial symbionts in these are contained within cell wall-bound fixation threads. (h) Branched indeterminate nodule on the Brazilian native tree Dimorphandra wilsonii (Caesalpiniae); these also contain fixation threads. Bars: (a, c) 4 mm; (b, f) 1 mm; (d, g) 10 mm; (e, h) 5 mm.

Fig. 17: Development of root nodules in soybean. (a) Rhizobium bacteria contact a susceptible root hair divide near it. (b) Upon successful infection of the root hair cause it to curl. (c) Infected thread carry the bacteria to the inner cortex. The bacteria are modified into rod-shaped bacteroids and cause inner cortical and pericycle cells to divide. Division and growth of cortical and pericycle cells lead to nodule formation. (d) A mature nodule is complete with vascular tissues continuous with those of the root.
2.3. Host Plant

Plants release a significant amount of organic carbon into the soil in the form of cell lysates, intact border cells, mucilage, and root exudates (Wagner, 1997). The amount and type of exudates depend on plant genotype and growth stage, vary across different environmental conditions (soil type, soil moisture, nutrient availability, or toxicity), and are greatly affected by the organisms living in the rhizosphere. Exudates are complex mixtures of low-molecular weight organic substances, like sugars, amino and organic acids, fatty acids, sterols, growth factors, and vitamins (Günter and Volker, 2007). It is well known that root exudates can influence the soil microbial community structure and biogeochemical cycles of key nutrients, such as nitrogen and phosphorous (Turner et al., 2013). The composition of exudates is highly varied between plant species and allows the recruitment of unique populations of prokaryotes and eukaryotes (Turner et al., 2013).

Plants can enrich their rhizosphere with specific microbiota by the secretion of particular carbon sources. For example, dicarboxylate in tomato root exudates favor the growth of Pseudomonas biocontrol strains (Kamilova et al., 2005; Kamilova et al., 2006). Pea plants select for their symbiont Rhizobium leguminosarum by the excretion of homoserine into the rhizosphere (Van Egeraat 1975; Vanderlinde et al., 2014). In fact, Rhizobium leguminosarum has been shown to contain a pea-rhizosphere-specific plasmid that is globally upregulated in the pea rhizosphere (Ramachandran et al., 2011). Root exudates also play an important role in plant defense through the secretion of phytochemicals that can inhibit the growth of certain microbes (Baetz and Martinoa, 2014). The ability to tolerate these chemicals can play an important role in the ability to colonize the plant. For example, the PGPR Pseudomonas putida is both tolerant of and attracted by the main antimicrobial benzoxazinoid produced by maize (Neal et al., 2012). Pseudomonads also possess specialized gene sets that allow them to overcome nonhost isothiocyanate resistance in Arabidopsis (Fan et al., 2011). Some legumes produce toxic amino acid derivatives (for example, mimosine and canavanine) that are harmful to the general root microbiota but can be resisted or even catabolized by their rhizobial symbionts (Soedarjo and Borthakur, 1997; Cai et al., 2009). Remarkably, some bacteria have the ability to modify the plant rhizosphere to favor their growth or the growth of their siblings. Agrobacterium strains contain genes on their tumor-inducing plasmids that encode the synthesis and catabolism of novel carbon and nitrogen compounds from the condensation of sugars and amino acids. Opine synthesis genes are transferred to the plant host upon invasion and result in the production of opines by the plant that provide a specialized ecological niche that favors the growth of Agrobacterium (Savka et al., 2002). Some strains of Sinorhizobium melloti and Rhizobium leguminosarum are capable of synthesizing inositol derivatives called rhizopines during nitrogen fixation in legume nodules (Murphy et al., 1995). The ability to catabolize these compounds has been proposed to provide a competitive advantage to their siblings in the rhizosphere (Gordon et al., 1996). Transgenic plants expressing opine biosynthesis genes have been generated and shown to reshape rhizosphere populations to increase the population densities of opine-catabolizing bacteria compared to wild type plants (Oger et al., 1997; Mondy et al., 2014). These findings provide proof of principle for the biased rhizosphere concept, bolstered by observations that changes in population density correlated with levels of opine production under a range of concentrations in the two phylogenetically distant plant species Lotus corniculatus and Arabidopsis thaliana (Mondy et al., 2014; Oger et al., 2000). Engineering of Pseudomonas to catabolize opines resulted in a competitive advantage for colonization compared to wild-type Pseudomonas during colonization of transgenic opine-producing plant roots (Savka and Farrand, 1997). Thus, biased rhizospheres and targeted rewards represent an exciting opportunity for engineering to both provide a competitive advantage to a symbiont in the rhizosphere and potentially provide dedicated carbon sources to energize nitrogen fixation. Plants have evolved several mechanisms for exerting additional control over the symbiont once symbiosis has been established. It has recently emerged that exopolysaccharides on the cell surface may serve as a second checkpoint for appropriate partner selection and are recognized by specific receptors in the plant (Kawaharada et al., 2015). Nod factors also serve as an important signal to suppress plant immunity and permit the invasion of partner rhizobia. Once successful invasion of the plant and nodule formation has occurred, there is some evidence that legumes are able to limit the proliferation of “cheater” bacteria that express the traits for successful invasion but not for efficient nitrogen fixation. This process is essential to guarantee the stability of cooperation in these mutualistic associations. It has been established that legumes are able to monitor symbiotic performance and sanction nodules that are ineffective (Kiers et al., 2003). Sanctioning may
be accomplished by restricting the supply of sugars to ineffective nodules, such that the plant only dedicates resources to nodules that supply a significant amount of nitrogen in return for the carbon they receive. This leads to premature senescence of nodules harboring low-quality symbionts. It has been proposed that *Parasponia* spp., some woody legumes, and actinorhizal plants control their symbionts by the production and storage of antimicrobial phenolic compounds in uninfected cells (Behm et al., 2006). There is also some evidence of control of cheaters in symbiosis with *Nostoc*. When the global nitrogen cycle regulator *ntrC* of *Nostoc* is mutated, the host *Anthoceros* limits the extent of infection (Adams and Duggan, 2008). Other mutualistic associations, such as the arbuscular mycorrhizal symbiosis, are stabilized through mutual and targeted rewards (Kiers et al., 2011). Several plant clades have evolved short defensin-like proteins that further control the behavior of the bacterial symbiont. Legumes in the inverted repeat-lacking clade (but not legumes in the related robusinoid clade) produce hundreds of small, nodule-specific, and cysteine-rich peptides. These peptides perturb the cell cycle, leading to endoreduplication of both plant and bacterial genomes, disruption of membrane stability, alteration of gene expression, and promotion of terminal differentiation of the *Rhizobium* species (Maróti et al., 2015). More recently, sets of defensin-like peptides with similar properties have been found in dalbergioid legumes Czernic et al., (2015) and in three genera of actinorhizal hosts (Carro et al., 2013).

### 2.4. Nutrient exchange.

The driving force of symbiosis between a plant and a nitrogen-fixing microorganism is the exchange of nutrients between the two partners. In return for fixed nitrogen, the plant typically provides its bacterial symbiont with a carbon source and, depending on the intimacy of the symbiosis, other crucial nutrients. Both organisms change their metabolic routines in order to accommodate to each other’s needs, a process that is monitored and regulated by both partners. Fig. (18). Bonic anhydrase, phosphoenolpyruvate carboxylase, and malate dehydrogenase are upregulated during nodule development, which directs carbon flow toward malate (Udvardi et al., 2013). The exchange of metabolites between the plant and bacteroids does not happen freely but is facilitated by specialized transporters. Analysis of the genomic inventory of *Medicago truncatula* transporters revealed that wide ranges of transporters are induced during nodule development (Benedito et al., 2010). Among these are genes encoding putative sugar transporters, amino acid transporters, and sulfate transporters (Udvardi et al., 2013). In *Rhizobium*-legume symbiosis, carbon is specifically supplied to the bacteroids in the form of dicarboxylic acids, such as malate (Udvardi et al., 1988). After crossing the symbiosome membrane that separates the bacteroids from the plant cell cytoplasm, dicarboxylates are taken up by DctA, a transporter of the major facilitator superfamily (Yurgel et al., 2004). Dicarboxylic acids are assimilated by gluconeogenesis or catabolized via enzymes of the tricarboxylic acid (TCA) cycle to provide the reductant and ATP required for nitrogen fixation (Finan et al., 1988; Finan et al., 1991).

### 2.5. Efficiency of nitrogen and its uptake by plants.

Ludwig et al., (2003) stated that despite carbon source, the exchange of fixed nitrogen is another nutrient important for the symbiosis to be commonly beneficial. Bacterial nitrogen metabolism specifically, must be altered so that nitrogen is excreted rather than incorporated into the microbial biomass. In all plant host appears to directly interfere with bacterial amino acid biosynthesis and thereby force the release of nitrogen. Udvardi et al., (2013) reported that, in nitrogen-fixing *Rhizobium* bacteroids, suggests that nitrogen metabolism is significantly adapted during bacteroids differentiation, and ammonia assimilation is effectively shut down *Rhizobium leguminosarum* bacteroids become symbiotic auxotrophs for branched-chain amino acid transport and become dependent on the plant for the supply of amino acids. Mulley et al., (2011) stated that mutants of the branched-chain amino acid ABC transporters Aap and Bra are unable to fix nitrogen for the host plant *R. leguminosarum* mutants of ammonium assimilation are unaltered in their capacity for symbiotic nitrogen fixation (Mulley et al., 2011; Udvardi et al., 1992).

The inactivation of ammonia assimilation in the bacteroid may be accomplished via an unknown and presumably plant-regulated posttranslational modification of the enzyme glutamine synthetase (GS) (Patriarca et al., 2002). In legume-*Rhizobium* symbiosis, ammonia produced by nitrogenase is delivered to the plant cell as NH$_4$ and/or NH$_3$ Fig. (19). Ammonia in its neutral lipophilic form probably crosses the bacteroids membranes via diffusion. The bacterial NH$_4$ transporter AmtB, which transports...
NH\textsubscript{4} in the opposite direction (i.e., into the bacteroid), is repressed in bacteroids, ensuring that NH\textsubscript{3} lost from the cell is not recovered by the bacterium but rather is taken into the plant cytoplasm. After entering the symbiosome space between the bacteroid and the symbiosome membrane, ammonia is protonated to NH\textsubscript{4} because of the acidic environment there (Day et al., 2001).

Fig. 18: Schematic representation of partnership between a diazotrophic bacterial cell and a modulating plant cell during symbiotic nitrogen fixation. Rhizobia induce the formation of nodules on legumes using either Nod factor-dependent or Nod factor-independent processes. In the Nod factor-dependent strategy, plants release signals, such as flavonoids, that are perceived by compatible bacteria in the rhizosphere. This activates the nodulation (nod) genes of rhizobia, which in turn synthesize and release bacterial signals, mainly lipochitooligosaccharides (LCOs) (Nod factors), which trigger early events in the nodulation process. Synthesis of the Nod factors backbone is controlled by the canonical nodABC genes, which are present in all rhizobia, but a combination of other nodulation genes (nod, nol, or noe) encode the addition of various decorations to the core structure. In the Nod factor-independent process, bacteria enter in the plant via cracks in the epidermis. Accumulation of cytokinin synthesized by the bacteria in these infection zones might trigger nodule organogenesis. In the mature nodule, bacteria progressively experience lower oxygen concentrations and differentiate into bacteroids, fixing diffused nitrogen gas using their nitrogenase enzyme complex. NH\textsubscript{3} produced by nitrogenase from the bacteria (nif, fix, and cytochrome bd) can be incorporated into amino acids via the glutamine synthetaseglutamate synthase (GS-GOGAT) pathway. NH\textsubscript{3} can also diffuse through the bacterial membrane and be transported to the plant cytoplasm via ammonia transporters (e.g., AmtB), where it is assimilated into nitrogen compounds (amino acids, proteins, and alkaloids) in exchange for food molecules, e.g., glucose, amino acids, and other saccharides. The plant provides amino acids to the bacterial cell and in return, the bacterial cell cycles amino acids back to the plant for asparagine synthesis. Other nutrients have to be made available for the microbe, including phosphorus, sulfur, molybdenum, and cobalt. Asn, asparagine; Asp, aspartate; KG, alpha ketoglutarate; AmtB, ammonia transporter; Co, cobalt; cyt bd, cytochrome bd; DctA, dicarboxylate transporter; Glu, glutamate; Gln, glutamine; GOGAT, glutamate synthase; GS, glutamine synthetase; HCO\textsubscript{3}, bicarbonate; Mo, molybdenum; NH\textsubscript{3}, ammonia; N2ase, nitrogenase; Nod factors, nodulation factors; NFR, Nod factor receptor; OAA, oxaloacetate; P, phosphorus; S, sulfur

Therefore, ammonium crosses the symbiosome membrane and enters the cytoplasm of the infected plant cell, where it is rapidly assimilated into organic form. For ammonium transport across the symbiosome, membrane two possible pathways are exists, one through an NH\textsubscript{3} channel Niemietz and Tyerman (2000), and other through a cation channel that transports K, Na, and NH\textsubscript{3} Tyerman et al., (1995) Fig. (20).
Fig. 19: Represents nitrogen uptake and assimilation process in plants. Nitrate (NRT) and ammonium transporters (AMT), respectively mediate the uptake of nitrate (NO$_3^-$) and ammonium (NH$_4^+$) ions. The NO$_3^-$ entered into the cell is reduced to nitrite ions (NO$_2^-$) by an enzyme nitrate reductase (NR). The nitrite ion then moves to plastid and reduced to ammonium ion by nitrite reductase (NiR) enzyme. The ammonium is then incorporated into amino acid by glutamine synthetase and glutamate synthase via GS/GOGAT cycle. The ammonium ion transported by ammonium transporters directly enters into GS/GOGAT cycle. The two additional enzymes glutamate dehydrogenase (GDH) and asparagine synthetase (ASN) also participates in ammonium assimilation. The GS, GDH and ASN are the key enzymes involved in synthesis of glutamine (Gln), Glutamate (Glu) and Asparagine (Asn).

Fig. 20: Characterized transport processes of the symbiosome membrane. Symbiosomes exist with in infected plant cells, where they act to partition nitrogen-fixing bacteroids from the celllysosol. Arange of transport processes have been characterized on the symbiosome membrane to facilitate movement of solutes between symbionts. These include (1) transport processes supporting the primary needs of symbionts (nitrogen, malate, and metal ions), (2) efflux processes (nitrogen), (3) secondary transport processes (nitrate sulfate, and IAA), and (4) regulatory transport processes (H$^+$-ATPase, calcium, and water flux).
Once inside the plant cell, ammonia is assimilated into amino acids mainly by the action of GS, glutamate synthase (GOGAT), and aspartate aminotransferase. The expression of genes encoding these enzymes is induced during nodule development (Colebatch et al., 2004). Interestingly, nodulin 26, which can transport NH$_3$ (Hwang et al., 2010), interacts physically with cytosolic GS that is responsible for the assimilation of ammonia to glutamine (Masalkar et al., 2010) Fig. (21).

Fig. 21: Schematic presentation of key enzymes involved in nitrogen management in (A) young and (B) senescing leaves. (A) Nitrate reductase (NR) and asparagine synthetase (AS) are localized in the cytosol, and nitrite reductase (NiR), glutamine synthetase 2 isoenzymes (GS2), glutamate synthase (GOGAT) and carbamoyl phosphate synthetase (CPSase) within the plastids of mesophyll cells. Glutamine synthetase isoenzymes 1 (GS1) and AS are located in the cytosol of companion cells. (B) Senescence-associated events include chloroplast degradation and translocation of plastid proteins to the central vacuole via senescence-associated vacuole (SAV) trafficking. Amino acid recycling occurred in mitochondria and cytosol of mesophyll cells and companion cells. Glutamate dehydrogenase (GDH), GS1 and AS are the major enzymes involved in the synthesis of glutamine, glutamate and asparagine in the phloem.

Several other genes encoding aquaporin-like proteins that potentially transport ammonia are induced in infected cells of Medicago truncatula nodules (Limpens et al., 2013). The symbiosome membrane NH$_4$ /K channels have not yet been identified genetically. In actinorhizal plant-Frankia symbiosis, the bacterial GS remains fully functional, but downstream components of amino acid biosynthesis are downregulated (unlike in legume-Rhizobium symbiosis). Fixed nitrogen is released to the plant in the form of amino acids or amides, with the exact chemical species varying according to the plant host. These are then broken back down to NH$_3$ which is then assimilated by the actinorhizal host by the action of GOGAT (Colebatch et al., 2004). In plant-Nostoc symbiosis, up to 80% of the cyanobacterial cells differentiate into heterocysts in order to maximize nitrogen fixation. The
percentage of differentiation varies according to the host, with the lowest rates in the associative symbiosis with *Azolla* (Peters and Meeks 1989) and the highest rates in endosymbiosis with *Gunnera* (Bergman and Osborne, 2002). In symbiosis with both *Azolla* and *Gunnera*, the bacterial GS is downregulated, unlike in legume-Rhizobium symbiosis, resulting in up to 40% of fixed nitrogen being released as ammonium. This ammonium is subsequently assimilated by the GS-GOGAT system of the plant host (Peters and Meeks 1989; Bergman and Osborne, 2002). In the bryophyte-*Nostoc* symbioses, up to 80% of fixed nitrogen is excreted to the host as NH₃, but the mechanisms leading to secretion by the bacterium and incorporation by the plant are still unknown (Adams and Duggan, 2008). In *Nostoc*-cycad associations, unlike other cyanobacterial symbioses, the GSGOGAT system of *Nostoc* is not downregulated, and nitrogen is transferred to the host in the form of citrulline, glutamine, or both, depending on the cycad host (Costa and Lindblad, 2002). Sugarcane infected with *Gluconacetobacter* has been reported to acquire up to 60% of its nitrogen from biological nitrogen fixation, although this seems highly varied depending on environmental conditions. *Gluconacetobacter* loses about 40% of its fixed nitrogen, probably in the form of NH₃, and this is likely assimilated by the GS-GOGAT pathway of the plant, although this has not yet been demonstrated conclusively (Eskin et al., 2014).

**Fig. 22:** Schematic illustration of important metabolic pathways in associations of nitrogen-fixing cyanobacteria and host plant. The cell on the left represents a vegetative cell, while the cell on the right represents a nitrogen-fixing heterocyst. Important metabolic pathways in associations of nitrogen-fixing cyanobacteria and host plant are glycolysis, carbon fixation, photosynthesis, respiration, and nitrogen fixation. The nitrogen fixed in the heterocyst is incorporated via the GS-GOGAT pathway and used for the synthesis of amino acids, although during symbiosis, most nitrogen is exported to the plant as NH₃. In exchange, the host plant provides sugars. GOGAT, glutamate synthase; GlnA, glutamine synthetase; HCO₃⁻, bicarbonate; NH₃, ammonia; N₂ase, nitrogenase; OAA, oxaloacetate; 3-PGA, polyglycolic acid; PGAL, phosphoglyceraldehyde.

### 3. Fertilization of Nitrogen in Agriculture

Nitrogen is a major nutrient, essential for all living organisms, although it is the main constituent of the air. Availability of nitrogen is the main limiting factor for productivity of terrestrial ecosystems, including agro ecosystems. Nitrogen is one of the most important inputs in crop production, it is well documented by the existence of a billion € fertilizer industry. The economic importance of nitrogen has been identified already more than 100 years ago (Lohnis, 1913). Erisman et al., 2008, reported that,
modern society would not exist without the invention of technical creation of reactive nitrogen used to fertilize agricultural land in order to ensure food security for the growing global population. However, application of mineral and organic fertilizer in excessive amounts leads to environmental problems, such as eutrophication of waters, loss of biodiversity, global warming and stratospheric ozone depletion, often found in high-income areas. In fact, the costs of environmental nitrogen pollution are exceeding the benefit due to nitrogen fertilization Sutton et al., (2011b) Fig. (23). Sutton et al., (2011a) stated that low nitrogen fertilization gradually leads to low yields and insufficient food supply, in many low-income areas. Several researchers are searching for improving soil fertility and nitrogen management at different type of climates, soils and crop conditions around the world. This includes increased yields, increased nitrogen use efficiency (NUE) as well as reducing nitrogen losses to the environment in both short and long-term. Intensive agricultural production systems is to combine intensive production with high NUE, since efficient use of nitrogen sources is the basis for combining future food security and minimized negative environmental impact. Several strategies for applications of nitrogen with organic and mineral fertilizers and strategies for soil and crop management in order to utilize nitrogen mineralization processes in the soil as well as avoiding risk of nitrogen losses. Nitrogen losses from agro ecosystems occur throughout leaching of nitrate (NO$_3^-$) and emission of nitrogen gaseous of which nitrous oxide (N$_2$O) and ammonia (NH$_3$) are of main environmental concern (Sutton et al., 2011a).

Fig. 23: Illustrates fertilizer in excessive amounts leads to environmental problems, which exceeding the benefit due to N fertilization.
Several research work have been done on this topic, where key management practices are e.g., manure and mineral fertilizer applications techniques and precision agriculture Sommer and Hutchings (2001); Robert (2002), different soil tillage strategies Myrbeck et al., (2012), cover crops Schipanski et al., (2014) and managing the soil microbial community (Richardson et al., 2009). Although much has been investigated, novel research is being conducted constantly in order to increase productivity and sustainability of agricultural soils.

3.1. Dynamics of nitrogen in farming systems

Nitrogen dynamics is highly dependent on the farming manure management are important factors for the farm N efficiency. Surveying more than nine hundred beef operations across Canada. Sheppard et al., (2018) showed that nitrogen budgets and NUE varied significantly among different types of beef operations. Forage based operations showed the largest nitrogen surplus and lower NH₃ emissions than confinement housing, while finisher operations had the highest NUE, but also the highest NH₃ emissions.

Einarsson et al., (2018) studied and compared three indicators for N emissions; all three indicators had considerable uncertainty, due to biases in the estimation of biological N fixation. Therefore, more research is needed to improve the N indicators, to avoid misleading information for decision-makers. In cropping systems, soil management is one factor that may influence the N dynamics. Laine et al., (2018) investigated the in situ gross N mineralization rates in barley fields to evaluate the potential benefit of no till on the soil N supply In Finland. Overall, no tilled soil showed a higher gross N mineralization, indicating higher soil N supply, than mouldboard ploughed soil. This was accompanied by an increased soil N retention such that ammonium immobilization was more enhanced than mineralization in no till at the expense of a decreased nitrification. Such a decreased gross nitrification relative to ammonium immobilization is an indicator for a decreased risk of N leaching Stockdale et al., (2002). Laine et al., (2018) concluded that no till of boreal arable soils decreases N leaching. In some farming systems, catch crops are grown during the season without a main crop as a management option to reduce N₂O and NO₃⁻ Leaching Schipanski et al., (2014). In a two-year field trial, Komainda et al., (2018) investigated the effect of two catch crops, rye and ryegrass, in a maize cropping system on yield, NO₃⁻ leaching and N₂O emissions. While the catch crops did affect neither the maize yield nor the N₂O emission, rye significantly reduced NO₃ leaching, despite the fact that NO₃ leaching was below the EU critical load even in the fields without catch crop. Therefore, the use of a suitable catch crop might further reduce NO₃ leaching even from crops that already optimized N fertilizer.

3.2. Root - rhizosphere management

Using less produces more, is becoming a promising characteristic for sustainability of modern agriculture despite the great contribution of intensive agriculture with ‘high input, high output’ to the growth of food production in the past. The status of agriculture today is more complex than before because of the increased demand for global food production while also protecting environmental quality and conserving natural resources in the coming decades. Simultaneously achieving high nutrient use efficiency and high crop productivity has become a challenge with increased global demand for food, depletion of natural resources, and deterioration of environmental conditions Cassman, (1999); Tilman et al., (2002); Cassman et al., (2003). In , China a cereal grain yields increased 3.5-fold from 1.2 to 5.4 t ha⁻¹; however, cereal grain yields increased by only 65% from 1980 to 2010, while the consumption of chemical fertilizers increased by 512% Zhang et al., (2011), (2012). Total crop yield in intensive farming systems has failed to increase in proportion by increasing the inputs of chemical fertilizers over the last 20 years, leading to low nutrient use efficiency and increasing environmental problems. This is mainly attributed to the overuse of chemical fertilizers while ignoring the intrinsic potential benefits of biological processes in crop exploitation of nutrient resources in the soil.

Plant roots via the rhizosphere take up soil nutrients, which is the key zone of interaction between plants and soils. Therefore, root growth and rhizosphere processes have a great influence on soil nutrient transformation, mobilization, and efficient use by plants. Plant roots can not only highly regulate morphological traits to adapt to soil environmental conditions, but also significantly modify rhizosphere processes through their physiological activities, particularly the exudation of organic acids, phosphatases, and some signalling substances, proton release, and redox changes (Hinsinger, 2001; Hinsinger et al., 2009; Zhang et al., 2010; (Marschner, 2012). The root-induced rhizosphere processes

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not only determine mobilization and acquisition of soil nutrients as well as microbial dynamics, but also control nutrient use efficiency by crops, and thus profoundly influence crop production and sustainability Zhang et al., (2010) Fig. (24).

Therefore, manipulating root growth and rhizosphere processes provides an effective approach to improve nutrient use efficiency and crop productivity simultaneously. The efficiency of root and rhizosphere processes can be enhanced with increasing intensity of soil nutrient supply. However, overuse of fertilizers may lead to high concentrations of nutrients in the rhizosphere, resulting in inhibition of root growth and rhizosphere processes (Li et al., 2008; Mi et al., 2010; Zhang et al., 2010).

Fig. 24: Illustrates Strategies of rhizosphere management for macadamia. The key components include 1) optimizing nutrient input to keep proper nutrient supply intensity in the root zone, 2) using localized nutrient supply by band fertilization to stimulate root proliferation and rhizosphere effects through optimizing nutrient placement and compositions, and 3) exploring root/rhizosphere biological interactions to maximize nutrient-use efficiency.

Synchronizing root-zone nutrient supply with crop demands spatially and temporally at an optimal level of nutrient supply in the rhizosphere is important for maximizing the efficiency of the root/rhizosphere in nutrient mobilization and acquisition. The main strategies of root/rhizosphere management are: (1) manipulating root growth in terms of both morphological and physiological traits; (2) intensifying rhizosphere processes in terms of acidification and carboxylate exudation; and (3) synchronizing root-zone nutrient supply with crop demand by integrated soil–crop system management Zhang et al., (2010); Chen et al., (2011).

3.3. Maximizing root efficiency

There is evidence indicating that plants exhibit large differences in their capacity to use soil nutrients through modifying root growth and root exudation. We found that root morphological development was greatly inhibited when excessive nitrogen (N) was supplied during intensive maize (Zea mays L.) production Fig. (25).

In principle, N deficiency increases root growth, resulting in longer axial roots (primary roots, seminal roots, and nodal roots) and this helps maize roots to explore a larger soil volume and thus increases the spatial N availability Tian et al., (2008); Marschner, (2012); however, long-term N deficiency stunts root growth due to insufficient N (Wang et al., 2003).

In addition, root elongation can be inhibited if the N supply is too high. In maize, for example, root length was found to be reduced when the nitrate concentration in culture solutions was more than 5
mmol l\(^{-1}\) (Tian et al., 2008). Only at the level of N supply was there optimal development of the root system, resulting in increased nutrient use efficiency Fig (26), (27), and (28).

**Fig. 25:** Represents root/rhizosphere interaction and management that demonstrated to be an effective approach to simultaneously enhance nutrient use efficiency and crop yields for sustainable crop production in intensive agriculture.

**Fig. 26:** Represents rhizosphere and its importance for plant nutrition starting from root exudates to the role of plant growth-promoting Rhizobium (PGPR), the role of different soil microbial communities, and the potential of soil organic matter in this area and effects of plant-soil-microbe interactions on the uptake of nutrients by plant root.
Fig. 27: Illustrates modifying root system architecture is one of the important strategies for plants to enhance N/P acquisition from N/P-limited soils. Plants have developed deeper root systems for efficient N acquisition, especially in response to nitrate leaching, while in response to P deficiency changes in root architecture include (i) the formation of proteoid or cluster roots that can have several different cluster shapes: Proteoid (arising from the pericycle of first order lateral roots), hairbrush shape cluster roots (develop more rootlets per centimeter length of secondary/tertiary roots where almost every pericycle cells gives rise to a rootlet); and (ii) dauciform or proliferation of shallow lateral roots for greater root growth in the topsoil where more of the P is fixed in P deficient soils.

Fig. 28: Represents a nutrient acquisition in agroforestry systems. Indicators (tree and crop root distribution and fine root functional traits) and processes (deep soil nutrient capture and rhizosphere chemical and microbial processes) are shown. The two boxes on the left depict nutrient acquisition strategies at the whole root system scale and the two boxes on the right depict nutrient acquisition strategies at the sites of uptake.
There was optimum lateral root growth when the nitrate supply was maintained at around 1 mmol l\(^{-1}\) in agar gel-based culture for maize (Guo et al., 2005). Roots in the field are exposed to changing N levels for a longer time, and therefore their responses to N supplies may differ from that found in solution culture systems where the growth period is relatively short. For example, Morell et al., (2011) found that grain yield but not root growth in barley was affected by N fertilization in field conditions. In maize, there are conflicting results concerning whether N application causes increase Maizlish et al., (1980) or decrease Eghball and Maranville, (1993) in root growth.

Nevertheless, in a 2-year experiment across three types of soils, a moderate but significant correlation was found between soil nitrate concentration and maize root length at silking stage Fig. (29). Similar responses were found in the roots of Arabidopsis seedlings growing on agar plates uniformly supplied with a range of KNO\(_3\) concentrations (0.01–50 mmol l\(^{-1}\) (Zhang and Forde, 1998; Zhang et al., 1999). Results reveal that high rates of nitrate supply have no effect on lateral root initiation but cause a pronounced delay in lateral root development at around the time of emergence from the parent root and thus result in 100% growth inhibition of the lateral roots by 50 mmol l\(^{-1}\) KNO\(_3\) (a concentration higher than would normally be found in the soil (Forde and Lorenzo, 2001).

Therefore, root growth can be manipulated or optimized via optimizing soil N status in the root zone or rhizosphere. Under field conditions, nitrate that moves down to deeper soil layers by heavy rainfall is not spatially available if plant roots cannot grow deep enough. Modelling work has concluded that root system architecture, with a larger investment in fine roots deep in the soil, will increase crop yields by accessing extra soil resources from the whole soil profile King et al., (2003) Fig. (30). Research comparing different maize genotypes suggests that root length density deep in the soil (30–150 cm) has a significant positive correlation with nitrate depletion (Wiesler and Horst, 1994). Therefore, increasing root proliferation in deep soil by crop improvement through breeding Mi et al., (2010); Lynch, (2007), (2011) or agronomic N management may be a promising way of enhancing N use efficiency under high N input conditions.

Fig. 29: (a) relative root depth distribution among maize IBM RILs. Means ± SE of the percent of total root mass located below 124 cm (deep) percent of total root mass in the top 30cm of media (shallow) among a subset of IBM RIL in the greenhouse, (HN blue, LN red). Few thick phenotypes classified as having a root cross-sectional area less than 1µm and a nodal root number greater than 30. Phenotypes not meeting these criteria excluded from these analyses. Mean with the same letter not significantly different (P ≤0.5), (b) correlation between the maize root/shoot ratio and nitrogen use efficiency (NUE) at silking. Only data published with root/shoot ratio at silking, grain yield at maturity and total N fertilizer were used in this analysis. Open circles indicate the results from Chinese farmlands and closed circles indicate data from western countries.
Results demonstrated that the vertical distribution of roots in the soil profile could be manipulated through N management to enhance nutrient capture and uptake by crops (Mi et al., 2010; Zhang et al., 2012). In intensive cropping systems characterized by high input and high output, chemical fertilizers are usually overused. However, over application of N cannot further increase grain yields in most cases. Plant N content is significantly correlated with shoot biomass (Reich et al., 2006). Under the same conditions, an N-efficient maize variety has higher shoot biomass and larger root size and takes up more N than does an N-inefficient variety (Kamprath et al., 1982; Peng et al., 2010). There is a positive relationship between grain yield and root size in maize (Barber and Mackay, 1986). Maize cultivars having high root length density enhanced the utilization of soil N and thus reduced the risk of nitrate leaching (Wiesler and Horst, 1994). However, in some cases with high soil fertility, the amount of N taken up by maize can be driven by the demand of shoot growth rather than by the size of the root system (Peng et al., 2010; Ning et al., 2012). In general, increasing application of N fertilizer will increase grain yield. However, this does not mean that the more N that is applied the higher the grain yield that can be achieved. A linear relationship between shoot N content and green leaf area has been reported for a wide range of crops (Lemaire and Gastal, 1997; Plénet and Lemaire, 1999; Lemaire et al., 2007). There is a critical N concentration, e.g. the minimum percentage of N in shoots required to produce the maximum aerial biomass at a given time (Plénet and Lemaire, 1999). Over application of N, cannot further increase shoot biomass and grain yield of maize plants (Boomsma et al., 2009). Under field conditions, our results demonstrate that in comparison with the optimized N application, conventional N supply (over application) inhibits root growth at both the early growth stage, the rapid growing stage cannot increase the final N content of the whole plant and thus the final grain yield. On the other hand, optimized N application involves not only controlling the total amount of chemical N fertilizer, but also synchronizing crop N demand and soil N supply by splitting the N applications (Peng et al., 2012). In addition, optimized N supply delayed root mortality at harvest, especially in the top 30 cm soil layer, compared with the conventional N supply. Thus, optimized N application can not only improve N use efficiency, but also reduce the risk of N leaching and potential environmental pollution (Ju et al., 2009; Vitousek et al., 2009; Mi et al., 2010; Zhang et al., 2012).

3.4. Maximizing rhizosphere efficiency
Rhizosphere processes reflect dynamic changes in rhizosphere biology and chemistry for the interactions between plants and soils. Controlling nutrient transformation, availability, and efficient use by plants Fig. (31).
Fig. 31: Rhizobiome diversity and effect on plant health. Photosynthates products of photosynthesis in the form of simple sugars for energy. Functions majorly in energy production. Phytosiderophores these enhance microbial activities in the soil. They relieve stress due to iron and zinc deficiencies through the acquisition of required iron and zinc for plant use. Polysaccharides the most important form in plant is starch. It is a form of energy storage that is more complex than simple sugars.

A better manipulation of rhizosphere processes may provide an effective approach for improving nutrient use efficiency and crop productivity simultaneously through exploiting biological potential for efficient acquisition and utilization of nutrients and reducing overreliance on the application of chemical fertilizers. The rhizosphere efficiency can be enhanced through optimizing nutrient supply. The rhizosphere efficiency, to some extent, can be repressed by either severe nutrient deficiency or excessive nutrient supply (Zhang et al., 2010).

3.5. Nitrogen management in farming system

In order to optimize fertilizer nitrogen inputs for high yields and minimized losses to the environment, accurate fertilizer requirements by the crop must be predicted. The fertilizer requirement is dependent on both crop requirement and nitrogen supply by the soil. The nitrogen supply from the soil completely varies between years and site, depending on soil and weather parameters. Ratjen and Kage (2018) studied the effects of climatic and site-related factors on the nitrogen mineralization during growing season in a large number of winter wheat experiments. The study revealed that both, climate and soil fertility were important factors for prediction of the effective nitrogen mineralization and have a significant effect on the soil nitrogen supply. Consequently, these factors carry valuable information for planning nitrogen fertilizer applications. The soil nitrogen supply does not only vary between farms and field, but also within the field. Córdova et al., (2018) studied the spatial correlation of nitrogen mineralization within an individual field, in order to guide future spatial sampling of nitrogen supply. Their data for a cereal cropping system in Chile showed that significant variation in net nitrogen mineralization existed, even at small spatial scale (1.5 m).

Importantly, almost all of the variation was accounted for at a 40.5 m sampling distance. Furthermore, as no difference in variation was observed between autumn and spring, concluding that sampling can be done at any time during the crop-growing season. Taking soil samples at high spatial resolution can be very expensive and time consuming. As an alternative, crop sensors can be used to
assess crop available nitrogen. These sensors, allowing quick and dense sampling and using crop parameters as an indicator of nitrogen supply, can be a good solution for assessing the site-specific N fertilizer requirements and its variation within individual fields. This was studied by Aranguren et al., (2018), who tested two commercially available proxy tools (crop sensors) for the nitrogen nutritional status of crops in winter wheat that had received manure at an earlier stage. The tools were shown to be as good indicators as soil mineral nitrogen or nutrition index, demonstrating their practical usefulness. Precision nitrogen management with organic fertilizers involves an additional challenge, as the organic fertilizer is usually not as well defined and predictable as the mineral fertilizers. Nitrogen Fertilizer Replacement Value (NFRV) is a measure for nitrogen supply from organic amendments to crops Jensen (2013), representing the amount of mineral N fertilizer replaced by the organic amendment. Hijbeek et al., (2018) Fig. (32).

Using data from long-term experiments in Europe and showed that NFRV were higher at high total nitrogen supply than at low total nitrogen supply.

Efficient use of nitrogen (N) requires careful matching of N supply to crop demand. Often application of mineral fertilizers is combined with application of organic amendments such as farmyard manure (FYM), slurries, and crop residues (also called organic manures, organic fertilizers or organic inputs). N in organic amendments generally has a lower availability to crops than N in mineral fertilizers, mainly depending on the C: N ratio of the amendment (Flavel and Murphy, 2006). Therefore, N in organic amendments must be carefully matched with mineral fertilizer N application to avoid leaching while making sure sufficient N is available for crop growth. This requires the characterization of the organic amendments by their Nitrogen Fertilizer Replacement Value (NFRV), also called the Mineral Fertilizer Equivalent Jensen (2013). NFRV can be based on the amount of organic amendment-N (kg/kg) while attaining the same crop yield (Herron and Erhart 1965; Schroder 2005a; Schilling, 1987). Crop yields can be expressed as fresh matter weights (FM), dry matter weights (DM) or N contents N yields, (Jensen, 2013). These are all valid procedures, with the difference that values of NFRV based on N yields are often slightly lower than those based on DM or FM weights (Jensen, 2013). N in organic amendments is always accompanied by other nutrients, such as phosphorus (P), potassium (K) or sulphur (S) which also affect crop yields. It is therefore important to exclude these effects when estimating values of NFRV based on yields either DM, FM or N yields, Schröder (2005a). Lory et al., (1995) have suggested calculating NFRV using economic optimal N rates (mineral fertilizer N application rates at which marginal crop yields offset marginal fertilizer costs) with and without organic amendments. Yields at economic N rates with and without organic amendments might however differ, which hampers comparison at equal yield levels. In addition, economic N rates are dependent on prices of fertilizers and harvested products,
which makes estimations less robust through time and space. Another manner to determine NFRV of organic amendments is by using isotope dilution techniques. Using $^{15}$N labelled materials, the fate of N from either organic amendments or mineral fertilizers is measured among plant and soil fractions and compared (Dickmann et al., 1993; Janzen et al., 1990). Additional methods to calculate NFRV (such as the analysis of near infrared reflectance spectra of organic amendments) have been proposed but need further development (Delin et al., 2012). Values for NFRV differ when estimated in the first year of application of organic amendments (short-term NFRV) or after repeated applications and several years (long-term NFRV, Gutser et al., 2005; Schroder (2005b), with higher values found for long-term NFRV. For FYM, NFRV ranges between 0.10 and 0.70 Birkmose 2009; Jensen (2013); Pikula et al., (2016); Webb et al., (2013). For slurry, NFRV ranges between 0.20 and 0.90 Birkmose (2009); Delin et al., (2012); Jensen (2013); Kundler et al., (1989); Langmeier et al., (2002); Webb et al., (2013). For straw, NFRV has been estimated to be around zero (Katyal, 1993). For green manures, NFRV has been estimated ca. 0.4 Janzen et al., (1990), but this will probably depend on the species of green manure cultivated. Factors known to affect NFRV (at a given dose of the amendment) include the form of N in the amendment, crop type cultivated, soil type, method of application, time of application and the manuring history which may govern N retention and losses (Birkmose, 2009; Jensen 2013; Katyal 1993; Kundler et al., 1989; Webb et al., 2013). Here we evaluate the effect of an additional factor on NFRV that is currently not taken into account: the total nitrogen supply. Therefore, requirements of mineral nitrogen fertilizer in fields with organic amendments might today be overestimated, causing an overuse of nitrogen fertilizers and leading to nitrogen losses to the environment.

4. Nitrogen assimilation by Plants

Nitrate is the principal nitrogen source for most wild and crop species, whatever the source of inorganic or organic nitrogen provided to the plant (Salsac, et al., 1989; Nåsholm, et al., 2009). It is taken up by means of specific high and low affinity transporters located in the root cell membrane (Miller et al., 2007; Dechorgnat et al., 2011). Nitrites are then reduced to nitrite through the reaction catalyzed by the enzyme nitrate reductase (NR; EC 1.6.6.1), Kaiser followed by the reduction of nitrite to ammonia catalyzed by the enzyme nitrite reductase (NiR; EC 1.7.7.1). Under particular environments, root ammonia transporters Sétif, et al., (2009), can allow a direct uptake of ammonia when available in the soil, in rice paddy fields or in acidic forest habitats (Salsac et al., 1989; Mae et al., 1997). Ammonia can be generated inside the plant by a variety of metabolic pathways such as photorespiration, phenylpropanoid metabolism, utilization of nitrogen transport compounds and amino acids catabolism. Symbiotically fixed nitrogen is also an important source of ammonia readily available to herbaceous plants or woody species that are able to form a symbiotic relationship with nitrogen-fixing microorganisms Fig. (33, 34).

Fig. 33: Relationship between ammonium and nitrate uptake and cytosolic pH. AMT1 is a plasma membrane (PM) ammonium transporter functioning either as an ammonia channel or as an ammonium uniporters or symporters with H.
4.1. Mechanisms of nitrogen transport

Transport of nitrogen from root to shoot takes place in the xylem, while nitrogen partitioning from source leaves to sinks occurs in the phloem. Sink organs generally display little xylem import because of their low transpiration rates van Bel, (1984). Phloem loading takes place in the collection phloem of the leaf minor vein networks van Bel, (1996) and phloem unloading happens in the release phloem of sink organs. The transport phloem interconnects the collection phloem with the release phloem, and represents the largest component of the phloem network. It accommodates loading and unloading as well as exchange of nitrogen compounds between the phloem and xylem along the transport path in roots, stem, leaf major veins and pod wall (van Bel, 1984).

Fig. 34: Represents mechanisms of transport and uptake of NO$_3^-$ (A), NH$_4^+$ (B) and urea (C) in Arabidopsis.

For the sequence of transfer from soil to source to sink, plasma membrane-localized transport proteins are essential in source and sink. They regulate nitrogen root uptake, root-to-shoot and leaf-to-sink transport, and seed loading (Tegeder, 2014; Fan et al., 2017). Nitrogen availability and utilization for vegetative growth mainly depend on nitrogen uptake from the soil, assimilation and transport to sinks. During the reproductive phase, nitrogen utilization by seeds relies on how much soil nitrogen is still available and taken up, as well as how much nitrogen is accessible from proteins and transient storage pools in source leaves, stems or roots and remobilized as amino acids (or ureides) for reproductive sink development (Masclaux-Daubresse et al., 2010).

Transporters involved in soil-root-to-leaf-to-seed allocation of inorganic nitrogen (i.e. nitrate and ammonium) and organic nitrogen (i.e. amino acids and ureides), their role in source and sink physiology, and their importance for plant productivity Fig. (35). Low- and high-affinity nitrate transporters Fan et al., (2017) mediate nitrate uptake from the soil. Nitrate transporters of the Nitrate Transporter1 (NRT1) family, recently renamed the Nitrate Transporter1/Peptide Transporter Family (NPF) Leran et al., (2014), are low-affinity systems, with the exception of Arabidopsis NPF6.3/NRT1.1 and rice (Oryza sativa) NRT1.1B, which have both a low and high affinity for nitrate (Wang et al., 1998; Huang et al., 1999; Liu et al., 1999). Of 53 NPF/NRT1 proteins in Arabidopsis, 16 have been characterized so far. High-affinity nitrate transporters belong to the NRT2 family, seven of which have been studied (Fan et al., 2017). Members of the NPF/NRT1 and NRT2 transporter families are proton-coupled importers, except for the bidirectional NPF7.3/NRT1.5 transporter Lin et al., (2008), and NPF2.7/Nitrate Excretion Transporter1 (NAXT1), which mediates nitrate efflux (Segonzac et al., 2007). Additional nitrate transporters are found within the Chloride Channel (CLC) family, which consists of either anion channels or anion proton exchangers (De Angeli et al., 2006). In Arabidopsis, at least six transporters are involved in root nitrate uptake Fig. (36). NPF6.3/NRT1.1 (also called Chlorate resistance Protein 1 (CHL1) and NPF4.6/NRT1.2 mainly operate under high nitrate supply, while NRT2.1, NRT2.2, NRT2.4 and NRT2.5 function under nitrate starvation Tsay et al., (1993); Huang et al., (1996), (1999); Liu et al.,
The four NRT2 transporters take up c. 95% of the total nitrate under limited N supply, with NRT2.1 and NRT2.2 being the main contributors (Lezhneva et al., 2014). Based on expression and localization studies, NRT2.4 and NRT2.5 seem mainly to be involved in direct nitrate acquisition from the soil via the epidermis and cortex at the root hair zone, while NRT2.1 and NRT2.2 additionally import apoplastic nitrate into cortical and endodermal cells (Kiba et al., 2012; Lezhneva et al., 2014). Nitrate transporters have also been functionally characterized in tomato (Solanum lycopersicum), rice and maize (Zea mays) (Garnett et al., 2013; Fu et al., 2015; Xia et al., 2015; Fan et al., 2017). Because excess ammonium is toxic to plant cells, its uptake and assimilation are tightly regulated. Saturable high-affinity (i.e. Ammonium Transporters (AMTs)) and nonsaturable low-affinity uptake systems (i.e. aquaporins or cation channels) control ammonium transport and homeostasis in plants. Six AMT genes were found in Arabidopsis Gazzarrini et al., 1999, in rice Sonoda et al., (2003), 14 in poplar (Populus trichocarpa) Couturier et al., (2007) and three in pine (Pinus pinaster) Castro-Rodriguez et al., (2016). In Arabidopsis, four AMTs function in ammonium root acquisition, with AMT1;1, AMT1;3 and AMT1;5 being involved in direct soil uptake Loque et al., (2006); Yuan et al., (2007a); Fig. (31). AMT1;2 is expressed in cortical and endodermal cells and mediates apoplastic absorption of ammonium. Collectively, AMT1;1, AMT1;2 and AMT1;3 import up to 95% of the ammonium. In rice, OsAMT1;1, OsAMT1;2 and OsAMT1;3 play a role in root ammonium uptake (Li et al., 2016). OsAMT1;1 and OsAMT1;2 expression is up-regulated in response to high ammonium concentrations, whereas OsAMT1;3 is expressed under N deprivation, suggesting its function in rice adaption to low-ammonium environments (Ferreira et al., 2015).

Fig. 35: Schematic representation of nitrogen Chloroplast (N) root uptake and partitioning from root to leaves. Inorganic N transporters for nitrate (NO$_3^-$; arrow with orange circle) and ammonium (NH$_4^+$; green circle), as well as organic N transporters for amino acids (AA; yellow circle) and ureides (Ur; blue circle) are shown at root, nodule, xylem, phloem and source leaf levels. Arrows for the respective transporters either refer to uptake of N from the apoplast and import into the cell, or indicate cellular efflux. Listed are characterized Arabidopsis nitrate, ammonium, amino acid and ureide transporters that are involved in N (1) root uptake, (2) root efflux, (3) movement from the root or nodule to the xylem and xylem loading, (4) root reimport, (5) xylem removal and xylem–phloem transfer, (6) import into leaf mesophyll cells, (7) import into leaf vacuoles or (8) exchange across the chloroplast envelope. Question marks indicate unknown transporters. For details, see Sections I, II and III in the main text. The nomenclature of the nitrate transporter genes has recently changed Leran et al., (2014); both the terms Nitrate Transporter/Peptide Transporter Family (NPF) and NitrateTransporter1 (NRT1) are used in the figure. AAP, Amino Acid Permease; AMT, Ammonium Transporter; AVT, Amino Acid Vacuolar Transporter; CLC, Chloride Channel; DiT, Dicarboxylate Transporter; LHT, Lysine/ Histidine-like Transporter; NAXT, Nitrate Excretion Transporter; ProT, Proline and Glycine Betaine Transporter; UmamiT, Usually Multiple Acids Move In and Out Transporter; UPS, Ureide Permease. After Tegeder and Masclaux-Daubresse (2018)
4.2. Amino Acid Uptake, Transport, and Distribution

4.2.1. Transporters mediating root amino acid uptake

Low and high affinity transporters mediate amino acid uptake from the soil. Following uptake or biosynthesis in the roots, amino acids then move from root hairs or epidermal cells to the vascular cylinder via the symplasm Fig. (36). Alternatively, transporter-mediated import into the root symplasm takes place at or before the endodermis, since the Casparian strip blocks apoplastic flow to the root vasculature.

![Diagram illustrating amino acid transport](image)

**Fig. 36:** Illustrates a model of amino acid transport in roots and the role of AAP1. Amino acid transport in roots may occur apoplastically until the organic nitrogen compounds reach the Casparian strip of the endodermis, which blocks apoplastic flow. For passage, the organic nitrogen has to be loaded into the symplast, presumably by transporters including AAP1, which are located in membranes of the root cortex and/or endodermis cells. After passing the endodermis, the amino acids are transported to the vascular cylinder and translocated in the xylem with the transpiration stream to the shoot. Amino acids are also predicted to be loaded into the phloem to supply the developing root tips with nitrogen. AAP2 and AAP3 are probably responsible for this loading step (Hirner et al., 1998; Okumoto et al., 2004). In addition, transport of amino acids can be via the symplast after import into the root epidermis cells, root hairs and root cap cells, respectively. Our studies indicate that AAP1 is involved in this process. Other amino acid transporters might also function in amino acid uptake via the epidermis and root cap, including LHT1 (Hammes et al., 2005; Hirner et al., 2006). After Yong-Hwa et al., (2007).

For xylem loading, amino acids are released into the apoplasm from the endodermis, pericycle, or xylem parenchyma cells using export proteins. In Arabidopsis, at least five amino acid transporters play a role in amino acid uptake in roots and belong to three families within the AAAP group: AAPs, LHTs, and
ProTs. Arabidopsis AAPs and LHTs are broad substrate transporters for neutral and acidic amino acids, whereas ProTs specifically transport proline, glycine, and amino butyric acid (GABA) (Grallath, et al., 2005; Fischer, et al., 2002). Arabidopsis AAP1 localizes to the root tip and epidermal cells, including root hairs, and transports glutamate and neutral amino acids Fischer et al (2002) Lee et al., (2007), Molly et al (2024) Fig. (37).

**Fig. 37**: Schematic overview of nitrogen (N) transport processes and source sink relationships at the whole-plant level. N fluxes from soil to root to leaf to sinks involve short- and long-distance transport of inorganic N (nitrate (NO$_3^-$), ammonium (NH$_4^+$) and di-nitrogen (N2)) and organic N (aminoacids (AA) and ureides (Ur)). The xylem and phloem connect sources with sinks and are essential for N mobilization. The smaller font size of xylem NH$_4^+$ and phloem NO$_3^-$ refers to their lower concentration compared with other transported N compounds. Gray arrows indicate feedback controls exerted by source and sink on N uptake and partitioning, respectively. After Tegeder (2014).

Arabidopsis AAP5 is expressed in the root and functions in the acquisition of basic amino acids Svennerstam, et al., (2011), Birnbaum et al., (2003), whereas Arabidopsis AAP3, expressed in the root vascular tissue, may be involved in amino acid uptake from the phloem or the soil (Fischer et al., 1998). LHTs are considered high affinity transport systems. Bush (1997) documented expression of Arabidopsis LHT1 at the root surface and assigned it a lysine and histidine-selective transporter function, although other studies described a role in the uptake of neutral and acidic amino acids into roots for LHT1 (Himer, et al., 2006; Ganeteg, et al., 2016). In addition, histochemical analysis of pLHT1: GUS (β-GLUCURONIDASE) reporter lines revealed that Arabidopsis LHT1 is preferentially expressed in the lateral root cap (Himer, et al., 2006). The Arabidopsis root expression map detects LHT1 expression in the root epidermis, cortex, and endodermis during early plant development, which supports a direct role for LHT1 in amino acid import into root cells (Brady, et al., 2007). In rice, LHT1 is expressed throughout the root, including root hairs, the epidermis, cortex, and stele, as demonstrated by GUS reporter lines. Knockout of OsLHT1 by genome editing in japonica rice exhibited reduced root uptake of amino acids (Guo, et al., 2020). Other studies showed that rice AAP3 and AAP6 are expressed in the elongation zone of lateral roots, root stele, and epidermis, and function as regulators of amino acid levels in roots Lu, et al., (2018), Peng et al., (2014). Arabidopsis LHT6 is highly expressed in root cells and contributes to the assimilation of acidic amino acids, glutamine, and alanine from the rhizosphere Molly et al., (2014). In addition, Arabidopsis ProT2 is expressed in the root epidermis and cortex, where the encoded protein functions in the import of the compatible solutes proline and glycine betaine (Grallath, et al., 2005; Peng et al., 2014; Lehmann, et al., 2011). Based on the specific expression patterns mentioned in the above studies of AAP and LHT genes in the root, we generated a model of amino acid uptake in the root Fig. (38). However, the expression profile of individual transporters does not explain all aspects of amino acid uptake, as several studies have confirmed that transporter activity in the root may vary depending on soil conditions and plant species. For example, LHT1 and AAP5 are crucial for amino acid uptake at concentrations in the soil below 50 µM Transport studies with aap1 mutants suggest that AAP1 may take up amino acids at high concentrations.
(Svennerstam et al. (2008; Lee et al. 2007 and Forsum et al., 2008). In addition, AAP1 functions in the acquisition of glutamate and neutral amino acids when present in the soil at ecologically relevant concentrations, whereas LHT6 is involved in the import of the acidic amino acids alanine and aspartate by roots at both low and high concentrations (Molly et al., 2014).

Fig. 38: Represents histochemical GUS staining of GmAAP6a:GmAAPa-GUS soybean plants. The GUS signal was observed in germinating radicles (a), taproots and lateral roots of 4- (b) or 10-day-old seedlings (c). The GUS signal was also found in vasculatures of leaves and newly developing buds (c). Bright-field images of cross sections showed that GUS signals were predominant in phloem and xylem parenchyma cells of the leaf (d, e) and stem (f, g) and in root phloem (h, i). In developing pods, GUS signals were predominant in vasculatures and peaked at 18 days after pollination (j-l). The GUS signal was also found in the funicle and the adjacent seed coat at 28 days after pollination (m), but not in developing embryos (n). Short black lines in (b, c) indicate the section site for (d-i). Scale bars = 1 cm (b-c, j-l), 3 mm (a, m and n), 100 lm (d, f and h) or 50 lm (e, g and i). Ph: phloem; Xy: xylem; Xp: xylem parenchyma. After Liu, et al., (2020).

4.2.2. Transporters Function in Xylem–Phloem Transfer and Intercellular Transport of Amino Acids

Identification of amino acid transporters is essential to understand how they regulate nitrogen root uptake, as well as root to shoot and leaf to sink transport. Root to shoot movement of amino acids occurs in the xylem, whereas amino acid partitioning from source leaves to sink organs takes place in the phloem Fig. (39).
Fig. (39) Summary of the role of the characterized amino acid transporters in plants. The role in Uptake by roots, Phloem export in root, Phloem loading, Xylem-Phloem transfer, Seed Development and Intracellular Transport is depicted for amino acid transporters recently identified and mentioned in the main text (in rounded boxes) and for amino acid transporters previously. Black arrows refer to direction of transport when known.

Fig. 40: Represents schematic diagram of nitrate transport steps within the plant.
However, some amino acids can also be removed from the long-distance transport mediated by the xylem and transferred to the phloem to supply fast growing sink organs, such as root tips and young leaves (Pate, et al., 1975; Atkins, and Biochemical, 2000). This transfer between xylem and phloem requires the retrieval of amino acids from the transpiration stream (xylem) to xylem parenchyma cells, with subsequent symplastic movement to phloem parenchyma cells. Ultimately, amino acids are released into the phloem sap (Tegeder and Masclaux-Daubresse, 2018; Tegeder, and. Transsporters 2014 and Offer et al., 2003). Arabidopsis AAP6 localizes to the xylem parenchyma, where it mediates N exchange between the xylem and phloem, as evidenced by the reduced amino acid concentrations in the phloem of aap6 mutants (Hunt et al., 2010). In addition, Arabidopsis AAP2 is expressed in phloem companion cells along the transport path, and aap2 mutants displayed reduced organic N supply to seed sinks, leading to reduced seed protein levels Zhang et al., (2010) Fig. (40). Once in the leaf, amino acids are imported into parenchyma or mesophyll cells surrounding the xylem by the action of Arabidopsis LHT1 (Himer, et al., 2006). In addition to long-range transport, amino acids synthesized inside cells move across various organelles, which requires intracellular transporters. Fusion proteins between transporters and the green fluorescent protein (GFP) have demonstrated that several amino acid transporters localize to organelar membranes rather than the plasma membrane.

Identification of vacuole transporters is the focus of much research in multiple plant species. In Arabidopsis, the cationic amino acid transporters CAT2 and CAT4 localize to the vacuolar membrane (tonoplast) and CAT2 is implicated in the regulation of amino acid levels in leaves (Yang, et al., 2014, Su, et al., 2004). Arabidopsis CAT8 localizes to both the plasma membrane and the tonoplast (Yang et al., 2010). In tomato, CAT9 was identified using quantitative proteomics of a tonoplast-enriched membrane fraction. Tomato CAT9 is a tonoplast exchanger that transports glutamine and aspartate into the vacuole lumen in exchange for amino butyrate (GABA), and plays a role in amino acid accumulation during fruit development (Snowden et al., 2015). Another tomato member of the CAT family, CAT2, localizes to the tonoplast in stamen cells, indicating a role in flower development Yang, et al., (2012) Outside of the CAT family, members of the Amino acid Vacuolar Transport (AVT) sub-group may also function at the tonoplast, since homologues from yeast can mediate amino acid transport across the vacuolar membrane Russnak, et al., (2001) One of the Arabidopsis homologues, AVT3, transports alanine and proline from the vacuole into the cytosol when expressed in yeast (Fujiki, et al., 2016).

In addition, the Arabidopsis transporters DICARBOXYLATETRANSPORT (DiT2.1) and the MITOCHONDRIALBASIC AMINO ACID CARRIERs (m BAC) mBAC1 and mBAC2 localize to the chloroplast and mitochondrial membranes. DiT2.1 function in malate/glutamate exchange during photorespiration (Petra, et al., 2003). The two m BACs transport arginine, lysine, ornithine, and histidine by an exchange mechanism (Palmieri et al., 2006).

4.2.3. Transporter Function in Phloem Loading of Amino Acids

In leaves, amino acids are synthesized from inorganic N and photosynthates. Alternatively, amino acids are also synthesized from photorespiration and the hydrolysis of leaf proteins Rentsch, (2010) Following synthesis, amino acids are released into the cytosol by transporters, transported in the phloem to sink tissues, or stored in the vacuole (Tegeder, 2012). To be exported out of leaves, amino acids are loaded into the SE/CC complexes of minor veins. Loading of amino acids into the sieve elements and companion cells of the phloem may follow an apoplastic or symplastic route, depending on the plant species and the number of functional plasmodesmata connecting phloem parenchyma and companion cells (Tegeder, 2014; Turgeon, and Wolf, 2009). In the symplastic pathway, amino acids diffuse between cells through plasmodesmata towards the Phloem. During exoplasmic loading, amino acids first need to be released into the cell wall space and subsequently taken up by neighboring cells. This pathway relies on plasma membrane-localized amino acid transporters (Tegeder and Hammes, 2018; Tegeder and Transsporters 2014; Lalonde, and Tegeder, 2003). The AAP family of transporters has been proposed to facilitate import into the phloem Tegeder et al. (2012), whereas the bidirectional transporters SIAR1/UMAMIT18 (siliques are RED1/ usually multiple acids move in and out transporters) and bidirectional amino acid transporter 1 (BAT1) may mediate amino acid export from leaf cells (Ladwig et al., 2012; Dündar et al., 2009). It is currently believed that AAPs play a major role in phloem loading, as they transport a broad spectrum of amino acids, although their exact function remains to be investigated. In Arabidopsis, AAP1, AAP2, AAP3, AAP4, AAP5, and AAP8 are expressed in mature leaves and may be involved in the phloem loading process (Rentsch, 2010) Liu,
and Bush, 2006; Fischer et al., 1995; Santiago, and Tegeder, 2016). AAP1 and AAP4 are present in the phloem of leaf minor and major veins, whereas AAP8 is expressed in source leaves during the vegetative and reproductive phases. Moreover, the aap8 mutant reduces source-to-sink transport of amino acids, demonstrating that AAP8 is indeed fundamental for the loading of a broad spectrum of amino acids into the phloem to supply sink organs with essential N (Santiago, and Tegeder, 2016; Santiago, and Tegeder, 2017). A potential role of AAPS in phloem loading has also been observed in other plants. In pea, overexpression of AAP1 increased phloem loading of amino acids, resulting in improved source-to-sink N transport, enhanced sink organ development, and higher seed yield (Zhang, et al., 2014). Based on expression and localization studies, members of other transporter families are suspected to function in phloem loading. This includes the Arabidopsis transporters CAT1, CAT6, and CAT9, as well as members of the ProT family (Zhang, et al., 2014; Grallath et al., 2005; Rentsch et al., 1996; Lehmann et al., 2011; Su et al., 2004; Hammes, et al., 2006). By contrast, AROMATIC AND NEUTRAL TRANSPORTER1 (ANT1) may participate, directly or indirectly, in phloem loading, as the amino acid content of ant1 mutant sieve tubes rose sharply over wild-type levels (Hunt, et al., 2006).

4.2.4. Transporter nitrate to Amino Acids via Phloem

Most crop plants are considered apoplastic phloem loaders. This involves release of amino acids, ureides and/or nitrate from the parenchyma or bundle sheath cells into the leaf apoplast by a passive transport mechanism, followed by import into the phloem (Okamoto and Pilot, 2011; Tegeder, 2012; Yadav et al., 2015 Peuke, 2010; Kiba et al., 2012; Rennie and Turgeon, 2009). This step gradually requires activity of transport proteins located in the plasma membrane of companion cells. Transporter that export nitrate into the leaf/ phloem apoplast have not yet been identified, but Arabidopsis proteins functioning in nitrate phloem loading have been characterized and include NPF2.13/NRT1.7 Fan et al., (2009), NRT2.4 Kiba et al., (2012) and NRT2.5 Lezhneva et al., (2014). NPF2.13/NRT1.7 is localized to the SEs/CCs of the leaf minor veins and facilitates phloem loading in older leaves. NRT2.4 is close to the phloem of leaf major veins (probably in the phloem parenchyma) and, under nitrogen starvation, might retrieve nitrate from the apoplast to enable nitrogen movement towards the SEs/CCs. While its cellular localization is still unknown, NRT 2.5 is expressed in minor veins of Arabidopsis leaves and, together with NRT2.4, affects leaf remineralization and phloem transport of nitrate. NPF1.2/NRT1.11 and NPF1.1/ NRT1.12 are localized to the companion cells of the leaf major veins, where they may function in phloem loading, in addition to having a role in xylem-to-phloem transfer (Hsu and Tsay, 2013). Relatively little is known about transporters involved in amino acid export from leaves (Tegeder, 2014). Release of amino acids from the bundle sheath or vascular parenchyma cells may involve UmamiTs, the bidirectional amino acid transporter (BAT1) Dündar and Bush, (2009), or other systems, but at present this efflux function has only been shown for UmamiT18/SIAR1 Ladwig et al., (2012) Fig. (41). UmamiT18 exports glutamine, and potentially other amino acids, and affects N delivery to sinks. Uptake of amino acids from the leaf apoplast into the SE/CC complex probably involves AAPS, as some have been localized to the phloem of Arabidopsis, pea and common bean (Phaseolus vulgaris) (Tegeder et al., 2007; Tan et al., 2008; Tegeder and Rentsch, 2010; Tegeder and Ward, 2012). In fact, recent work in Arabidopsis demonstrated that AAP8 plays a key role in amino acid phloem loading and that its function strongly affects sink size and number (Santiago and Tegeder, 2016, 2017). Similarly, bean UPS1 transporters are expressed in the leaf phloem and are predicted to control source-to-sink partitioning of ureides (Pelisser and Tegeder, 2007).

A few studies have been done on the uptake of organic nitrogen by commercial crops: e.g., corn Biernath, et al., (2008), agricultural grasses including species of clover Näsholm, (2000) and wheat. Despite these limited studies, they demonstrate the ability of plants to directly take up organic N, but have not established the importance and significance of organic N as a source of crop N, for example, when they are grown under organic farming conditions. Main reactions involved in nitrogen assimilation in higher plants. \( \text{NO}_3^- \) (nitrate); \( \text{NO}_2^- \) (nitrite); \( \text{NH}_4^+ \) (ammonium), \( \text{N}_2 \) (atmospheric dinitrogen). This indicates that the spectrum of nitrogen compounds that can be taken up by the roots is quite diverse, indicating that the relationships existing between the soil fauna and the plant for nitrogen capture is more complex than originally thought (Paungfoo-Lonhienne et al., 2007). Urea is a low molecular weight organic molecule containing nitrogen that exists in natural systems and is applied as a synthetic fertilizer in conventional agriculture.
Nitrate reduction and ammonia assimilation are the main enzymes indicating in italics: NR - nitrate reductase; NiR - nitrite reductase; Nase - nitrogenase; GS - glutamine synthetase; GOGAT - glutamate synthase. The ultimate source of inorganic N available to the plant is ammonium, which is incorporated into organic molecules in the form of Glutamine and Glutamate through the combined action of the two enzymes GS and GOGAT. Plants can take organic nitrogen; there is also an interesting report in which it has been shown that herbaceous species can use protein as a nitrogen source without the assistance of other organisms. It is well known that urea is absorbed as an intact molecule by plant leaves and roots Tan et al., (2000) by means of specific root transporters (Kojima et al., 2006, 2007). Excellent progress has been made over the years in dissecting factors essential for plant nitrogen nutrition with special focus on inorganic nitrogen transport, metabolism and their regulation.

In comparison, organic nitrogen partitioning has received relatively little consideration, even though amino acids and ureides are the main long-distance N transport forms and they regulate inorganic nitrogen uptake and assimilation. Bearing in mind these essential roles, it seems sensible that increased transporter function in source-to-sink partitioning of amino acids and ureides has serious consequences for nitrogen acquisition, source metabolism and sink strength Fig. (41). Obviously, accumulation of specific amino acids (or ureides) and negative feedback on inorganic nitrogen transporter expression and metabolic enzymes can be avoided by increasing organic nitrogen flux towards sinks. Plant growth strongly relies on the relationships between source and sink, and associated feedback and feed-forward regulatory mechanisms, both source and sink strengths are influenced by nitrogen transporter activity. Nitrogen supply to sources affects leaf carbon fixation, assimilation and allocation to sinks, and controls short and long-term nitrogen storage pools. Sink strength is influenced by phloem nitrogen concentrations affecting sink number (e.g. fruits and seeds), and by seed nitrogen loading regulating sink size and protein content. Relatively few studies on nitrogen transport have

Fig. 41: Schematic representation of organic and inorganic nitrogen (N) partitioning from source leaves to seeds. Transporters of nitrate (NO₃; arrow with orange circle), amino acids (AA; yellow circle) and ureides (Ur; blue circle) are indicated. Arrows for the respective transporters refer to either uptake of N from the apoplast and import into the cell, or cellular efflux. Listed are Arabidopsis nitrate and amino acid transporters that are involved in N (1) leaf export and phloem Loading, (2) phloem unloading, (3) seed loading, or (4) seed vacuolar storage. Question marks indicate unknown transporters. The nomenclature of the nitrate transporter genes has recently changed Leran et al., (2014); both the terms Nitrate Transporter1/Peptide Transporter Family (NPF) and Nitrate Transporter1 (NRT1) are used in the figure. AAP, Amino Acid Permease; CAT, Cationic Amino Acid Transporter; GOGAT, Glutamine Oxoglutarate Aminotransferase; GS, Glutamine Synthetase; NIR, Nitrite Reductase; NR, Nitrate Reductase; UmamiT, Usually Multiple Acids Move In and Out Transporter After Tegeder and Masciaux-Daubresse (2018).
considered that the origin of nitrogen used by sinks may shift throughout the plant’s life cycle, from initial soil or atmospheric nitrogen to remobilized nitrogen resources. While root uptake and root to shoot transport systems for inorganic nitrogen are crucial for vegetative growth, and to build nitrogen storage pools, their contribution to plant nitrogen nutrition during the reproductive phase is less clear. Amino acid (and ureide) transporter function in source-to-sink nitrogen distribution is important throughout development, but most certainly requires adjustments during leaf senescence and the seed-filling phase to maximize nitrogen remobilization and seed protein yields. Although both inorganic and organic nitrogen transporters are critical bottlenecks for root to leaf to sink nitrogen flux, and source and sink metabolism, little is known about their relative contributions to plant performance and how they affect each other’s function. Crop improvement strategies will need to simultaneously consider inorganic and organic nitrogen transport systems, their temporal expression, localization, and interrelationship, to fine-tune nitrogen uptake, metabolism, and transient storage and completely plant partitioning. Modern tools including CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) - Cas (CRISPR-associated proteins) technology, high-throughput sequencing, metabolomics and phenomics will help to further understand and integrate N partitioning processes with source and sink physiology and development, and select for the most promising candidates for optimized productivity and efficient N use. Although the use of urea is mainly as a source of N fertilizer, the contribution of plant urea uptake and metabolism in a physiological and agricultural context is still not investigated. However, plants possess urea transporters, and can hydrolyze and use urea very efficiently (Witte, 2010). The importance of AM fungi for nutrient uptake by plants is well-documented (Smith, and Read, 2008; Peay, 2010; Several studies have shown that AM fungi-infected plants can take up organic N compounds Hodge, et al., 2000; Näsholm, et al., (1998, 2000). Thus, AM fungi can be used as a source of biological fertilization, since they are able to develop symbiotic associations with most terrestrial plants. They are able to alleviate the effects of different stresses on both growth and yield, by significantly increasing the uptake of water and nutrients (including N) by the host plant (Tanaka, and Yano, 2005; Miransari, 2011). In particular, it has been reported that the hyphae of AM are able to use inorganic N more efficiently, thus enabling the host plant to indirectly have access to soil N through its fungal collaborate Tobar et al., (1994). Miransari, (2011), reported that the quantitative contribution of AM fungi to the direct uptake of organic N by plants is not well established even though recent progress have been made in this field of research. Nevertheless, Tian et al., (2010) showed that AM fungi were able to absorb both organic and inorganic N and synthesize organic N molecules such as arginine that are further released by the fungal hyphae and then absorbed by the host plant. Makarov, (2019) reported that, role of mycorrhizal symbiosis as a control of the biogeochemical cycle of nitrogen in soils and in the nitrogen nutrition of plants is considered. The contribution of ericoid mycorrhiza (ErM) and ecto mycorrhiza (ECM) to nitrogen (N) supply of host plants is well known, whereas the role of arbuscular mycorrhiza (ArM) is insufficiently understood.

The contribution of ericoid mycorrhiza (ErM) and ecto mycorrhiza (ECM) to nitrogen (N) supply of host plants is well known, whereas the role of arbuscular mycorrhiza (ArM) is insufficiently understood. Exoenzymes released into the soil from the ErM and EcM mycelium favor the hydrolysis of high-molecular-weight N-containing organic compounds of plant litter and soils to or amino acids that are then transported toward plant roots and are absorbed by them. ArM-producing fungi have a limited capacity to release hydrolytic enzymes capable to decompose high-molecular-weight organic compounds into the soil (or do not have it at all). Therefore, they are specialized on the absorption of inorganic forms of N and amino acids appearing in the soil in the course of decomposition of high molecular weight N-containing compounds by saprotrophic microorganisms. The activity of hydrolytic Exoenzymes and the role of mycorrhiza in the nitrogen nutrition of plants become more significant under conditions of the low supply with mineral N compounds and decrease upon the rise in availability of mineral N compounds Fig. (42).
Fig. 42: Schematic diagram showing material transport and signal transduction-mediated source–sink interaction of cereal crops after heading. Root is the only source of inorganic nitrogen, and a major source of CKs. Root can produce a proportion of organic nitrogen and transport it to the shoot through the xylem, but it may also import amino acids from the shoot through the phloem. The growth and functional maintenance of the root depend on sugar supply from the shoot. Mature leaf is the major source of carbon and organic nitrogen. Root, grains, and the leaf itself through source–sink interaction with or without environmental stresses (see the text) can regulate leaf senescence. During leaf senescence, a large amount of organic nitrogen can also be remobilized and reused. Storage cells located in the stem, leaf sheath (e.g. phloem parenchyma cells) form the temporary reserve pool, which can store carbon and nitrogen when the phloem carbon and nitrogen level is high, and release them when the opposite is the case. Grains gradually become the major sink of carbon and nitrogen after anthesis. Carbon and nitrogen supply can greatly influence grain number during panicle formation, floret fertility at anthesis, and grain setting during grain filling. During anthesis and grain filling, a substantial amount of CKs can be synthesized to regulate grain cell proliferation and starch accumulation. Developing florets and/or grains may produce certain (unknown) signal(s) to regulate leaf senescence directly, in spite of their drain of leaf nitrogen.

At the same time, mycorrhizal fungi and host plants may compete for the limited resource. The isotopic composition of N in plants and the fractionation of $^{15}$N isotope between the mycorrhizal fungi and host plants are considered indicative of the participation of mycorrhiza in the nitrogen nutrition of plants. Interestingly, the occurrence of a transfer of symbiotically fixed N to a crop such as maize via vesicular-AM hyphae has been demonstrated Frey, and Schüpp, (1992) Bonfante and Genre, (2010), indicating that associated or continuous cover cropping systems could be an alternative way to rationalize plant nitrogen nutrition by optimizing field conditions favorable to mycorrhizal colonization Fig. (43), (44).
Fig. 43: Illustrates Plant uptake and mycorrhizal uptake pathway. Plants can take up nutrients by transporters that are located in epidermis or root hairs (yellow symbols) or via the mycorrhizal uptake pathway that comprises the uptake of nutrients by fungal transporters in the extraradical mycelium (red or green symbols), the transport through the hyphae from the ERM to the IRM (see mycorrhizal interface), and the uptake from the mycorrhizal interface by mycorrhiza-inducible plant transporters in the peri arbuscular membrane (orange symbols). Indicated by the red and green fungal structures is the colonization of one host root by multiple fungal species that can differ in their efficiency with which they are able to take up nutrients from the soil and transfer these nutrients to their host.
Ammonia, is the form of inorganic nitrogen available to the plant, is then incorporated into the amino acid glutamate through the action of two enzymes. First reaction by enzyme glutamine synthetase (GS; EC 6.3.1.2) Lea, and Miflin, (2011), that is considered to be the major route which facilitating the incorporation of inorganic N into organic molecules in conjunction with the second enzyme glutamate synthase (GOGAT; EC 1.4.7.1) (Suzuki, and Knaff, 2005). The amino acids glutamine and glutamate are used as amino group donors to all the other N-containing molecules notably. Other amino acids used for storage, transport and protein synthesis and to nucleotides using as basic molecules for both RNA and DNA synthesis Suzuki, and Knaff, 2005; Hirel et al. 2001). The two enzymes GS and GOGAT are present in the plant in several isoenzymic forms located in different cellular compartments and differentially expressed in a particular organ or cell type according to the developmental stage. The GS enzyme exists as a cytosolic form (GS1) present in a variety of organ and tissues such as roots, leaves, phloem cells and a plastidic form (GS2) localized in the chloroplasts of photosynthetic tissues and the plastids of roots and etiolated tissues. It has also been proposed that GS2 is located in the mitochondria (Taira et al., 2004). However, in numerous studies using immunocytolocalization techniques, the presence of the enzyme in the mitochondria has never been reported (Dubois, et al., 1996). The relative proportions of GS1 and GS2 vary within the organs of the same plant and between plant species, each GS isoform playing a specific role in a given metabolic process, such as photo respiratory ammonia assimilation, nitrate reduction, N translocation and recycling (Lea and Miflin, 2011; Cren and Hirel 1999). The enzyme GOGAT also exists as two forms particularly in during primary N assimilation or N recycling. A ferredoxin-dependent isoenzymes (Fd-GOGAT) is mainly involved, in conjunction with
GS2, in the reassimilation of photo respiratory ammonia and a pyridine nucleotide-dependent isoenzymes (NADH-GOGAT; EC 1.4.1.14) involved in the synthesis of glutamate in either photosynthetic and non-photosynthetic organs or tissues to sustain plant growth and development (Lea, and Miflin 2011; Hirel et al. 2001). Moreover, by virtue of their differential mode of expression regulated either at the transcriptional and post transcriptional levels, both GS and GOGAT isoenzymes have been shown to play a specific role at particular stages of the plant life cycle and under particular environmental conditions related mainly to the mode of N nutrition (Lea and Miflin, 2011; Suzuki and Knaff, 2005; Cren, and Hirel, 1999). The reversible reaction catalyzed by the enzyme glutamate dehydrogenase (GDH; EC 1.4.1.2) (Lea and Miflin, 2011), which has theoretically the capacity to incorporate ammonia into 2-oxoglutarate to form glutamate, was originally thought to be the main enzyme involved in inorganic N assimilation in plants. Later on, a number of experiments using 15N labeling techniques and mutants deficient in GS and GOGAT have demonstrated that over 95% of the ammonia made available to the plant is assimilated via the GS/GOGAT pathway Lea and Miflin, (2011), Lea, and Ireland, (1999)A number of 15N labeling experiments followed by GCMS or NMR spectroscopy analysis have shown that GDH operates in the direction of glutamate deamination to provide organic acids notably when the cell is C-limited (Aubert et al. 2001; Labboun, et al., 2009). The finding that under certain physiological conditions GDH is able to assimilate ammonia also needs to be taken into consideration, although the rate of glutamate synthesis is probably far lower than that formed through the GS/GOGAT pathway (Skopelitis et al. 2006). Recently the hypothesis that GDH plays an important role in controlling glutamate homeostasis has been put forward (Labboun, et al., 2009). This function, which may have a signaling role at the interface of C and N metabolism, may be of importance under certain phases of plant growth and development when there is an important release or accumulation of ammonia (Masclaux, et al., 2001; Tercê-Laforge, et al., 2004).

4.2.5. Physiological and morphological functions of nitrogen in plants

Mild nitrogen deficiency leads to elongation of lateral roots and the primary root, while severe or prolonged nitrogen deficiency inhibits primary root growth and total root length (Gruber et al., 2013) Fig. (45). Stimulation of lateral root growth during mild N deficiency has been shown to be auxin-dependent (Ma et al., 2014). During prolonged N deficiency, inhibition of lateral root growth is controlled by NRT1.1 that removes auxin from lateral root primordia inhibiting their growth. By contrast, high NO3- inhibits auxin transport away from lateral root primordia, thereby stimulating root growth (Krouk et al., 2010; Bouguyon et al., 2015). The local inhibition of lateral root growth during N starvation is, in addition to auxin, also regulated by CLAVATA3 (CLV3)/EMBRYOSURROUNDING REGION-related (CLE) peptides, which are induced to specifically inhibit emergence of lateral roots through a CLAVATA1-dependent signalling pathway Fig. (46) (Araya et al., 2014).

Two systemic Signalling pathways known as the nitrogen demand and nitrogen supply pathways promote root growth and N uptake in root parts exposed to high nitrogen (Poitout et al., 2018). In the nitrogen demand pathway, CTERMINALLY ENCODED PEPTIDES (CEP) peptides are produced in low nitrogen root parts that, via shoot-acting receptors and phloem-mobile Class III glutaredoxins polypeptides (CEPD1/CEPD2), promote root growth and N uptake in high N root parts (Tabata et al., 2014; Ohkubo et al., 2017).

Nitrogen supply pathway is initiated by synthesis and translocation of cytokinin from the root to the shoot, which both induces N-dependent leaf expansion and increased expression of NO3- transporters in high N roots (Poitout et al., 2018) NH4+ toxicity inhibits primary root growth, while local NH4+ may stimulate lateral root branching (Liu & von Wiren,2017). Nitrogen deficient plants exposed to a local supply of NH4+ respond by developing more second and third order lateral roots through a mechanism dependent on the NH4+ transporter AMT1;3 (Lima et al., 2010).
Fig. 45: Represents RSA responses to nitrogen availability. (A), Excess supply of ammonium ($NH_4^+$) or nitrate ($NO_3^-$) leads to a systemic repression of root growth, where high ammonium inhibits mostly PR elongation and high nitrate represses mainly LR elongation. Compared with sufficient nitrogen supply, mild nitrogen deficiency (2N) increases the lengths of PR and LRs, whereas severe nitrogen deficiency inhibits PR elongation as well as LR emergence and elongation. These distinct RSA responses likely reflect different strategies of the plants to cope with limited nitrogen availability. Figure based on Gruber et al., (2013). (B), Examples of signaling pathways involved in modulating RSA responses to the supply of nitrate to otherwise nitrogen-deficient plants (Vidal et al., 2010, 2013) and mild (Ma et al., 2014) or severe (Krouk et al., 2010; Araya et al., 2014) nitrogen deficiency. Details are in the text. Aux/IAA, AUXIN RESISTANT/ INDOLE-3-ACETIC ACID INDUCIBLE; miR393, microRNA393.
Fig. 46: Schematic model showing how nitrogen (N) affects plant growth and physiology. (1) NRT1.1 (NPF6.3) is a dual-affinity nitrate transporter that senses external nitrate concentrations. At high NO$_3^-$ the calcineurin B-like interacting protein kinase 8 (CIPK8) regulated dephosphorylation of NRT1.1, causing dimerization in a low-affinity state. Cytosolic Ca$^{2+}$ concentrations increase and the Ca$^{2+}$ signal is further transmitted by three calcium-sensor protein kinases (CPK): CPK10, CPK30 and CPK32 that phosphorylate Nin-like protein 7 (NLP7) in the nucleus. NLP7 induces expression of hundreds of genes as part of the primary nitrate response (PNR). At low NO$_3^-$, CIPK23 phosphorylates NRT1.1 resulting in a high-affinity monomeric state. (2) Spatial distribution and concentration of N affects root development. Mild N deficiency results in a root foraging response, that is existing lateral roots grow longer and deeper, a response caused by high auxin in root tips. At severe deficiency, root growth is inhibited through auxin removal from root tips. In addition, CLAVATA3/Embryo Surrounding Region-Related (CLE) peptides inhibit lateral root growth locally. Under conditions with heterogeneous N distribution, systemic signalling via CTERMINALLY ENCODED PEPTIDES (CEP-CEPD) from low N roots induces lateral root growth and N uptake in high N roots. Cytokinin promotes root proliferation in high N roots via root-shoot-root signalling. (3) N levels affect flowering time. (a) Mild N deficiency promotes flowering compared with severe starvation and high N levels. (b) Nitrogen availability and flowering are correlated via different pathways related to ageing, photoperiod, gibberellic acid (GA) and vernalisation, as well as the autonomous pathway. Central to this regulation is the flowering gene FLOWERING LOCUS T (FT). In the photoperiod pathway, low and high NO$_3^-$ oppositely affect expression of Ferredoxin-NADP$^+$-oxidoreductase 1 (FNR1) that positively affects FT via Constans (CO) and Cryptochrome 1 (CRY1). In the ageing pathway, the balance between miR156 and miR172 oppositely affects FT via expression of APATELA2 (AP2) and SPL. In the GA pathway, NO$_3^-$ modulates GA1 levels and DELLA protein activity that in turn affects flowering via AP2 and SPL in the ageing pathway. The vernalisation pathway and the autonomous pathway both inhibit FLOWERING LOCUS C (FLC) that in turn inhibits FT. (4) (left) Glutamate is the precursor for chlorophyll synthesis and is stimulated by nitrogen; (right) Nitrogen deficiency results in proteolysis and released amino acids are translocated through the phloem from old source leaves to young sink leaves. (5) Leaf growth and shoot branching are regulated by cytokinin in a NO$_3^-$ dependent manner. Biosynthesis of the root cytokinin trans-zeatin (tZ) and the cytokinin precursor trans-zeatineriboside (tZR) is correlated with nitrate levels. Root-derived cytokinin positively regulates shoot apical meristem (SAM) size and activity, and breaks auxiliary bud dormancy. Collectively this leads to more shoot branches and more leaves. In addition, cytokinin delays senescence and activates photosynthesis resulting in improved carbohydrate assimilation. (6) In some species, N deficiency induces anthocyanin biosynthesis in stems and leaves. Low N induces the expression of PRODUCTION OF ANTHOCYANIN PIGMENT (PAP) transcription factors via miR165-mediated down regulation of SQUAMOSA PROMOTOR BINDING PROTEIN-LIKE 9 (SPL9). High N represses the expression of PAP genes via NLP7-mediated expression of LATERAL ORGAN BOUNDARY DOMAIN (LBD) genes. Furthermore, DELLA proteins positively affect anthocyanin accumulation during N deficiency through repression of negative effects of gibberellic acid (GA) on anthocyanin biosynthesis. After, Thomas Christian de Bang (2020).
Nitrogen supply pathway is initiated by synthesis and translocation of cytokinin from the root to the shoot, which both induces N-dependent leaf expansion and increased expression of NO$_3^-$ transporters in high N roots (Poitout et al., 2018). NH$_4^+$ toxicity inhibits primary root growth, while local NH$_4^+$ may stimulate lateral root branching (Liu & von Wiren, 2017). Nitrogen deficient plants exposed to a local supply of NH$_4^+$ respond by developing more second and third order lateral roots through a mechanism dependent on the NH$_4^+$ transporter AMT1;3 Lima et al., (2010).

5. Visual symptoms of nitrogen on plants

Low N leads to developmental responses such as stunted growth, early flowering, reduced tillering in cereals, shoot branching and smaller leaves due to decreased cell division and expansion. The most prominent visual symptom of nitrogen deficiency is chlorotic leaves, where the chlorosis spreads uniformly (general chlorosis) across the entire leaf due to either reduced chlorophyll synthesis or breakdown of existing chlorophyll binding proteins in the photosystems Fig. (47). Nitrogen assimilation in the chloroplast is tightly coupled with chlorophyll biosynthesis via the GS/GOGAT pathway, as glutamate is the precursor for all chlorophylls produced in the tetrapyrrole biosynthetic pathway. Hence, there is a strong correlation between the nitrogen status of plants and the chlorophyll content, which can be used to optimize nitrogen fertilizer application Hudson et al., (2011). Although the mutual regulation of chlorophyll biosynthesis and breakdown largely remains unknown, it is well established that nitrogen deficiency induces chlorophyll breakdown via proteolysis leading to the release of amino acids, amides and NH$_4^+$. Have et al., (2016). All released nitrogen compounds are highly mobile in the phloem. Older leaves consequently act as source tissue during low nitrogen situations supplying young and developing tissue like leaves, flowers and seeds with nitrogen. Therefore, visual symptoms are first visible on the oldest leaves (acropetal stratification). Reduced shoot branching, stunted growth and inhibition of leaf expansion constitute central physiological responses to nitrogen deficiency (Rahayu et al., 2005) Fig (47.).

Subsequent root-to-shoot translocation of trans-zeatin (a cytokinin) and trans-zeatin-riboside (a cytokinin precursor) affects leaf growth through stimulation of leaf expansion and meristem activity (Osugi et al., 2017). In addition, root-derived trans-zeatin riboside directly stimulates shoot apical meristem (SAM) growth and thereby overall shoot biomass (Landrein et al., 2018). Shoot branching is controlled by formation and activation of axillary buds. Axillary buds are activated by NO$_3^-$ due to a stimulatory effect on cytokinin production, whereas low NO$_3^-$ maintains bud dormancy and thereby reduce branching (M€uller et al., 2015). This effect further relies on interaction with auxin and strigolactone (de Jong et al., 2014). Another prominent visual symptom of N deficiency is reddish coloration due to anthocyanin production Fig (48.). This is only seen in some species and may be influenced by other abiotic factors such as high light intensity, low temperatures and P deficiency. In A. thaliana, low nitrogen induces expression of key transcription factors positively regulating anthocyanin synthesis, including PRODUCTION OF ANTHOCYANIN PIGMENT1 (PAP1), PAP2 and GL3 (Xu et al., 2015). Repression of GA signalling by DELLA proteins is required for the induction of several biosynthesis genes, including genes related to anthocyanin accumulation during N deficiency (Zhang et al., 2017). By contrast, three members of the LATERAL ORGAN BOUNDARY DOMAIN (LBD) protein family LBD37/38/39, which areNO$_3^-$ induced, act as negative regulators of anthocyanin synthesis by repressing PAP1 and PAP2 expression (Rubin et al., 2009). In parallel, the SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL) transcription factor, SPL9, is a negative regulator of anthocyanin synthesis, as it represses PAP1, PAP2 and other transcription factors that positively regulate anthocyanin production. SPL9 is repressed by miR156, which itself is induced by N deficiency, thus linking low nitrogen conditions to anthocyanin production by increasing expression of PAP1 and the biosynthetic gene DFR (Cui et al., 2014).
Fig. 47: Represents nitrogen (N) deficiency always appears on the oldest leaves first characterized by a general chlorosis. (a) Leaf of healthy maize plant to the left and nitrogen deficient leaf with general chlorosis to the right. (b) Anthocyanin accumulation in a nitrogen deficient oilseed rape leaf with general chlorosis. (c) General chlorosis in tomato, with anthocyanosis on veins and on the abaxial side of leaves.

Nitrogen is an essential nutrient for plants life cycle. However, the excessive use of this element causes serious problems in agriculture. This is can disrupt the development of many important plants. *Sesamum indicum* or sesame represents one of the most economically important and ancient oil crops in the world. In fact, its seeds are used for many biological activities. Farmers occasionally need to add nitrogen fertilizer to their farms and gardens to make available just the precise nutrients for their plants growth. The applications of inorganic nitrogen fertilizers to various crops have been continuously increasing since last many decades globally. Although nitrogen fertilizer contributes substantially to yield enhancement, but excessive use of this manure has posed serious threats to environment and human health. Rate of nitrogen fertilizers application has a close relationship with nitrate accumulation in surrounding environment, groundwater, as well as leafy and root vegetables.

In addition, in the last years, the use of fertilizers has increased considerably in the objective to increase the mass of crop per land area for ensuring the increased need of world population. However, excessive nitrogen fertilization is a major problem for both agriculture and environment. Overmuch accumulation of N in plants may cause toxicity problems for human health like methemoglobinemia Hord- et al., (2009), nitrous oxide emissions, and, groundwater’s nitrate pollution Ju et al., (2006). Moreover, Jeppsson (2000) has shown the negative effect of high fertilization on the composition and quality of some plants. The rational use of nitrogen supply can prevent pollution and ensure the quality and sustainability of agriculture. In this logic, farmers need to understand the importance of optimization of the mineral fertilization. Several authors have tried to create awareness among public, farming community, health practitioners, and agricultural scientists for the risk involved with excessive...
use of nitrogen fertilizers to human health to nitrogen fertilizer runoff from arable lands. Fig. (48) illustrates some symptoms toxicity of nitrogen.

**Fig. 48:** Illustrates symptoms of nitrogen toxicity in some different crops

**Conclusion**

The agronomic and molecular approaches altogether have potential to improve nitrogen use efficiency. Nitrogen losses can be minimized by precision agriculture, cut off nitrogen dose, intercropping of legume and nonlegume crops, improving plant populations and introducing nitrogen efficient genotypes.

A large number of studies have been carried out over the last two decades to identify by means of agronomic, physiological and genetic studies, the rate limiting steps of NUE both in model and crop species, as a function of environmental conditions. For abiotic stress improvement in crops, NUE has become the second priority after drought both in the private and in the public sector. To decipher the genetic and physiological basis of NUE, many tools are available for most crops and for cereals in particular. They include mutant collections, wide genetic diversity, recombinant inbred lines (RILs) or Doubled Haploid Line populations (DHLs), straightforward transformation protocols and physiological, biochemical and genomic data for systems biology development. Considering both the economic and environmental challenge represented by reducing both the cost and application of N fertilizers, all major maize seed breeding companies such as Monsanto, moreover, improvement in yield for most crops over the last 50 years has been estimated to be 40%, due to improvements in cultural practices and 60% due to genetic gains, thus indicating that breeding for improved NUE is still possible.
Improving NUE through either genetic engineering or marker assisted breeding is still at the stage of proof of concept. Therefore, very little information is currently released from both the private and public sector in consideration of the potential economic value of crop NUE improvement. However, both on the genetic and physiological side, the identification of key steps involved in the control of NUE from gene expression to metabolic activity remains incomplete. It is likely because the regulatory mechanisms involved in the control of the two components of NUE (N uptake and utilization efficiencies) are species-specific. Moreover, they are subjected to changes or adaptation in a constantly changing soil and aerial environment during plant growth and development that require the taking into account the various genotypic/environment interactions.

Over the last two decades, the construction of cereals that can fix atmospheric N has always been a challenge for plant scientists, in order to reduce the need for mineral N fertilization. Although, the signaling pathway for recognition of N-fixing bacteria is present in cereals, complex genetic modification will be necessary to allow bacterial colonization and nodule organogenesis. At the field level, only agronomic predictive models using the appropriate biogical and environmental parameters should be able to take into account interactions between plants and their environment to obtain an integrated view of the various inputs or outputs, influencing crop NUE. One of the main challenges in the future will be to develop reliable decision support systems with the help of and biological diagnostic tools in precision agriculture, in order to optimize the application of N under organic or conventional conditions in a more sustainable manner. Moreover, the establishment of such models will need to be scaled up at the ecological level in order to obtain a better understanding as to how N cycling is occurring from organisms to the whole ecosystem.

A proposed strategy for integrating multidisciplinary approaches for improving crop NUE, that strategy highlights the necessity to develop an integrated approach between the public and private sectors to improve our understanding and control of the biological and agronomic basis of NUE in crops of major economic importance. However, the nature of an agronomic trait such as NUE is complex, due to the intervention of multiple elements interacting with each other as a function of both plant development and environmental constraints. Therefore, improvement of this understanding will require the development of a multi-disciplinary approach, integrating expertise from fundamental and more applied studies in crop developmental biology, physiology, genomics, genetics, physiology, modeling, agronomy and breeding. In addition, taking advantage of the genetic variability that already exists or that can be created, will provide a valuable contribution to the genetic and physiological dissection of NUE under mineral and organic N nutrition conditions and an evaluation of the genes or group of genes involved.

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