The Nitrogen Cycle: Volatilization and its Relationship to N_2 fascination

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Abstract— **The nitrogen cycle is the biogeochemical cycle by which nitrogen is converted into multiple chemical forms as it circulates among atmospheric, terrestrial, and marine ecosystems. The conversion of nitrogen can be carried out through both biological and physical processes. Important processes in the nitrogen cycle include fixation, ammonification, nitrification, and denitrification. The majority of Earth's atmosphere (78%) is atmospheric nitrogen, making it the largest source of nitrogen. However, atmospheric nitrogen has limited availability for biological use, leading to a scarcity of usable nitrogen in many types of ecosystems.**

The nitrogen cycle is of particular interest to ecologists because nitrogen availability can affect the rate of key ecosystem processes, including primary production and decomposition. Human activities such as fossil fuel combustion, use of artificial nitrogen fertilizers, and release of nitrogen in wastewater have dramatically altered the global nitrogen cycle. Human modification of the global nitrogen cycle can negatively affect the natural environment system and also human health. Volatilization and its Relationship to N2 fascination in Nitrogen Cycle in agriculture flield is discuss in this paper.

***Index Terms*- Carbon metabolism, drought, environmental stresses, nitrogen fixation, nodules, sucrose synthase, dinitration**

I. INTRODUCTION

All living cells require nitrogen for the synthesis of many of their biomolecules. The assimilation of nitrogen occurs via the incorporation of the ammonium ion. In Nature, however, nitrogen is present in many other oxidation states. The biologically most important compounds are nitrate, nitrite, nitric oxide, nitrous oxide, dinitrogen, and ammonium. The free concentration of each of these nitrogencompounds is mostly determined by the production and consumption rates of bacterial metabolic processes. Together, these processes drive the global nitrogen cycle and ensure a balanced recycling of the nitrogen compounds (Ferguson, 1998;Moreno-Vivian and Ferguson, 1998; Moura and Moura, 2001; Richardson andWatmough, 1999).

The fixation of atmospheric nitrogen is in part achieved by the chemical reaction of dinitrogen (N2) and dioxygen (O2) induced by lightning, which gives rise to nitric oxide (NO). In the O2-rich atmosphere, NO is then oxidized to nitrogen dioxide (NO2) and taken up in the oceans in the form of nitrate ions. Biological nitrogen fixation into ammonium as carried out by certain bacteria, however, is much more efficient and makes most of the nitrogen available to all living cells.

The production of gaseous N2 is mainly carried out by denitrifying species. Denitrification is an anaerobic respiratory process carried out by many bacterial species and some fungi and yeasts whereby N-oxides substitute for O2 as the terminal electron acceptor of respiration. In a sequence of four reactions, nitrate is reduced to N2 via the intermediates, nitrite (NO2 –), nitric oxide (NO), and nitrous oxide (N2O), successively (Ferguson, 1994; Stouthamer, 1991; Stouthamer, 1992).

The key reaction that distinguishes denitrification from nitrate respiration is the reduction of nitrite, which yields nitric oxide rather than ammonium. In the past few years, three-dimensional structures have been solved for the majority of these enzymes, revealing the architecture of the active metal sites as well as global structural and mechanistic aspects. Bacteria that are able to denitrify are confronted with a paradox. On the one hand, the potential to denitrify enhances their metabolic flexibility because it allows them to grow in the absence of O2. On the other hand, there is the potential risk that the free concentrations of the toxic intermediates, nitrite and nitric oxide, reach levels that are lethal to the cell. Due to the sequential order of reactions during denitrification, the reaction products of three of the four enzymes are substrates for the next enzyme. The toxicity of nitrite and nitric oxide imply that concentrations and activities of each of the enzymes should be well tuned in order to keep these steady-state concentrations in the cell low. It has now become evident that denitrifying organisms regulate the activity of denitrification both by means of the expression of each of the gene clusters involved (DNA level, long-term adaptation) and through the specific properties (Km and kcat) of the participating enzymes (protein level, short-term adaptation).

Denitrification and aerobic respiration have some features in common.
These are:
(i) cd1-type nitrite reductase and bc-type nitric oxide reductase, which are capable of reducing O2 to water;
(ii) subunit I of the haem copper oxidases and the large subunit from nitric oxide reductase have similar topologies;
(iii) a CuA site,which is active in electron transfer, is present in nitrous oxide reductase, in subunit II of aa3-type cytochrome c oxidases, and in some quinol-oxidizing nitric oxide reductases; and

(iv) subunit III of aa3-type cytochrome c oxidases has homology with NorE, which is encoded by the nitric oxide reductase gene cluster. It is, therefore, tempting to speculate that some of the building blocks of the denitrification apparatus have been used and/or rearranged giving rise to the evolution of the aerobic respiratory system in a time where the O2 concentration in the earth's atmosphere increased as a result of photosynthetic activity (Saraste, 1994; Saraste and Castresana, 1994).

Products of denitrification have manifold, mainly adverse, effects on the atmosphere, soils, and waters and thus have both agronomic and environmental impact. When nitrate is converted to gaseous nitrogen by denitrifying bacteria in agricultural soils, nitrogen is lost as an essential nutrient for the growth of plants. In contrast to ammonium, which is tightly bound in soil, nitrate is easily washed out and flows into the groundwater where it (and its reduction product, nitrite) adversely affects water quality. In addition, nitrogenous oxides released from soils and waters are, in part, responsible for the depletion of the ozone layer above the Antarctic and, in part, for the initiation of acid rain and global warming. Thus, the impact of products of denitrification in soils, waters, and the atmosphere is of extreme relevance for human welfare and makes a detailed knowledge of this process essential.

In a key innovation to CMIP5, the majority of earth system models (ESMs) of the latest generation that contribute to CMIP6 (Eyring et al., 2016) include a nitrogen cycle to better represent the terrestrial carbon cycle (Arora et al., 2020; Davies-Barnard et al., 2020). Nitrogen is a key nutrient requirement for plants to take up carbon and, in its bioavailable inorganic form, is highly liable to losses via gaseous and water processes (Thomas et al., 2013; Vitousek and Howarth, 1991). Over the last few decades, terrestrial carbon uptake has sequestered around a quarter of anthropogenic carbon emissions (Friedlingstein et al., 2020). However, previous assessments of ESMs have suggested that future projections of terrestrial carbon storage are decreased by 37 %–58 % if nitrogen availability is accounted for (Wieder et al., 2015; Zaehle et al., 2014). Therefore, the accuracy of ESMs, which help guide policy on preventing further climate change, is partly determined by the functioning of the nitrogen cycles within them.

The uptake of new carbon by plants is reliant on new sources of nitrogen as existing nitrogen may not be bioavailable. The sources of this new input of nitrogen vary by biome, including anthropogenic inputs via addition of 70– 108 Tg of fertilizer per year (Lu and Tian, 2017; Potter et al., 2010) and increased deposition and natural sources such as lightning 3.5–7 Tg N yr−1 (Tie et al., 2002), atmospheric 3492 T. Davies-Barnard et al.: Biological nitrogen fixation in CMIP6 models N deposition 63 Tg N yr−1 (Lamarque et al., 2013), weathering (Holloway and Dahlgren, 2002), and biological nitrogen fixation (BNF) 40–141 Tg N yr−1 (Davies-Barnard and Friedlingstein, 2020; Vitousek et al., 2013). In many natural ecosystems BNF is likely the largest natural or anthropogenic source of new nitrogen to the terrestrial biosphere. But because of the intricate processes that control fixation and the lack of global estimates from observations, it is also the most uncertain (Meyerholt et al., 2016; Reed et al., 2011). Therefore, continued carbon sequestration in critical natural ecosystems that are present-day and future carbon sinks is reliant on BNF. We need to know how well models are representing the current quantity and distribution of BNF to assess the reliability of the functions and therefore the robustness of future projections of terrestrial carbon uptake. Studies of individual models suggest differences in representation of BNF can lead to widely differing future terrestrial carbon sequestration (Meyerholt et al., 2016; Peng et al., 2020; Wieder et al., 2015). Therefore inaccuracies in BNF representation could lead to errors in allowable emissions (Jones et al., 2013) for targets such as constraining warming to 1.5 or 2 ◦C (Millar et al., 2017).

BNF is performed by a large range of bacteria in virtually all parts of the terrestrial environment, including soil, litter, leaf canopy, and decaying wood and in association with bryophytes, lichens, and angiosperms (Davies-Barnard and Friedlingstein, 2020; Reed et al., 2011; Son, 2001; Tedersoo et al., 2018). BNF is frequently classified into symbiotic (higher plant association) and free-living pathways (Cleveland et al., 1999; Reed et al., 2011). Symbiotic BNF makes up around two-thirds of BNF and free-living BNF around one-third (Davies-Barnard and Friedlingstein, 2020) or as much as 49 Tg N yr−1 (Elbert et al., 2012).

Despite the complexity of BNF, most models have a simple BNF representation based on either (i) a linear relationship with net primary productivity (NPP) or (ii) a linear relationship with evapotranspiration (ET), both derived from Cleveland et al. (1999) (see Table 1). However, recent analyses show that in non-agricultural biomes ET and NPP are poor predictors of both symbiotic and free-living BNF (Davies-Barnard and Friedlingstein, 2020; Dynarski and Houlton, 2018). Models with more complex representations are mainly based on plant nitrogen demand, physiological limits, or optimality approaches (Fisher et al., 2010; Meyerholt et al., 2016; Wang et al., 2007) (see Table 1). While single model assessments have shown the importance of BNF to carbon sequestration, affecting the terrestrial carbon sink by up to a third (Meyerholt et al., 2016, 2020; Wieder et al., 2015), the performance of multiple models has hitherto not been assessed against observed BNF values.

II. THE NITROGEN CYCLE

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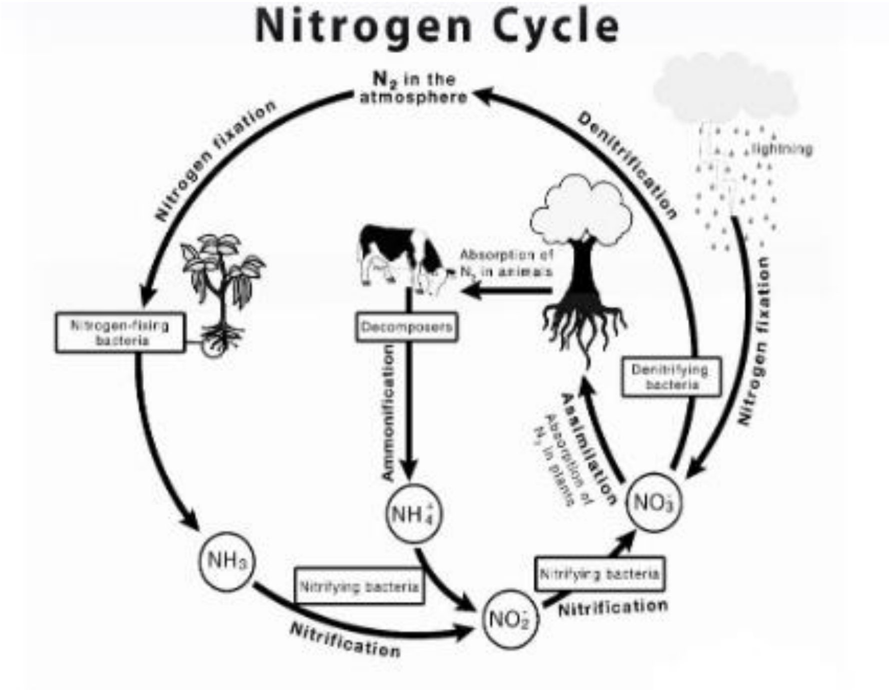

Figure-1

The N cycle illustrates how N from manure, fertilizers and plants moves through the soil to crops, water and the air. Understanding the N cycle will help you make the best use of manure and fertilizers to meet crop needs while safeguarding the environment. In general, the N cycle processes of fixation, mineralization and nitrification increase plant available N. Denitrification, volatilization, immobilization, and leaching result in permanent or temporary N losses from the root zone. Read on for specifics about each of the N cycle processes

III. FIXATION

Fixation refers to the conversion of atmospheric N to a plant available form. This occurs either through an industrial process, as in the production of commercial fertilizers, or a biological process, as with legumes such as alfalfa and clover. Nitrogen fixation requires energy, enzymes and minerals, so if a plant available form of N is present, the crop will use it instead of fixing it from the air.

ƒ 1. Mineralization readily occurs in warm (68-95°F), well-aerated and moist soils.

ƒ 2. In New York State, approximately 60—80 lbs of N per acre is mineralized on average from soil organic matter each year.

IV. NITRIFICATION

Nitrification is the process by which microorganisms convert ammonium to nitrate to obtain energy. Nitrate is the most plant available form of N, but is also highly susceptible to leaching losses.

ƒ Nitrification is most rapid when soil is warm (67- 86°F), moist and well-aerated, but is virtually halted below 41°F and above 122°F.

V. DENITRIFICATION

Denitrification occurs when N is lost through the conversion of nitrate to gaseous forms of N, such as nitric oxide, nitrous oxide and dinitrogen gas. This occurs when the soil is saturated and the bacteria use nitrate as an oxygen source. ƒ De-nitrification is common in poorly drained soils.

VI. VOLATILIZATION

Volatilization is the loss of N through the conversion of ammonium to ammonia gas, which is released to the atmosphere. The volatilization losses increase at higher soil pH and conditions that favor evaporation (e.g. hot and windy). ƒ

1. **Volatilization** losses are higher for manures and urea fertilizers that are surface applied and not incorporated (by tillage or by rain) into the soil. ƒ

2. Manure contains N in two primary forms: ammonium and organic N. If manure is incorporated within one day, 65% of the ammonium N is retained; when incorporated after 5 days the ammonium N will have been lost through volatilization. Organic N in manure is not lost through volatilization, but it takes time to mineralize and become plant available.

VII. IMMOBILIZATION

Immobilization is the reverse of mineralization. All living things require N; therefore microorganisms in the soil compete with crops for N. Immobilization refers to the process in which nitrate and ammonium are taken up by soil organisms and therefore become unavailable to crops. ƒ

Incorporation of materials with a high carbon to nitrogen ratio (e.g. sawdust, straw, etc.), will increase biological activity and cause a greater demand for N, and thus result in N immobilization ƒ

Immobilization only temporarily locks up N. When the microorganisms die, the organic N contained in their cells is converted by mineralization and nitrification to plant available nitrate.

Leaching is a pathway of N loss of a high concern to water quality. Soil particles do not retain nitrate very well because both are negatively charged. As a result, nitrate easily moves with water in the soil. The rate of leaching depends on soil drainage, rainfall, amount of nitrate present in the soil, and crop uptake. ƒ

The EPA has set the maximum contaminant level for drinking water at 10 ppm N as nitrate. ƒ

Well-drained soils, unexpected low crop yield, high N inputs (especially outside of the growing season) and high rainfall are all conditions that increase the potential for nitrate leaching.

VIII. CROP UPTAKE

Crop Uptake is the prime goal of N management on farms. The greatest efficiency occurs when adequate N is applied at a time when the crop is actively taking it up. Efficient N use also depends on a number of other factors including temperature, soil moisture, pest pressure, and soil compaction. ƒ

In the moist Northeast climate, nitrate remaining in the soil after the growing season will be lost to leaching or denitrification between crop harvest and the next planting season. ƒ

Efficient N use during the growing season and the use of cover crops can minimize such losses.

IX. NITROGEN FIXATION

Nitrogen is a critical limiting element for plant growth and production. It is a major component of chlorophyll, the most important pigment needed for photosynthesis, as well as amino acids, the key building blocks of proteins. It is also found in other important biomolecules, such as ATP and nucleic acids. Even though it is one of the most abundant elements (predominately in the form of nitrogen gas (N2) in the Earth’s atmosphere), plants can only utilize reduced forms of this element. Plants acquire these forms of “combined” nitrogen by: 1) the addition of ammonia and/or nitrate fertilizer (from the Haber-Bosch process) or manure to soil, 2) the release of these compounds during organic matter decomposition, 3) the conversion of atmospheric nitrogen into the compounds by natural processes, such as lightning, and 4) biological nitrogen fixation (Vance 2001).

Biological nitrogen fixation (BNF), discovered by Beijerinck in 1901 (Beijerinck 1901), is carried out by a specialized group of prokaryotes. These organisms utilize the enzyme nitrogenase to catalyze the conversion of atmospheric nitrogen (N2) to ammonia (NH3). Plants can readily assimilate NH3 to produce the aforementioned nitrogenous biomolecules. These prokaryotes include aquatic organisms, such as cyanobacteria, free-living soil bacteria, such as Azotobacter, bacteria that form associative relationships with plants, such as Azospirillum, and most importantly, bacteria, such as Rhizobium and Bradyrhizobium, that form symbioses with legumes and other plants (Postgate 1982). These organisms are summarized in Figure 2.

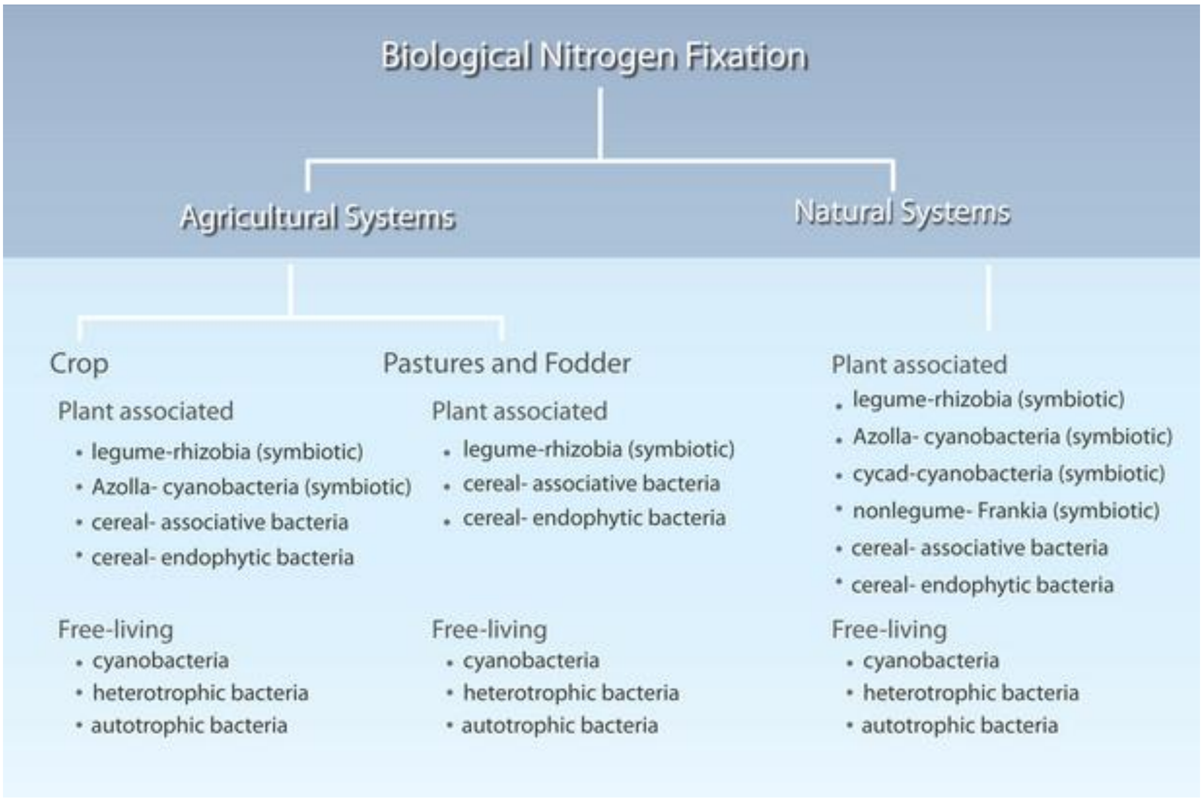

Figure-2

X. THE PROCESS

The reduction of atmospheric nitrogen is a complex process that requires a large input of energy to proceed (Postgate 1982). The nitrogen molecule is composed of two nitrogen atoms joined by a triple covalent bond, thus making the molecule highly inert and nonreactive. Nitrogenase catalyzes the breaking of this bond and the addition of three hydrogen atoms to each nitrogen atom.

Microorganisms that fix nitrogen require 16 moles of adenosine triphosphate (ATP) to reduce each mole of nitrogen (Hubbell & Kidder, 2009). These organisms obtain this energy by oxidizing organic molecules. Non-photosynthetic free-living microorganisms must obtain these molecules from other organisms, while photosynthetic microorganisms, such as cyanobacteria, use sugars produced by photosynthesis. Associative and symbiotic nitrogen-fixing microorganisms obtain these compounds from their host plants'rhizospheres(NationalResearchCouncil1994,Hubbell&Kidder2009).

Industries use the Haber-Bosch process to reduce nitrogen essentially in the same way. Conventional agriculture has depended upon this process to produce the commercial fertilizer needed to grow most of the world's hybrid crops. But this approach comes with many consequences, including using fossil fuels for the energy needed to produce this fertilizer, the resulting carbon dioxide emissions and pollution from burning these fuels, and adverse affects on human health (Vitousek 1997).

Overuse of these chemical fertilizers has led to an upset in the nitrogen cycle and consequently to surface water as well as groundwater pollution. Increased loads of nitrogen fertilizer to freshwater, as well as marine ecosystems, has caused eutrophication, the process whereby these systems have a proliferation of microorganisms, especially algae. This "greening" of the water column has caused decreased levels of dissolved oxygen (DO) in bottom waters as planktonic algae die and fuel microbial respiration. These depleted DO levels result in massive mortality of aquatic organisms and create so-called dead zones, areas where little or no aquatic life can be found (Figure 2). Since the 1960's, dead zones have increased exponentially worldwide, and have now been documented from over 400 systems, affecting more than 245,000 square kilometers of coastal regions (Diaz & Rosenberg 2008, Figure 3). This phenomenon is now deemed the key stressor on marine ecosystems.

Nitrogen Fixation by Free-Living Heterotrophs

Many heterotrophic bacteria live in the soil and fix significant levels of nitrogen without the direct interaction with other organisms. Examples of this type of nitrogen-fixing bacteria include species of Azotobacter, Bacillus, Clostridium, and Klebsiella. As previously noted, these organisms must find their own source of energy, typically by oxidizing organic molecules released by other organisms or from decomposition. There are some free-living organisms that have chemolithotrophic capabilities and can thereby utilizeinorganiccompounds as a source of energy.

Because nitrogenase can be inhibited by oxygen, free-living organisms behave as anaerobes or microaerophiles while fixing nitrogen. Because of the scarcity of suitable carbon and energy sources for these organisms, their contribution to global nitrogen fixation rates is generally considered minor. However, a recent study in Australia of an intensive wheat rotation farming system demonstrated that free-living microorganisms contributed 20 kilograms per hectare per year to the long-term nitrogen needs of this cropping system (30-50% of the total needs; Vadakattu & Paterson 2006). Maintaining wheat stubble and reduced tillage in this system provided the necessary high-carbon, low-nitrogen environment to optimize activity of the free-living organisms.

Associative Nitrogen Fixation

Species of Azospirillum are able to form close associations with several members of the Poaceae (grasses), including agronomically important cereal crops, such as rice, wheat, corn, oats, and barley. These bacteria fix appreciable amounts of nitrogen within the rhizosphere of the host plants. Efficiencies of 52 mg N2 g-1 malate have been reported (Stephan et al. 1979). The level of nitrogen fixation is determined by several factors, including soil temperature (Azospirillum species thrive in more temperate and/or tropical environments), the ability of the host plant to provide a rhizosphere environment low in oxygen pressure, the availability of host photosynthates for the bacteria, the competitiveness of the bacteria, and the efficiency of nitrogenase (Vlassak & Reynders, 1979).

XI. SYMBIOTIC NITROGEN FIXATION

Many microorganisms fix nitrogen symbiotically by partnering with a host plant. The plant provides sugars from photosynthesis that are utilized by the nitrogen-fixing microorganism for the energy it needs for nitrogen fixation. In exchange for these carbon sources,themicrobe provides fixed nitrogento the host plant for its growth.

One example of this type of nitrogen fixation is the water fern Azolla's symbiosis with a cyanobacterium Anabaena azollae. Anabaena colonizes cavities formed at the base of Azolla fronds. There the cyanobacteria fix significant amounts of nitrogen in specialized cells called heterocysts. This symbiosis has been used for at least 1000 years as a biofertilizer in wetland paddies in Southeast Asia. Rice paddies are typically covered with Azolla "blooms" that fix up to 600 Kg N ha-1 yr-1 during the growingseason(Postgate1982,Fattah2015).

Another example is the symbiosis between actinorhizal trees and shrubs, such as Alder (Alnus sp.), with the actinomycete Frankia. These plants are native to North America and tend to thrive in nitrogen-poor environments. In many areas they are the most common non-legume nitrogen fixers and are often the pioneer species in successional plant communities. Actinorhizal plants are found in many ecosystems including alpine, xeric, chapparal, forest, glacial till, riparian, coastal dune, and arctic tundra environments(Benson&Silvester,1993).

Even though the symbiotic partners described above play an important role in the worldwide ecology of nitrogen fixation, by far the most important nitrogen-fixing symbiotic associations are the relationships between legumes and Rhizobium and Bradyrhizobium bacteria. Important legumes used in agricultural systems include alfalfa, beans, clover, cowpeas, lupines, peanut, soybean, and vetches. Of the legumes in agricultural production, soybeans are grown on 50% of the global area devoted to legumes, and represent 68% of the total global legume production (Vance 2019).

Legume Nodule Formation

In the future scenarios, the multiple sources of uncertainty as to how and to what extent BNF will change make any definitive statements about the capacity of models to capture BNF changes difficult. While increased atmospheric carbon dioxide tends to increase BNF (Liang et al., 2016), nitrogen addition in the form of deposition or fertilization tends to suppress BNF (Zheng et al., 2019) and effects from land use change (Zheng et al., 2020), increased temperature, reduced precipitation, and other climate change as well as the potential effects of climate-induced land cover change that may alter the composition and location of biomes. It is challenging to predict which of these factors will predominate over the coming century. Regardless of the change in BNF in future, it is revealing that, while single parameter perturbation experiments suggest BNF significantly affects terrestrial carbon storage (Meyerholt et al., 2016; Wieder et al., 2015), when in a dynamic system the effects of BNF are subsumed by structural differences in the nitrogen and carbon models as well as the larger effects of increasing carbon dioxide. In terms of confidence in model results, the process-based models have clear advantages. However, that increased complexity does not necessarily translate into increased fidelity in the representation of model BNF. This could be due to issues with the process-based representation of BNF, systematic problems with the model representation of the wider nitrogen cycle which BNF previously compensated for, or inaccuracies in the observational upscaled data.

The Rhizobium or Bradyrhizobium bacteria colonize the host plant's root system and cause the roots to form nodules to house the bacteria (Figure 4). The bacteria then begin to fix the nitrogen required by the plant. Access to the fixed nitrogen allows the plant to produce leaves fortified with nitrogen that can be recycled throughout the plant. This allows the plant to increase photosynthetic capacity, which in turn yields nitrogen-rich seed. The consequences of legumes not being nodulated can be quite dramatic, especially when the plants are grown in nitrogen-poor soil. The resulting plants are typically chlorotic, low in nitrogen content, and yield very little seed (Figure 3 and 4)

Figure-3

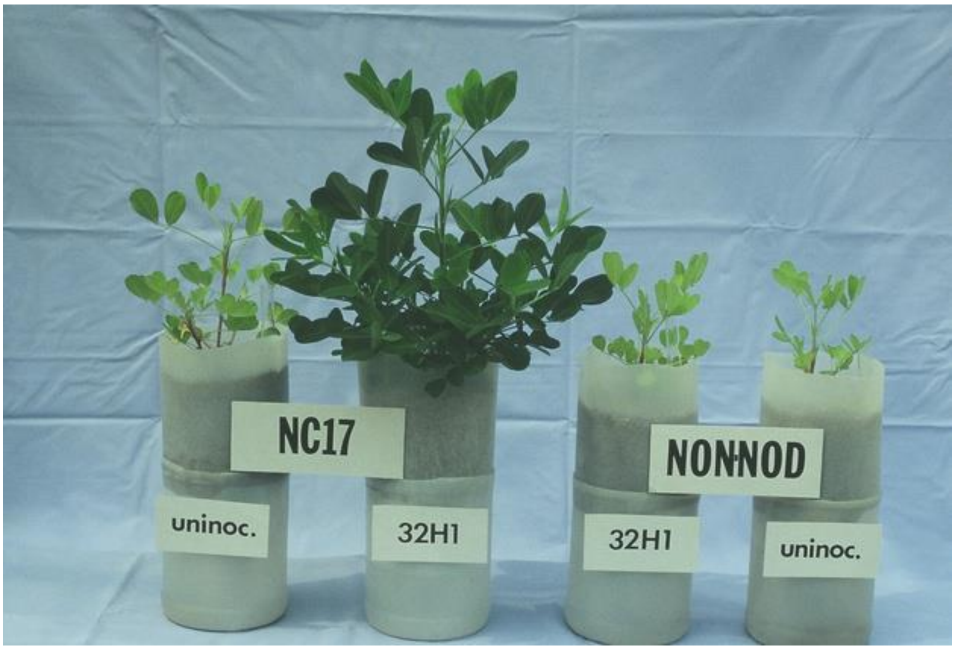

Figure-4

XII. SUMMARY

Nitrogen is an essential nutrient for plant growth and development but is unavailable in its most prevalent form as atmospheric nitrogen. Plants instead depend upon combined, or fixed, forms of nitrogen, such as ammonia and nitrate. Much of this nitrogen is provided to cropping systems in the form of industrially produced nitrogen fertilizers. Use of these fertilizers has led to worldwide, ecological problems, such as the formation of coastal dead zones. Biological nitrogen fixation, on the other hand, offers a natural means of providing nitrogen for plants. It is a critical component of many aquatic, as well as terrestrial ecosystems across our biosphere

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