ISSN print: 1224-5984
ISSN online: 2248-3942

Rom Biotechnol Lett. 2021; 26(4): 2834-2845
doi: 10.25083/rbl/26.4/2834.2845

Received for publication, February, 2, 2021
Accepted, May, 13, 2021

Original paper

Nitrogen Fixation and Diazotrophs – A Review

WENLI SUN[1#], MOHAMAD HESAM SHAHRAJABIAN[1#], QI CHENG[1,2*]

[1]Biotechnology Research Institute, Chinese Academy of Agricultural Sciences, Beijing, 100081, PR China
[2]College of Life Sciences, Hebei Agricultural University, Baoding, Hebei, 071000, China; Global Alliance of HeBAU-CLS&HeQiS for BioAl-Manufacturing, Baoding, Hebei, 071000, PR China

#These authors equally contributed to this paper

Abstract Nitrogen fixation involves formation of ammonium from N_2, which needs a high input of energy. Biological nitrogen fixation utilizes the enzyme nitrogenase and ATP to fix nitrogen. Nitrogenase contains a Fe-protein and a Mo-Fe-protein and other metal cofactors. Soil diazotrophs possess the function of fixing atmospheric N_2 into biologically available ammonium in ecosystems. In Aechaea, nitrogen fixation has been reported in some methanogens such as *Methanobacteriales*, *Methanococcales*, and *Methanosarcinales*. Community structure and diversity of diazotrophic are correlated with soil pH. All known organisms which involve in nitrogen-fixing which are called diazatrophs are prokaryotes, and both bacterial and archaeal domains are responsible for that. Diazotrophs are categorized into two main groups namely: root-nodule bacteria and plant growth-promoting rhizobacteria. Diazotrophs include free living bacteria, such as *Azospirillum*, *Cupriavidus*, and some sulfate reducing bacteria, and symbiotic *diazotrophs* such *Rhizobium* and *Frankia*. Two important parameters which may affect diazotroph communities are temperature and soil moisture in different seasons. To have sustainable agriculture, replacing expensive chemical nitrogen fertilizers with environmentally friendly ways is the most accepted practice.

Keywords Diazotrophs, Nitrogenase, Nitrogen Fixation.

To cite this article: SUN W, SHAHRAJABIAN MH, CHENG QI. Nitrogen Fixation and Diazotrophs – A Review. *Rom Biotechnol Lett*. 2021; 26(4): 2834-2845. DOI: 10.25083/rbl/26.4/2834-2845

*Corresponding author: QI CHENG, Biotechnology Research Institute, Chinese Academy of Agricultural Sciences, Beijing, 100081, PR China
E-mail: chengqi@caas.cn

Introduction

Nitrogen (N) is a vital parameter for crop productivity (BROUMAND et al, 2010; SOLEYMANI et al, 2011a,b). Nitrogen is most important limiting nutrient for crop production and plant productivity in many part of the world (MITCHELL et al, 2018; SUN et al, 2019). It is also a manin part in chlorophyll as well as key parameter in amino acids and proteicn (DIXON et al, 1997; KUMAR et al, 2017; MAHATO and KAFLE, 2018). Bacteria and archaea inhabit the most inhospitable environments and have unique roles in metabolic pathways and genes to cope with different environmental conditions (SMITH-MOORE and GRUNDEN, 2018). They are fund inhabiting the rhizosphere with numerous interactions with the plant host (ODELADE and BABALOLA, 2019). The goal of this review is to survey on role of diazotrophs in nitrogen fixation.

Nitrogen fixation

Nitrogen fixation has also significant role in biochemical pathways which play an important role in controlling oceanic nitrogen inventory (COTTA et al, 2014). Without any doubt, nitrogen fixation is an ancient way which is essential for surviving life, and play a key role during the beginning of microbial life when abiotic nitrogen sources become scarce (GABY and BUCKLEY, 2014). Nitrogenase plays an important part in global nitrogen cycle (SOLEYMANI et al, 2012; SHAHRAJABIAN and SOLEYMANI, 2017), and understanding of nitrogenase expression and regulation is important to utilize potential diazotrophs under various ecological niches to gain agricultural and environmental sustainability at the same time (SUYAL et al, 2018). Nitrogen fixation is divided into two parts abiotic methods (lightning), and biotic (nitrogen fixers) to fix nitrogen to the ground. In the abiotic fixation, N_2 would have been oxidized with CO_2 by lightning, and then NO gets converted to soluble nitrosyl hydride (HNO) (NAVARRO-GONZALEZ et al, 2011). Certain bacteria and archaea are responsible for biological nitrogen fixation. Although, there is large atmospheric reservoir, bioavailability of nitrogen mostly relies on biological nitrogen fixation (BNF) (PRAYITNO and ROLFE, 2010).

Two main drivers of universal nitrogen cycling are ammonia-oxidizing bacteria (AOB), and archaea (AOA) (LONG et al, 2012). Metabolic pathways usually are common between archaea and bacteria (ODELADE et al, 2019), and almost all genes involved in this process are founder under these domains (TATUSOV et al, 2003). It has been found that a lone bilayer lipid which makes the cell structural formation of archaea is very close to the bacterial cell which is gram-positive within prokaryota (MAKAROVA et al, 2001). One of the most important proteins which are common to both archaea and gram-positive bacteria are glutamine synthetase I and Hsp70 (MAKAROVA et al, 2001; KOCH, 2003). Nitrogen fixation is energetically expensive because it consumes 16 moles of ATP per mole of N fixed (AISALBIE and DESLIPPE, 2013). Nitrogen fixation also has been considered as the limiting factor for both crop and natural ecosystem productivity which has shown scholars the importance of this process in agricultural system (DIXON and KAHN, 2004; WANG et al, 2013). Relying on chemical fertilizer, especially nitrogen may lead to both serious health issues and environmental concern (SHAHRAJABIAN et al, 2011; SHAHRAJABIAN et al, 2019), and it is the barrier for the goal of having sustainable agriculture and organic life (SOLEYMANI and SHAHRAJABIAN, 2012a,b). Nitrogenase is an ATP-hydrolyzing, redox-active complex of two component proteins, the dinitrogenase reductase γ_2 homodimer (NifH protein), and the dinitroenase $\alpha_2\beta_2$ heterotetramer, where α is NifD, and β is NifK proteins (RAYMOND et al, 2004; CHENG et al, 2005; ZHAN et al, 2016).

The molybdenum nitrogenase is an oxygen sensitive complex dinitrogenase (NifDK heterotetramer), and dinitrogenase reductase (NifH homodimer) (ORTIZ-MARQUEZ et al, 2014). $MoFe_7S_9$ metal cluster is the active site for dinitrogen reduction for α, however, is some organisms Mo is replaced by either Fe or V, which is called Anf and Vnf, respectively instead of Nif (RAYMOND et al, 2004). It has been reported that FeMo nitrogenase has been recognized to be more efficient in binding dinitrogen and reducing it to ammonia compare with alternative nitrogenase (Nif > Vnf > Anf) (MILLER and RADY, 1988). Via the enzyme nitrogenase, microorganisms catalyze nitrogen fixation, which has been highly conserved throughout evolution (HRYNKIEWICZ et al, 2019). All N_2 fixers carry the *nif* (nitrogen fixation) genes, which encoded the nitrogenase complex (ARGANDONA et al, 2005; CHENG, 2008; SUN and CHENG, 2018). Nitrogenase is definitely sensitive to oxygen, which is why a specific oxygen barrier is formed around the infected cells by a cell layer which may reduce oxygen level in nodule cortex (RIBEIRO et al, 2015; ZHAN et al, 2019). KESHRI et al. (2013) also reported that the key functional genes namely cbbL, nifH, amoA, and apsA involced in various nutrient cycling.

The genes which encoding of enzymes in nitrification process are ammonium monooxygenase (*amo*), hydroxylamine oxidoreductase (*hao*), and nitrite oxidoreducatse (*nx*r), whereas those that conduct denitrification consist of nitrite reductases (*nir*K, *nir*S), nitric oxide reductase (*nor*B), and nitrous oxide reductase (*nos*Z) (BRAUMAN et al, 2015). The genes which are most commonly used as functional markers to assess both the nitrification and denitrification processes are *amo*, *nir*K, *nir*S and *nos*Z (LEVY-BOOTH et al, 2014). The *nif* operon includes the nitrogenase structural gene *nif*H, which has been sequenced to provide a large database from different environments (ARGANDONA et al, 2005). The additional of external organic matter provides a good source of energy and nutrients to support growth, because many

of the microorganisms participating in N2 fixation are heterotrophic or mixotrophic (RAHAV et al, 2016; TANG et al, 2017). Also, *nif*H has been used as a molecular marker to determine diazotroph indices, which encodes a nitrogenase iron protein (CHEN et al, 2019).

The characterization of diazotroph communities by *nif*H genes could be a potential indirect approach to the assessment of levels of biological N fixation in soils (REARDON et al, 2014). TSOY et al. (2016) stated that in most nitrogen-fixing bacteria NifA is the master regulator of nitrogen fixation as it works in relationship with the RNA-plymerase sigma factor RpoN (SCIOTTI et al, 2003). Both phosphorus (P) deficiency and potassium (K) deficiency resulted in significant decreases in *nif*H gene expression and N_2-fixation activity, and P deficiency exhibited more restricted impacts (TANG et al, 2017). Dinitrogenase reductase (azoferredoxin), and dinitrogenase (molybdoferredoxin) are two principal subunits of the nitrogenase complex, and Nif (nitrogen fixing) proteins NifH (γ_2 homodimeric azoerredoxin), and NifD/K ($\alpha_2\beta_2$ heterotetrameric molybdoferredoxin) are the structural components of these subunits (KNEIP et al, 2007). Three types of nitrogenase are iron and molybdenum (Fe/Mo), iron and vanadium (Fe/V) or iron only (Fe) (BISHOP et al, 1986; CHISNELL et al, 1988; BISHOP and PREMAKUMAR, 1992). The Mo-nitrogenase has a higher specific activity which is expreseed better when Mo is available (BETANCOURT et al, 2008). Although, all bacteria which have role in nitrogen fixation possess the Mo-nitrogenase, but just some of them have the genes for the V- and Fe-nitrogenase or both (BELLENGER et al, 2014). TSOY et al. (2016) noted that all known nitrogenases need a FeS-cluster and some other metal-dependent cofactors for transduction. The most common metal-dependent cofactor is the molybdenum-dependent nitrogenase which is encoded by the *nifHDK* genes (BOYD and PETERS, 2013).

Other notable nitrogenases are vanadium- and iron-dependent nitrogenases encoded by the *vnfHDGK* and *anfHDGK* genes, respectively (SEEFELDT et al, 2009; HARTMANN and BARNUM, 2010). Different ways of nitrogen fixing from unavailable gaseous forms are divided into 4 parts which is shown in Table 1. Biological nitrogen fixation is divided into two parts, 1) agricultural systems, 2) natural systems, both agricultural systems and natural systems are included plant associated and free living nitrogen fixation. Biological nitrogen fixing agents in both agricultural and terrestrial natural systems is presented in Figure 1. Global nitrogen fixation is divided into biological and non-biological nitrogen fixation, biological fixation consists of symbiotic, associative, and free-living nitrogen fixation, and non-biological fixation consists of natural and chemical fertilizers. Main sources of biologically available nitrogen is indicated in Figure 2.

Table 1. Different ways of nitrogen fixing from unavailable gaseous forms in the atmosphere to usable forms for plants and other organisms (NH_4^+ or NO_3^-) (TIMOTHY, 1999)

Bacteria in symbiotic relationships with vascular plants
Symbioses between cyanobacteria and fungi (lichens) or plants
Free living heterotrophic or autotrophic bacteria which are typically related to soil or detritus
Abiotic reactions occur without microbes in the atmosphere related to lightening

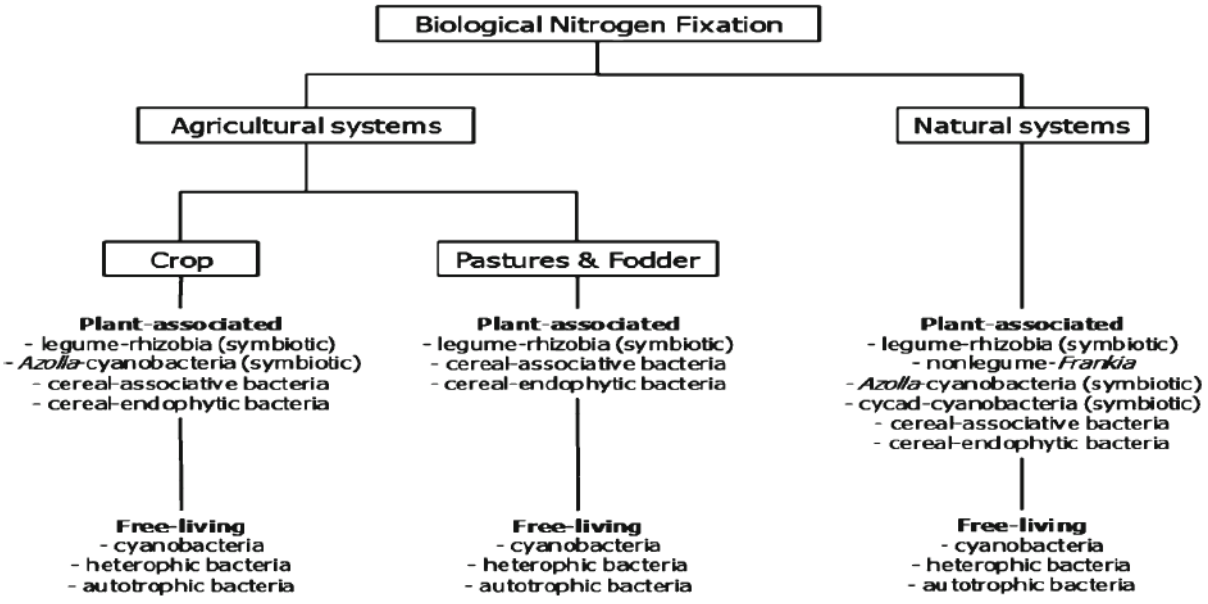

Figure 1. Biological nitrogen fixing agents in both agricultural and terrestrial natural systems (HERRIDGE et al, 2008)

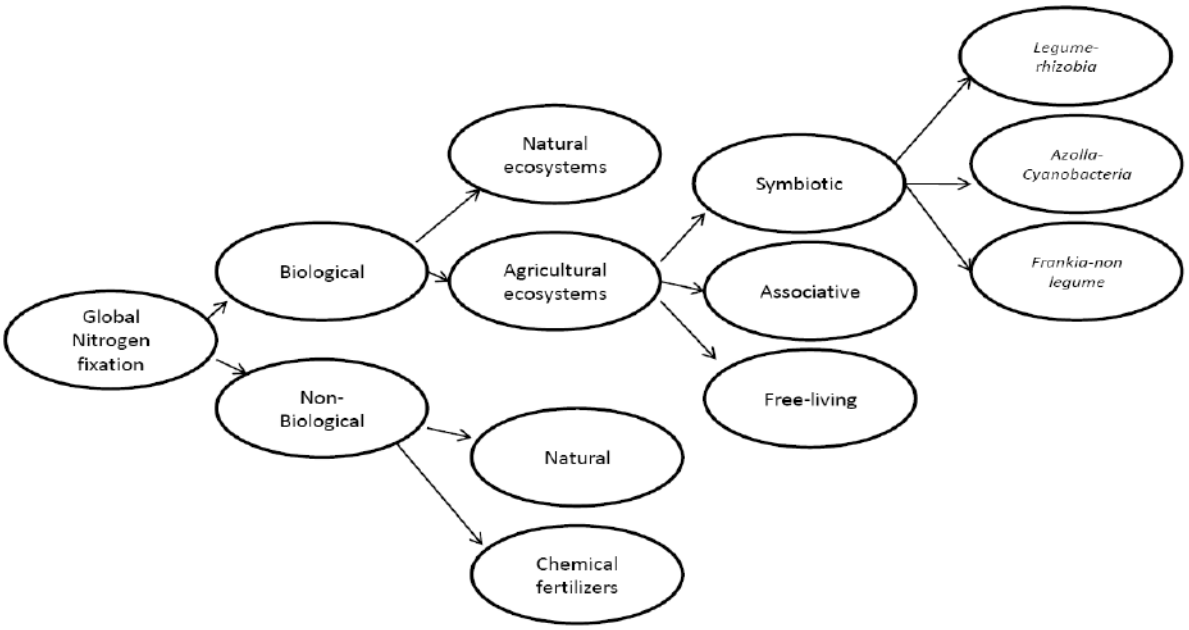

Figure 2. Main sources of biologically available nitrogen

Diazotrophs are categorized into two main groups namely: root-nodule bacteria and plant growth-promoting rhizobacteria (PGPR). Root-noduce bacteria cosnsit of rhizobia and *Frankia*. Rhizobia which include alpha-and betaproteobacteria enter into a symbiotic association with legumes and *Frankia* with actinorhizal plants. Some other palnts develop endosymbiotic interactions with nitrogen-fixing cyanobacteria (*Nostoc*). PGPRs consist of proteobacteria (alpha-, beta-, and gammaproteobacteria), actnobacteria, bacilli, and cyanobacteria (MUS et al, 2016). The oxidation of ammonia is done by ammonia oxidizers (both archaea and bacteria), and the nitrite produced is finally oxidized by nitrite-oxidising bacteria. In bacteria, ammonia is oxidized to nitrite via the intermediate hydroxylamine and the enzyme hydroxylamine oxidoreductase (HAO). Aerobic ammonia-oxidizing bacteria are known to have an important function in the marine nitrogen cycle. Anaerobic ammonium oxidation (anammox) carried out by some members of Planctomycetales which is also an important process in marine ecosystems. Aerobic ammonia oxidation during nitrification in presented in Figure 3. The nitrogen cycle is the set of biogeochemical processes, and it has changed thorugh Earth's history. It has been reported that there was no denitrification during the Archean, and after this period, nitrogen cycle and other biochemical cycles started. A simplified modern nitrogen cycle with known fractionimpacts for the nitrogenous reaction product (Left) and the proposed nitrogen cycle operating during the Archean (Right) are presented in Figure 4. Functional characterization of upregulated and downregulated selected proteins during low temperature N depletion condition is presented in Table 2. Homologs of the nifH gene can divided into five main phylogenetic clusters which are cluser I contains a diverse group of *nifH*, cluser II contains *anfH*, cluster III contain *nifH*, and cluster IV and V contain paralogous. Homologs of the nifH gene can be divided into five main phylogenetic clusters is indicated in Table 3.

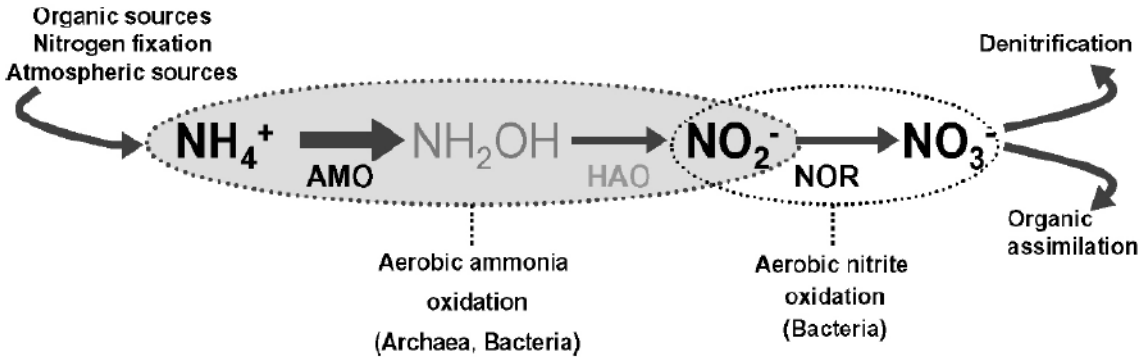

Figure 3. Aerobic ammonia oxidation during nitrification

Table 2. Functional characterization of upregulated and downregulated selected proteins during low temperature N depletion condition by *Pseudomonas palleroniana* N26 as revealed by LC-MS/MS analysis (SUYAL et al, 2018)

Genes	Proteins	Biological functions
Upregulated proteins (BM)		
*nif*H	Nitrogenase iron protein	Nitrogen fixation
*nif*A	*nif*-specific regulatory protein	Activation of most *nif* operons
*nif*L	Nitrogen fixation regulatory protein	Regulation of nitrogen fixation
*nif*B	FeMo cofactor biosynthesis protein	Biosynthesis of the iron-molybdenum cofactor
*nif*D	Nitrogenase molybdenum-iron protein	Nitrogen fixation
*nif*K	Nitrogenase molybdenum-iron protein	Nitrogen fixation
*nir*S	Nitrite reductase	Nitrite reduction
*hem*E	Uroporphyrinogen decarboxylase	Porphyrin biosynthesis
*gua*A	GMP synthase	Purine biosynthesis
*pyr*G	CTP synthase	Glutamine metabolic process
*pol*A	DNA polymerase I	DNA replication
*phe*T	Phenylalanine-tRNA ligase beta subunit	Phenylalanyl-tRNA aminoacylation
*gro*EL	60 kDa chperonin	Protein refolding
*gyr*B	DNA gyrase subunit B	DNA topological change
*rpl*E	50S ribosomal protein L1	Ribosomal large subunit assembly
*rpl*A	50S ribosomal protein L1	Translation regulation
*mur*D	UDP-*N*-acetylmuramoylalanine-D-glutamate ligase	Cell division
*uvr*B	UvrABC system protein B	DNA damage
*glm*S	Glutamine-fructose-6-phosphate aminotransderase	Glutamine metabolic process
*ala*S	Alanine-tRNA ligase	Alanyl-tRNA aminoacylation
*rps*F	30S ribosomal protein S6	Translation
tig	Trigger factor	Cell division
*tru*B	tRNA pseudouridine synthase B	tRNA processing
*tru*A	tRNA pseudouridine synthase A	tRNA processing
*pro*S	Proline-tRNA ligase	Prolul-tRNA aminoacylation
*gln*S	Glutamine-tRNA ligase	Glutamyl-tRNA aminoacylation
*his*S	Histidine-tRNA ligase	Protein biosynthesis
*sec*A	Protein translocase subunit SecA	Protein transport
*gly*A	Serine hydroxymethyltransderase	Amino-acid biosynthesis
*atp*G	ATP synthase gamma chain	ATP synthesis
*trm*D	tRNA (guanine-N91)-)-methyltransderase	tRNA processin
*mf*d	Transcription-repair-coupling factor	DNA repair
leuC_2	3-isopropylmalate dehydratase large subunit 2	Amino-acid biosynthesis
*sdh*A	Succinate dehydrogenase flavoprotein subunit	Electron transport
*yac*G	DNA gyrase inhibitor	Stress control
*cow*N	N(2)-fixation sustaining protein CowN	Protecting nitrogenase from CO
*coo*A	Carbon monoxide oxidation transcription regulator	CO regulator

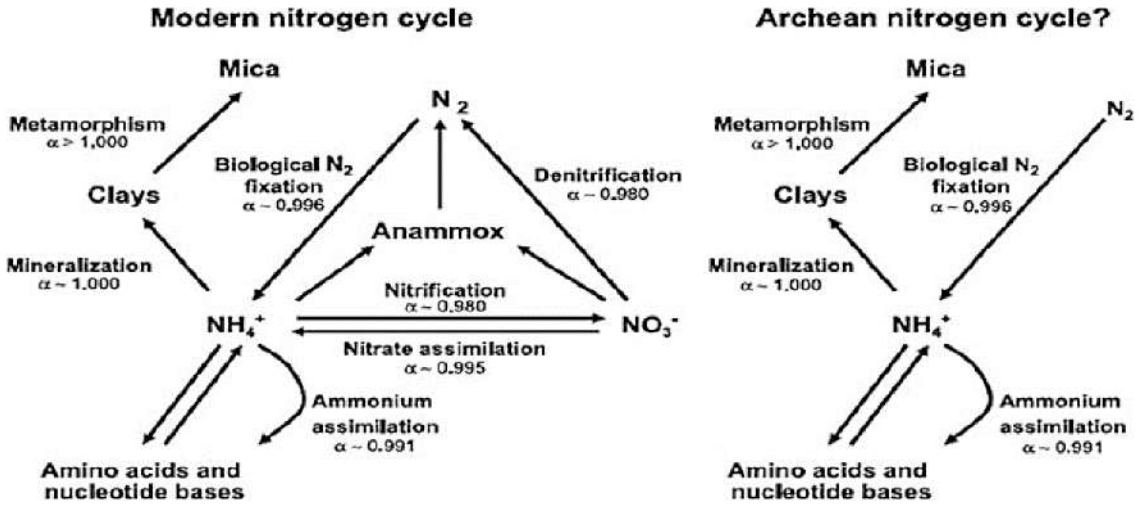

Figure 4. A simplified modern nitrogen cycle with known fractionimpacts for the nitrogenous reaction product (Left). The proposed nitrogen cycle operating during the Archean (Right) (PAPINEAU et al, 2005)

Table 3. Homologs of the nifH gene can be divided into five main phylogenetic clusters

Cluster I contains a diverse group of *nifH* genes primarily from aerobic and facultatively anaerobic organisms which belong to phyla including *Proeobacteria*, *Cyanobacteria*, *Firmicutes* and *Actinobacteria* (CHIEN and ZINDER, 1994).
Cluster II contains *anfH*, alternative nitrogenase which are paralogs of *nifH* and use an Fe-Fe cofactor in place of the Fe-Mo cofactor used by *nifH* (MASEPOHL et al, 2002). There also exist V-Fe alternative nitrogenases encoded by *vnfH*, and the alternative nitrogenases appear to be found only in the genomes of organisms which also contain *nif* genes (MASEPOHL et al, 2002).
Cluster III contains *nifH* genes that are almost exclusively found in obligate anaerobes including methanogenic *Archaea*, *Treponema*, *Clostridium* and sulfate-reducing and sulfur-reducing species of *Deltaproteobacteria* (CHIEN and ZINDER, 1994).
Cluster IV and V contain paralogous genes which do not participate in nitrogen fixation (RAYMOND et al, 2004; NOMATA et al, 2006; STAPLES et al, 2007).

Diazotrophs

Diazotrophs have a vital role in fixing atmospheric nitrogen (N) in terrestrial ecosystems (XIAO et al, 2020). The estimate areas of biological nitrogen fixation and related factors controlling BNF is done by diazotroph distribution (LIN et al, 2018; YANG et al, 2019), which contributes to the sustainability of agricultural ecosystems (REED et al, 2011). Diazotrophic community structure and diversity also mostly correlated with soil pH (FENG et al, 2018). MOSIANDER et al. (2012) showed that the free-living diazotrophs contributing to nitrogen fixation changes considerably and is mostly dependent on the soil nitrogen content. Diazotrophs are highly diverse an include members of α-, β-, and δ-Proteobacteria, Firmicutes, Cyanobacteria, and Archaea (ROSCH et al, 2002; REARDON et al, 2014).

Diazotrophs include free living bacteria, such as *Azospirillum*, *Cupriavidus*, and some sulfate reducing bacteria, and symbiotic *diazotrophs* such *Rhizobium* and *Frankia* (SELLSTEDT and RICHAU, 2013; YIN et al, 2018). DIXON and KAHN (2004) found that diazotrophs are found in a broad diversity of habitats: free-living in water and soil, symbiotic association in termite guts, associative symbioses with grasses, cyanobacterial symbioses with different plants, actinorhizal relationship with woody plants, and root-nodule symbioses with legumes. Biological nitrogen fixation by diaotophic bacteria in seagrass rhisozphere and leaf epiphytic community is also another considerable source of this process (LEE et al, 2007; GARCIAS-BONET et al, 2016). Nitrogen fixing plants can provide diverse impacts on diazotrophs under both nitrogen limitation or saturation (BISWAS and GRESS-HOFF, 2014; XIAO et al, 2020). A range of diazotrophic plant growth-promoting rhizobacteria which meaningfully boost the vegetative growth and final grain yield, participate in interactions with C_3 and C_4 crop plants such as rice, wheat, maize, sugarcane and cotton (KENNEDY et al, 2004).

The combination of intracellular symbiotic nitrogen fixation, may lead to increase rates of photosynthesis and presence of supplementary plant growh factors in cereals and other non-legumes (MOMOSE et al, 2013; DENT and COCKING, 2017). XIAO et al. (2020) concluded that diazotroph abundance may respond to differences in the density with leguminous plants. KE et al. (2019) revealed that soil compartment and different inoculation treatments were the main factors affecting the distribution of the diazotrophic community. PEREIRA et al. (2013) noted that two important parameters which may affect

diazotroph communities are temperature and soil moisture in different seasons. CHE et al. (2018) also noted that among all environmental factors, the soil moisture, organic carbon, available phosphorus, and inorganic nitrogen contents could be the main drivers of diazotroph distribution. Agronomic practices may also have impact on soil diazotrophs, such as application of nitrogen fertilizer which may reduce the diversity of diazotrophs (TAN et al, 2003). It has been reported that nitrogen supply is closely connected to soil diazotrophs, which shows the nitrogen supply capacity of soil (REED et al, 2011). CHEN et al. (2014) showed that the unicellular diazotrophs are important N_2 fixers and contributed significantly to N_2 fixation in the tropical marginal seas. CHEN et al. (2019) also confirmed that diazotrophic activity of heterotrophic Proteobacteria should be considered as an important part of nitrogen cycle in oceanic systems. *Trichodesmium* spp. and diatom-symbiotic *Calothrix rhizosoleniae* and *Richelia intracellularis* are important marine diazotrophs (GOMEZ et al, 2005), and it is believed that most of the biological nitrogen fixation in the ocean is performed by them (FOSTER et al, 2007; SHIOZAKI et al, 2014). The best hosts for *Rhizobium legumiosarum* are pisum, vicia, lathyrus and lens; Trifolium for *R. trifolii*; *Phaseolous vulgaris*, *P. angustifolia* for *R. phaseoli*; Medicago, Melilotus and Trigonella for *R. meliloti*; Lupinus and Ornithopus for Lupini, and *Glycine max* for *R. japonicum*. LAROCHE and BREITBARTH (2005) found that *Trichosemium* is one of the superior marine diazotrophs. Nitrogen is the basic component of aminoacids and nucleobases which has shown its importance for all living organisms on the Earth. Phylogenetic tree of the three domains of life is shown in Figure 5. DNA sequence comparisons and structural and biochemical comparisons consistently caregorize all living organisms into three primary domains, namely, Bacteria, Archaea and Eukarya. Bacteria and archaea are the smallest independently-living, single-celled organisms on earth. Most lack a true membrane-bound nucleus, so their DNA lies free in the cell cytoplasm, and their genome typically consists of a single circular molecule of double-stranded DNA. A cell membrane made of phospholipids surrounds the cell and outside is the cell wall, usually made up proteins, carbohydrates and lipids. Both bacteria and archaea require carbon to provide the building blocks for cell materials, and energy to drive the reactions involved in cell synthesis and metabolism. Archaea were originally thought to exist only in harsh environments and Archaea belonging to Crenarchaeota have been implicated in nitrogen cycling in soils. Fungi are eukarya and more closely related to plants and animals than to bacteria and archaea. Fungal cell contain membrane-bound nuclei with chromosomes which contain DNA. Fungi and archaea are closely associated with plant health and have significant biodiversity in the rhizosphere. The archaea possess the following characteristics: archaea are prokaryotic cells, they have membranes composed of branched hydrocarbon chains attached to glycerol by ether linkages, the cell walls of Archaea contain to peptidoglycan, Archaea are not sensitive to some antibiotics which affect the bacteria, but are sensitive to some antibiotics which influence the Eukaya, Archaea contain rRNA that is unique to the Archaea. The bacteria re prokaryotic cells, like the Eukarya, they have membranes composed of unbranched fatty chains attached to glycerol by ester linkages, the cell walls of Bacteria, unlike the Archaea and the Eukarya, contain peptidoglycan, Bacteria are sensitive to traditional antibacterial antibiotics but are resistant to most antibiotics which affect Eukarya, Bacteria contain rRNA which is unique to the Bacteria as showed by the presence molecular regions distinctly different from the rRNA of Archaea and Eukarya. The Eukarya have eukaryotic cells and their membranes composed of unbranched fatty acid chains attached to glycerol by ester linkages, some of them have a cell wall, and that wall contains no peptidoglycan, Eukarya are resistant to traditional antibacterial antibiotics but are sensitive to most antibiotics which influence eukaryotic cells, Eukarya contains rRNA which is unique to the Eukarya. Microbial domains comparisons are indicated in Table 4.

Table 4. Microbial domains comparisons (WANG et al, 2007)

Property	Bacteria	Archaea	Fungi
Cell Membrane	Made up of peptidoglycan and lipids are linked via ester molecule	Made up of pseudo-peptidoglycan and lipids are linked via ether molecule	Made up of different structures and lipids are linked via ester molecule
Gene Structure and Configuration	Chromosomes are circular, translation and transcription are unique	Chromosomes are circular, translation and transcription are similar to eukaryotes (fungi)	Chromosomes are multiple and linear, translation and transcription are similar to archaea
Structure of Internal Cell	The nucleus or organelles has no membrane bound	The nucleus or organelles has no membrane bound	There is membrane bound nucleus and organelles
Metabolic Reaction	There are several, including aerobic and anaerobic respiration, photosynthetic, autotrophic reactions and fermentation	There are several with methanogenic reaction specifically unique to this domain	Cellular respiration, fermentation and photosynthetic reaction
Reproduction	Reproduction is asexual and transfer of genes is horizontal	Reproduction is asexual and transfer of genes is horizontal	Reproduction is sexual and asexual

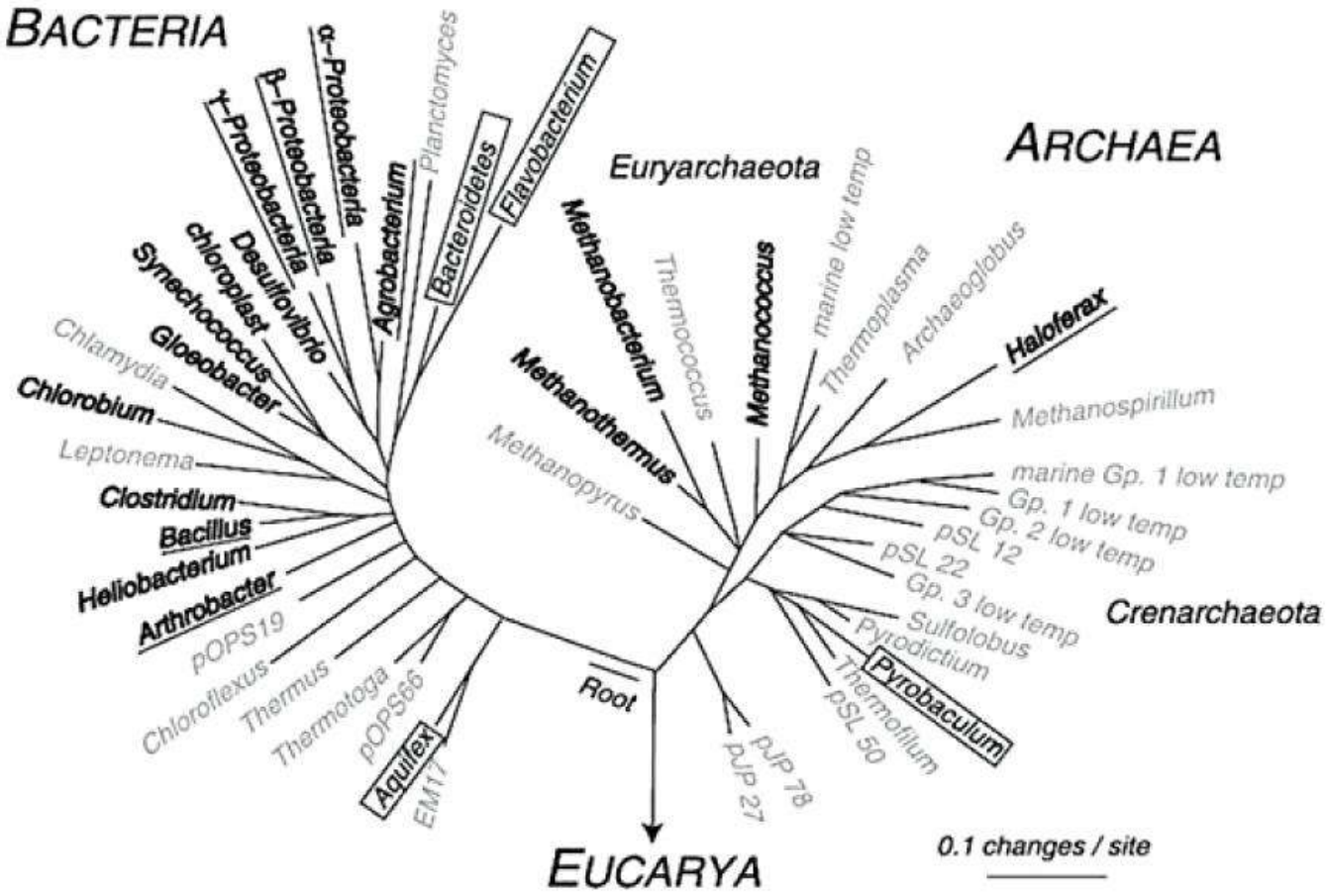

Figure 5. Phylogenetic tree of the three domains of life (Bacteria, Archaea and Eucarya) (BARNS et al, 1996)

Free-living and symbiotic nitrogen fixing bacteria are **a)** archeae which have two divisions, methanosarcinales, and methanobacteriales, **b)** bacteria which consists of divisions namely cyanobacteria, actinobacteria, proteobacteria, firmicutes (Clostridia), bacteroidetes/chlorobiales, spirochaetales and chloroflexi (KNEIP et al, 2007). Phylogenetic affinities of symbiotic and non-symbiotic nitrogen fixing bacteria. *Azotobacter* species (*Azotobacter vinelandii* and *A. chroococcum*) are free-living, aerobic heterotrophic diazotrophs that rely on an adequate supply of reduced C compounds like sugars for energy (KENNEDY et al, 2004). *Azospirillum* species aerobic heterotrophs that fix N_2 under microaerobic conditions (ROPER and LADHA, 1995), which grow widely in the rhizosphere of gramineous plants (KENNEDY and TCHAN, 1992). *Acetobacter* (Gluconacetobacter) diazotrophicus is an acid-tolerant endophyte which grows best on sucrose-rich medium (JAMES et al, 1994). *Azorhizobium* caulinodans increased the dry weight and N content of wheat plants in a green house experiment (MATTHEWS et al, 2001). Herbaspirillum is an endophyte which colonises sugarcane, rice, maize, sorghum and other cereals (JAMES et al, 2000).

Conclusion

Nitrogen availability often restricts biological productivity in ecosystems. Certain bacteria and archaea are responsible for biological nitrogen fixation. Metabolic pathways usually are common between archaea and bacteria. Nitrogen fixation is energetically expensive because it consumes 16 moles of ATP per mole of N fixed. Paying more attention on biological nitrogen fixation (BNF) has increased by growing concern about sustainability of agricultural production by considering population growth, environment and energy. Biological nitrogen fixation carried out by diazotrophs converts atmospheric N_2 to plant available ammonium.

Community structure and diversity of diazotrophic are correlated with soil pH. All known organisms which involve in nitrogen-fixing which are called diazatrophs are prokaryotes, and both bacterial and archaeal domains are responsible for that. DNA sequenc comparisons and structural and biochemical comparions consistenly categorize all living organisms into three primary domains, such as Bacteria, Archaea and Eukarya. Archaea and Bacteria are unicellular organisms, their cells lack organelles or other internal membrane-bound structures,

they have a single circular chromosome, they reproduce through fission and almost all prokaryotes have a cell wall, and the prokaryotes especially Archaea can survive in extreme environments which are inhospitable for most living things. Archaea are more closely related to eukaryotes than to bacteria, although superficially archaea appear to be much more similar to bacteria than eukaryotes. Diazotrophs are categorized into two main groups namely: root-nodule bacteria and plant growth-promoting rhizobacteria. Diazotrophs include free living bacteria, such as *Azospirillum*, *Cupriavidus*, and some sulfate reducing bacteria, and symbiotic *diazotrophs* such *Rhizobium* and *Frankia*.

Two important parameters which may affect diazotroph communities are temperature and soil moisture in different seasons. Nitrogenase is an ATP-hydrolyzing, redox-active complex of two component proteins, the dinitrogenase reductase γ_2 homodimer (NifH protein), and the dinitroenase $\alpha_2\beta_2$ heterotetramer, where α is NifD, and β is NifK proteins. Three types of nitrogenase are iron and molybdenum (Fe/Mo), iron and vanadium (Fe/V) or iron only (Fe). The Mo-nitrogenase has a higher specific activity which is expreseed better when Mo is available. Biological nitrogen fixation is the most important element in sustainable plants production and surviving life.

Funding

This work was supported by the National Key R&D Program of China (Research grant 2019YFA0904700).

Acknowledgments

Not applicable.

Authors' Contribution

All authors contributed equally to literature research, writing manuscript, etc.

Availability of data and materials

Not applicable.

Ethics approval and consent to participate

Not applicable.

Consent for publication

The authors consent for the publication of this review.

Competing interests

The authors declare that they have no potential conflicts of interest.

References

1. ARGANDONA M, FERNANDEZ-CARAZO R, LLAMAS I, MARTINEZ-CHECA F, CABA JM, QUESADA E. et al. The moderately halophilic bacterium *Halomonas Maura* is a free-living diazotroph. *FEMS Microbiol Lett*. 2005; 244: 69-74.
2. BARNS SM, DELWICHE CF, PALMER JD, PACE NR. Perspectives on archaeal diversity, thermophily and monophyly from environmental rRNA sequences. *Proc Natl Acad Sci U S A*. 1996; 93: 9188-9193.
3. BELLENGER JP, XU Y, ZHANG X, MOREL FMM, KRAEPIEL AML. Possible contribution of alternative nitrogenase to nitrogen fixation by asymbiotic N_2-fixing bacteria in soils. *Soil Biol Biochem*. 2014; 69: 413-420.
4. BETANCOURT DA, LOVELESS TM, BROWN JW, BISHOP PE. Characterization of diazotrophs containing Mo-independent nitrogenase, isolated from diverse natural environments. *Appl Environ Microbiol*. 2008; 74: 3471-3480.
5. BISHOP PE, PREMAKUMAR R, DEAN DR, JACOBSON MR, CHNISNELL JR, RIZZO TM, KOPCZYNSKI J. Nitrogen fixation by Azotobacter vinelandii strains having deletions in structural genes for nitrogenase. *Science*. 1986; 232: 92-94.
6. BISHOP PE and PREMAKUMAR R. Alternative nitrogen fixation systems. In Biological nitrogen fixation Edited by: Stacey G, Burris RH, Evans DJ. New York: Chapman & Hall. 1992; 736-762.
7. BISWAS B and GRESSHOFF PM. The role of symbiotic nitrogen fixation in sustainable production of biofuels. *Int J Mol Sci*. 2014; 15: 7380-7397.
8. BOYD ES and PETERS JW. New insights into the evolutionary history of biological nitrogen fixation. *Front Microbiol*. 2013; 4: 201.
9. BRAUMAN A, MAJEED MZ, BUATOIS B, ROBERT A, PABLO AL, MIAMBI EDOUARD. Nitrous oxide (N_2O) emissions by termites: does the feeding guild matter? *PLoS ONE*. 2015; 10(12): e01443440.
10. BROUMAND P, REZAEI A, SOLEYMANI A, SHAHRAJABIAN MH, NOORY A. Influence of forage clipping and top dressing of nitrogen fertilizer on grain yield of cereal crops in dual purpose cultivation system. *Res Crops*. 2010; 11(3): 603-613.
11. CHE R, DENG Y, WANG F, WANG W, XU Z, HAO Y, XUE K, ZHANG B, TANG L, ZHOU H, CUI X. Autotrophic and symbiotic diacotrophs dominate nitrogen-fixing communities in Tibetan grassland soils. *Sci Total Environ*. 2018; 639: 997-1006.
12. CHEN YLL, CHEN HY, LIN YH, YONG TC, TANIUCHI Y, TUO SH. The relative contributions of unicellular and filamentous diazotrophs to N_2 fixation in the South China Sea and the upstream Kuroshio. *Deep-Sea Res Part I*. 2014; 85: 56-71.
13. CHEN TY, CHEN YLL, SHEU DS, CHEN HY, LIN YH, SHIOZAKI T. Community and abundance of heterotrophic diazotrophs in the northern South China Sea: revealing the potential importance of a new alphaproteobacterium in N_2 fixation. *Deep-Sea Res Part I*. 2019; 143: 104-114.
14. CHENG Q, DAY A, DOWNSON-DAY M, SHEN G-F, DIXON R. The Klebsiella pneumonia nitrogenase Fe protein gene (nifH) functionally substitutes for the chlL gene in Chlamydomonas reinhardtii. *Biochem Biophysic Res Commun*. 2005; 329: 966-975.
15. CHENG Q. Perspectives in biological nitrogen fixation research. *J Integr Plant Biol*. 2008; 50: 786-798.

16. CHIEN YT and ZINDER SH. Cloning, DNA-sequencing, and characterization of a nifD-homologous gene from the archaeon methanosaticina barkeri-227 which resembles nifD from the eubacterium clostridium pasteurianum. *J Bacteriol*. 1994; 176: 6590-6598.
17. CHISNELL JR, PREMAKUMAR R, BISHOP PE. Purification of the second alternative nitrogenase from a nifHDK deletion strain of Azotobacter vinelandii. *J Bacteriol*. 1988; 170: 27-33.
18. COTTA SR, DIAS ACF, MARRIEL IE, ANDREOTE FD, SELDIN L, ELSAS JFV. Different effects of transgenic maize and nontransgenic maize on nitrogen-transforming archaea and bacteria in tropical soils. *Appl Environ Microbiol*. 2014; 80(20): 6437-6445.
19. DENT D and COCKING E. Establishing symbiotic nitrogen fixation in cereals and other non-legume crops: The Greener Nitrogen Revolution. *Agric Food Secur*. 2017; 6: 7.
20. DIXON R, CHENG Q, GUI G-F, DAY A, DOWSON-DAY M. Nif gene transfer and expression in chloroplasts: prospects and problems. *Plant Soil*. 1997; 194:193-203.
21. DIXON R and KAHN D. Genetic regulation of biological nitrogen fixation. *Nature Rev Microbiol*. 2004; 2:621.
22. FENG M, ADAMS JM, FAN K, SHI Y, SUN R, WANG D, GUO X, CHU H. Long-term fertilization influences community assembly processes of soil diazotrophs. *Soil Biol Biochem*. 2018; 126: 151-158.
23. FOSTER R, SUBRAMANIAM A, MAHAFFEY C, CARPENTER E, CAPONE D, ZEHR J. Influence of the Amazon River plume on distribution of free-living and symbiotic cyanobacteria in the western tropical North Atlantic Ocean. *Limnol Oceanography*. 2007; 52(2): 517-532.
24. GABY JC and BUCKLEY DH. A comprehensive aligned nifH gene database: a multipurpose tool for studies of nitrogen-fixing bacteria. Database. Article ID, bau001. 2014. doi: 10.1093/database/bau001
25. GARCIAS-BONET N, ARRIETA JM, DUARTE CM, MARBA N. Nitrogen-fixing bacteria in Mediterranean seagrass (Posidonia oceanica) roots. *Auqtic Botany*. 2016; 131: 57-60.
26. GOMEZ F, FURUYA K, TAKEDA S. Distribution of the cynaobacterium Richelia intracellularis as an epiphyte of the diatom Chaetoceros compressus in the western Pacific Ocean. *J Plankton Res*. 2005; 27: 323-330.
27. HARTMANN LS and BARNUM SR. Inferring the evolutionary history of Mo-dependent nitrogen fixation from phylogenetic studies of nifK and nifDK. *J Mol Evol*. 2010; 71: 7-85.
28. HERRIDGE DF, PEOPLES MB, BODDEY RM. Global inputs of biological nitrogen fixation in agricultural systems. *Plant Soil*. 2008; 311: 1-18.
29. HRYNKIEWICZ K, PATZ S, RUPPEL S. Salicornia europaea L. as an underutilized saline-tolerant plant inhabited by endophytic diazotrophs. *J Advan Res*. 2019; 19: 49-56.
30. JAMES EK, REIS VM, OLIVARES FL, BALDANI JI, DOBEREINER J. Infection of sugar cane by the nitrogen-fixing bacterium Acetobacter diazotrophicus. *J Experim Bot*. 1994; 45: 757-766.
31. JAMES EK. Nitrogen fixation in endophytic and associative symbiosis. *Field Crops Res*. 2000; 65: 197-209.
32. KE X, FENG S, WANG J, LU W, ZHANG W, CHEN M, LIN M. Effect of inoculation with nitrogen-fixing bacterium Pseudomonas stutzeri A1501 on maize plant growth and the microbiome indigenous to the rhizosphere. *Syst Appl Microbiol*. 2019; 42: 248-260.
33. KENNEDY IR and TCHAN Y. Biological nitrogen fixation in non-leguminous field crops: recent advances. *Plant Soil*. 1992; 141: 93-118.
34. KENNEDY IR, CHOUDHURY ATMA, KECSKES ML. Non-symbiotic bacterial diazotrophs in crop-farming systems: can their potential for plant growth promotion be better exploited? *Soil Biol Biochem*. 2004; 36: 1229-1244.
35. KESHRI J, MISHRA A, JHA B. Microbial population index and community structure in saline-alakine soil using gene targeted metagenomics. *Microbiol Res*. 2013; 168: 165-173.
36. KNEIP C, LOCKHART P, VO B, MAIER UG. Nitrogen fixation in eukaryotes- new models for symbiosis. *BMC Evol Biol*. 2007; 7: 55.
37. KOCH AL. Were Gram-positive rods the first bacteria? *Trends Microbiol*. 2003; 11: 166-170.
38. KUMAR U, PANNEERSELVAM P, GOVINDASAMY V, VITHALKUMAR L, SENTHILKUMAR M, BANIK A, ANNAPURNA K. Long-term aromatic rice cultivation effect on frequency and diversity of diazotrophs in its rhizosphere. *Ecol Engin*. 2017; 101: 227-236.
39. LAROCHE J and BREITBARTH E. Importance of the diazotrophs as a source of new nitrogen in the ocean. *J Sea Res*. 2005; 53: 67-91.
40. LEE KS, PARK SR, KIM YK. Effects of irradiance, temperature, and nutrients on growth dynamics of seagrasses: a review. *J Experim Marine Biol Ecol*. 2007; 350: 144-175.
41. LEVY-BOOTH DJ, PRESCOTT CE, GRAYSTON SJ. Microbial functional genes involved in nitrogen fixation, nitrification and denitrification in forest ecosystems. *Soil Biol Biochem*. 2014; 75: 11-25.
42. LIN Y, YE G, LIU D, LEDGARD S, LUO J, FAN J, YUAN J, CHEN Z, DING W. Long-term application of lime or pig manure rather than plant residues suppressed diazotroph abundance and diversity and altered community structure in an acidic Ultisol. *Soil Biol Biochem*. 2018; 123: 218-228.
43. LONG X, CHEN C, XU Z, OREN R, HE J-Z. Abundance and community structure of ammonia-oxidizing bacteria and archae in a temperate forest ecosystem under ten-years elevated CO_2. *Soil Biol Biochem*. 2012; 46: 163-171.
44. MAHATO S and KAFLE A. Comparative study of Azotobacter with or without other fertilizers on growth

and yield of wheat in Western hills of Nepal. *Annal Agar Sci*. 2018; 16: 250-256.
45. MAKAROVA KS, ARAVIND L, WOLF YI, TATUSOV RL, MINTON KW, KOONIN EV, DALY MJ. Genome of the extremely radiation-resistant bacterium Deinococcus radiodurans viewed from the perspective of comparative genomics. *Microbiol Mol Biol Rev*. 2001; 65: 44-79.
46. MASEPOHL B, SCHNEIDER K, DREPPER T et al. Alternative nitrogenase. In: Leigh, G.J. (ed). Elsevier Science B.V., Amsterdam, Netherlands, pp. 191-222. 2002.
47. MATTHEWS SS, SPARKES DL, BULLARD MJ. The response of wheat to inoculation with the diazotroph Azorhizobium caulinodans. *Aspects Appl Biol*. 2001; 63: 35-42.
48. MILLER RW and RADY RR. Molybdenum and vanadium nitrogenases of Azotobacter chroococcum. Low temperature favours N_2 reduction by vanadium nitrogenase. *Biochem J*. 1988; 256: 429-432.
49. MOISANDER PH, CHESHIRE LA, BRADDY J, CALANDRINO ES, HOFFMAN M, PIEHLER MF, PAERL HW. Facultative diazotrophy increases Cylindrospermopsis raciborskii competitiveness under fluctuating nitrogen availability. *FEMS Microbiol Ecol*. 2012; 79: 800-811.
50. MOMOSE A, HIYAMA T, NISHIMURA K, ISHIZAKI N, ISHIKAWA S, YAMAMOTO M et al. (2013) Characteristics of nitrogen fixation and nitrogen release from diazotrophic endophytes isolated from sugarcane stems. *Bullet Facul Agric Niigata Uni*. 2013; 66(1): 1-9.
51. MUS F, CROK MB, GARCIA K, COSTAS AG, GEDDES BA, KOURI ED, PARAMASIVAN P, RYU M-H, OLDROYD GED, POOLE PS, UDVARDI MK, VOIGT CA, ANE J-M, PETERS JW. Symbiotic nitrogen fixation and the challenges to its extension to nonlegumes. *Appl Environ Microbiol*. 2016; 82(13): 3698-3710.
52. NAVARRO-GONZALEZ R, MCKAY CP, MVONDO DN. A possible nitrogen crisis for Archaean life due to reduced nitrogen fixation by lightning. *Nature*. 2001; 412: 61-64.
53. NOMATA J, MIZOGUCHI T, TAMIAKI H et al. A second nitrogenase-like enzyme for bacteriochlorophyll biosynthesis-reconstitution of chlorophyllide a reductase with purified x-protein (bchX) and yz-protein (bchY-bchZ) from Rhodobacter capsulatus. *J Biol Chem*. 2006; 281: 15021-15028.
54. ODELADE KA and BARBALOLA OO. Bacteria, fungi and archaea domains in rhizospheric soil and their effects in enhancing agricultural productivity. *Int J Environ Res Public Health*. 2019; 16: 3873.
55. ORTIZ-MARQUEZ JCF, NASCIMENTO MD, CURATTI L. Metabolic engineering of ammonium release for nitrogen-fixing multispecies microbial cell-factories. *Metab Eng*. 2014; 23: 154-164.
56. PAPINEAU D, MOJZSIS SJ, KARHU JA, MARTY B. Nitrogen isotopic composition of ammoniated phyllosilicates: case studies from Precambrian metamorphosed sedimentary rocks. *Chem Geol*. 2005; 216(1-2): 37-58.
57. PEREIRA MES, SCHOLTER-HAI B, SCHLOTER, SALLES J. Temporal dynamics of abundance and composition of nitrogen-fixing communities across agricultural soils. *PLoS One*. 2013; 8: e74500.
58. PRAYITNO J and ROLFE B. Characterization of endophytic diazotroph bacteria isolated from rice. *HAYATI J Biosci* . 2010; 17(2): 73-78.
59. RAHAV E, GIANNETTO MJ, BAR-ZEEV E. Contribution of mono and polysaccharides to heterotrophic N_2 fixation at the eastern Mediterranean coastline. *Sci Rep*. 2016; 6: 27858.
60. RAYMOND J, SIEFERT JL, STAPLES CR, BLANKENSHIP RE. The natural history of nitrogen fixation. *Mol Biol Evol*. 2004; 21(3): 541-554.
61. REARDON CL, GOLLANY HT, WUEST SB. Diazotroph community structure and abundance in wheat-fallow and wheat-pea crop rotations. *Soil Biol Biochem*. 2014; 69: 406-412.
62. REED SC, CLEVELAND CC, TOWNSEND AR. Functional ecology of free-living nitrogen fixation: a contemporary perspective. *Annu Rev Ecol Evol Syst*. 2011; 42: 489-512.
63. RIBEIRO CW, ALLOING G, MANDON K, FRENDO P. Redox regulation of differentiation in symbiotic nitrogen fixation. *Biochimica et Biophysica Acta*. 2015; 1850: 1469-1478.
64. ROPER MM and LADHA JK. Biological N_2 fixation by heterotrophic and phototrophic bacteria in association with straw. *Plant Soil*. 1995; 174: 211-224.
65. ROSCH C, MERGEL A, BOTHE H. Biodiversity of denitrifying and dinitrogen-fixing bacteria in an acid forest soil. *Appl Environ Microbiol*. 2002; 68: 3818-3829.
66. SCIOTTI MA, CHANFON A, HENNECKE H, FISCHER HM. Disparate oxygen responsiveness of two regulatory cascades that control expression of symbiotic genes in Bradyrhizobium japonicum. *J Bacteriol*. 2003; 185: 5639-5642.
67. SEEFELDT IC, HOFFMAN BM, DEAN DR. Mechanism of Mo-dependent nitrogenase. *Annu Rev Biochem*. 2009; 78: 701-722.
68. SELLSTEDT A and RICHAU KH. Aspects of nitrogen-fixing actinobacteria, in particular free-living and symbiotic Frankia. *FEMS Microbiol Lett*. 2013; 342: 179-186.
69. SHAHRAJABIAN MH, SOLEYMANI A, NARANJANI L. Grain yield and forage characteristics of forage sorghum under different plant densities and nitrogen levels in second cropping after barley in Isfahan, Iran. *Res Crops*. 2011; 12(1): 68-78.
70. SHAHRAJABIAN MH and SOLEYMANI A. Responses of physiological indices of forage sorghum under different plant populations in various nitrogen fertilizer treatments. *Int J Plant Soil Sci*. 2017; 15(2): 1-8.

71. SHAHRAJABIAN MH, KHOSHKHARAM M, SUN W, CHENG Q. Exploring responses of berseem clover cultivars in low input cultivation management for agricultural sustainability. *World Sci News*. 2019; 131: 197-206.
72. SHIOZAKI T, CHEN Y-LL, LIN Y-H, TANIUCHI Y, SHEU D-S, FURUYA K, CHEN H-Y. Seasonal variations of unicellular diaztroph groups A and B, and Trichodesmium in the northern South China Sea and neighboring upstream Kuroshio current. *Cont Shelf Res*. 2014; 80: 20-31.
73. SMITH-MOORE C and GRUNDERN AM. Bacteria and archaea as the sources of traits for enhanced plant phenotypes. *Biotechnol Adv*. 2018; 36: 1900-1916.
74. SOLEYMANI A, SHAHRAJABIAN MH, NARANJANI L. Changes in qualitative characteristics and yield of three cultivars of Berseem clover intercropped with forage corn in low input farming system. *J Food Agric Environ*. 2011; 9(1): 345-347.
75. SOLEYMANI A, SHAHRAJABIAN MH, NARANJANI L. Study the effect of plant densities and nitrogen fertilizers on yield, yield components and grain protein of grain sorghum. *J Food Agric Environ*. 2011; 9(3&4): 244-246.
76. SOLEYMANI A and SHAHRAJABIAN MH. Forage yield and quality in intercropping of forage corn with different cultivars of berseem clover in different levels of nitrogen fertilizer. *J Food Agric Environ*. 2012; 10(1): 602-604.
77. SOLEYMANI A and SHAHRAJABIAN MH. Effects of different levels of nitrogen on yield and nitrate content of four spring onion genotypes. *Int J Agric Crop Sci*. 2012B; 4(4): 179-182.
78. SOLEYMANI A, SHAHRAJABIAN MH, MODARESI M. Influence of irrigation intervals and different levels of nitrogen fertilizer on yield and prussic acid content in second cropping of forage sorghum grown after wheat. *Res Crops*. 2012; 13(2):498-502.
79. STAPLES CR, LAHIRI S, RAYMOND J et al. Exprssion and association of group IV nitrogenase *nifD* and *nifH* homologs in the non-nitrogen-fixing archaeon *Methanocaldococcus jannaschii*. *J Bacteriol*. 2007; 189: 7392-7398.
80. SUN W and CHENG Q. Expression of nitrogenase structural scaffold genes in higher plant. *BAOJ Biotechnol*. 2018; 4: 038.
81. SUN W, SHAHRAJABIAN MH, CHENG Q. Anise (*Pimpinella anisum* L.), a dominant spice and traditional medicinal herb for both food and medicinal purposes. *Cogent Biol*. 2019; 5(1673688): 1-25.
82. SUYAL DC, KUMAR S, JOSHI D, SONI R, GOEL R. Quantitative proteomics of psychotrophic diazotroph in response to nitrogen deficiency and cold stress. *J Proteomics*. 2018; 187: 235-242.
83. TAN Z, HUREK T, REINHOLD-HUREK B. Effect of N-fertilization, plant genotype and environmental conditions on nifH gene pools in roots of rice. *Environ Microbiol*. 2003; 5: 1009-1015.
84. TANG Y, ZHANG W, ZHANG M, CHEN A, WEI W, SHENG R. Impact of fertilization regimes on diazotroph community compositions and N_2-fixation activity in paddy soil. *Agric Ecosyst Environ*. 2017; 247: 1-8.
85. TATUSOV RL, FEDOROVA ND, JACKSON JD, JACOBS JD, KIRYUTIN B, KOONIN EV, KRYLOV DM, MAZUMDER R, MEKHEDOV SL, NIKOLSKAYA AN. The COG database: An updated version includes eukaryotes. *BMC Bioinformatics*. 2003; 4: 41.
86. TIMOTHY CE. The presence of nitrogen fixing legumes in terrestrial communities: Evolutionaty vs ecological considerations. *Biogeochemistry*. 1991; 46: 233-246.
87. TSOY OV, RAVCHEEV DA, CUKLINA J, GELFAND MS. Nitrogen fixation and molecular oxygen: comparative genomic reconstruction of transcription regulation in Alphaproteobacteria. *Front Microbiol*. 2016; 7: 1343.
88. WANG Q, GARRITY GM, TIEDJE JM, COLE JR. Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl Environ Microbiol*. 2007; 73: 5261-5267.
89. WANG X, YANG J-G, CHEN L, WANG J-L, CHENG Q, DIXON R, WANG Y-P. Using synthetic biology to distinguish and overcome regulatory and functional barriers related to nitrogen fixation. *PLoS ONE*. 2013; 8: e68677.
90. XIAO D, TAN Y, LIU X, YANG R, ZHANG W, HE X, XU Z, WANG K. Responses of soil diazotrophs to legume species and density in a karst grassland, southwest China. *Agric Ecosyst Environ*. 2020; 288: 106707.
91. YANG L, BAI J, ZENG N, ZHOU X, LIAO Y, LU Y, REES RM, NIE J, CAO W. Diazotroph abundance and community structure are reshaped by straw return and mineral fertilizer in rice-rice-green manure rotation. *Appl Soil Ecol*. 2019; 136: 11-20.
92. YIN Y, GU J, WANG X, ZHANG K, HU T, MA J, WANG Q. Impact of copper on the diazotroph abundance and community composition during swine manure composting. *Bioresour Technol*. 2018; 255: 257-265.
93. ZHAN Y, YAN Y, DENG Z, CHEN M, LU W, LU C, SHANG L, YANG Z, ZHANG W, WANG W, LI Y, KE Q, LU J, XU Y, ZHANG L, XIE Z, CHENG Q, ELMERICH C, LIN M. The novel regulatory ncRNA, NfiS, optimizes nitrogen fixation via base pairing with the nitrogenase gene *nifK* mRNA in *Pseudomonas stutzeri* A1501. *Proc Natl Acad Sci U S A*. 2016; 113: 201604514.
94. ZHAN Y, DENG Z, YAN Y, ZHANG H, LU C, YANG Z, SHANG L, HUANG Y, LV F, LIU Y, LIU Y, WANG S, CHEN S, ZHANG X-X, CHENG Q, LIN M. NfiR, a new regulatory noncoding RNA (ncRNA) is required in concert with the NfiS ncRNA for optimal expression of nitrogenase genes in *Pseudomonas stutzeri* A1501. *Appl Environ Microbiol*. 2019; 85: e00762-19.