

Comparative Study of Nitrogen-Fixing Bacteria in Terrestrial and Aquatic Habitats: Insights and Future Prospects

Soniya Goyal¹, Amit Kumar¹, Pooja Sharma^{1*}, Anita Rani Gill²,
Anil Kumar Sharma³, Ashwanti Devi¹ and Chahat Sharma¹

¹Department of Biosciences and Technology, Maharishi Markandeshwar
(Deemed to be University), Mullana-Ambala-133207, India.

²Guru Jambheshwar Science and Technical University, Hissar (Haryana), India.

³Amity University, Mohali, Punjab, India.

<http://dx.doi.org/10.13005/bbra/3235>

(Received: 22 February 2024; accepted: 05 June 2024)

Nitrogen is an essential nutrient utilized by living organisms to produce a number of organic molecules such as nucleic acid, amino acid and protein. A special class of microorganisms known as diazotrophs that are able to fix atmospheric nitrogen into biological form of nitrogen which is directly utilized by plants. Moreover, they stimulate plant growth by decreasing or preventing deleterious effects of pathogens through synthesis of antibiotics, competition for nutrients through production of siderophores. Along with terrestrial diazotrophs, various marine bacterial species are well known which involves in biological nitrogen fixation process. In an open oligotrophic ocean, pelagic and sympagic nitrogen fixation has been shown a significant source of biological nitrogen for survival of oceanic living organisms. A different range of diazotroph have been reported at low temperature ice sea environment. The community of nitrogen fixing bacteria under deep anoxic bottom water is gradually increase. It may be possible that due to genetic variation population of nitrogen fixing bacteria increases under sea water. In this review article, authors have focused on nature of diazotrophs in terrestrial as well as in aquatic conditions. Moreover, the bacterial habitat and their classification, latest approaches and future prospects is also included in the manuscript. Thus, the study would be helpful for understanding the mode of action, their behavior and survival rate in different environment and beneficial for improvement in agricultural sustainability.

Keywords: Biological Nitrogen Fixation, Diazotroph, Marine, Symbiotic; Terrestrial; Pelagic.

In the terrestrial ecosystem, biological nitrogen fixation in the rhizosphere is directly influenced by the exudates of root secretions. This is the major contribution for nitrogen economy of biosphere i.e 30-50% of the total nitrogen used in the crop fields^{1,2}. Nitrogen is an essential nutrient which is taken by all organisms for production of a variety of organic molecules such as nucleic acids, proteins and amino acids. It is a limiting

factor that is responsible for growth of plant and yield production as nitrogen is a major constituent of chlorophyll (most vital pigment mandatory to carry out photosynthesis). Plants can acquire only combined form of nitrogen by three different ways such as Haber-Bosch process or manure to soil through decomposition of an organic matter, conversion of nitrogen present in an atmosphere to usable compounds through natural processes

*Corresponding author E-mail: pooja0029@gmail.com



such as lightning and biological nitrogen fixation (BNF)^{3,4,5}. A BNF system is the most suitable process to sustain the agricultural land naturally. BNF was initially discovered by Beijerinck⁶ which belongs to a special class of prokaryotic bacteria⁷. There are special types of microorganisms known as diazotrophs that are able to grow without external source and can fix atmospheric nitrogen into biological form of nitrogen which is directly utilized by plants. It is a natural process that is till date limited to prokaryotes but research has been going on to find the potent eukaryotes. These microorganisms can perform a variety of transformation reactions such as oxidation of nitrogen compounds to nitrite or nitrate, reduction of oxidized nitrogen compounds to ammonium or fixing of atmospheric nitrogen into ammonia encoding by nitrogenase enzyme^{8,9}.

Although diazotrophs are phylogenetically diverse with different physiological properties but still it is observed that rate of nitrogen fixation affected by various physiological and environmental factors like temperature, water holding capacity, nitrogen level, pH, salinity, water stress etc. The ability of BNF has been observed in phototrophic microorganisms include aerobic phototrophic cyanobacteria^{10,11,12}, anaerobic green sulphur phototrophs like *chlorobium* and anaerobic purple sulphur phototrophs like *chromatium*¹³. *Pseudomonadales* and *Rhizobiales* are two dominant diazotrophs are reported in bulk paddy soil rhizosphere¹⁴. It was found that due to increasing contamination of PCB (polychlorinated biphenyl) in paddy crops diazotroph community increases¹⁵. *Gluconacetobacter spp.*, *Herbaspirillum*, *Bradyrhizobium sp.*, *Burkholderia spp.* and *Rhizobium sp.* are also examined as predominant diazotrophs from sugarcane field¹⁶. Furthermore, some of the *Agrobacterium* and *Bacillus* species are also found in coastal saline soils of Sundarbans, West Bengal which depicts the BNF capacity of diazotroph under saline conditions well¹⁷. There is a huge bacterial diversity in switch grass (*Panicum virgatum*). It is characterized that *Methylobacterium* and *Rhizobium* belong to alpha proteobacteria, *Burkholderia*, *Geobacter* and *Desulfuromonas* belonging to delta proteobacteria and *Azoarcus* belonging to beta proteobacteria as predominant diazotrophs in switch grass on the basis of *nifH* RNA sequences¹⁸. Besides nitrogen

fixation, diazotrophs can also affect growth of plant directly *via* production of plant hormones and vitamins, inhibition of ethylene synthesis, enhancement in stress resistance, improvement in uptake of nutrients, mineralization of organic phosphate and solubilization of inorganic phosphate. However, diazotrophs indirectly affect plant growth by decreasing or preventing harmful effects of pathogens through synthesis of antibiotics, competition for nutrients *via* production of siderophores.^{19,96}

Diazotrophs are not only fix nitrogen in terrestrial but it can also fix nitrogen in marine water²⁰. In an open oligotrophic ocean, the dominant source of nitrogen is a pelagic nitrogen fixation. Various research studies have revealed that biological nitrogen fixation plays a major role in ocean²¹⁻³⁰. Microbial mediated redox reactions are the main source of available biological nitrogen in the ocean. The whole process is regulated by denitrification, anaerobic ammonium oxidation and nitrogen fixation^{31,32}. Although all the processes having equally important to fix the nitrogen under water conditions but nitrogen fixation is one of the most important pathways which plays a significant role in control of oceanic nitrogen. In marine ecosystem *Trichodesmium* is a non-heterocystous cyanobacterium providing fixed nitrogen to the oligotrophic sea. *Trichodesmium* can occur at a depth of 200 m with extensive filaments. These filaments can cluster into puff or tuff colonies *to form* extensive annual blooms. Mainly diazotrophic bacteria under sea water form water blooms regulated by temperature. Some of the nitrogen fixing microorganisms in the sub-tropical north pacific gyre can fuel to surface phytoplankton blooms over several month³³. *Trichodesmium* have unique capacity in forming visible bloom which are very dense in nature³⁴. They are probably the best studied marine N₂ fixer bacteria. N₂ fixation by *Trichodesmium spp.* has been shown to be an important source of nitrogen for tropical as well as for subtropical marine systems^{35,19,20,36}. It can help nitrogen fixation under marine via direct as well as indirect, through synthesis of amino acids, dissolved organic nitrogen (DON), NH₄⁺ and indirect through regeneration of Dissolved Inorganic Nitrogen (DIN) respectively^{37,38}. Moreover, it is also reported that at high-latitude water of the Danish Strait and Western Arctic region, a unicellular cyanobacteria

symbiont (UCYN-A) alga sustained the marine N₂-fixation to water at low temperature with high concentration of fixed inorganic nitrogen²⁷.

In addition to this, diversity of nitrogen fixing bacteria can also depend on physiological criteria such as nutrient regime preferences, temperature, tolerance range and iron requirement under sea^{39, 20, 96}. Some of the basic requirements for the diazotrophic bacteria in sea water is stable water column with an upper mixed layer around 100 m, low nutrients, very clear water and depth light penetration. Furthermore, other factors such as range and optimum temperature, salinity and light influences provide an empirical framework for the growth of bacteria. In comparison with photosynthesis, the nitrogen fixation phenomenon is quite related as both the processes are strongly tied to a circadian rhythm⁴⁰. Diazotroph grows well in subtropical as well as tropical surface water due to high light photon flux and acclimatize themselves to the photosynthesis characteristics as per its position in the water column and availability of light¹⁴. Community of Diazotroph in marine also depends on their environmental or sea conditions (Coastal Canadian Arctic, Arctic Tundra and Glacial Antarctica lakes). One of the important sources of nitrogen is atmosphere which is utilized by diazotrophs for providing bioavailable N₂ in pelagic ecosystems⁴¹. In an open oligotrophic ocean, pelagic and sympagic nitrogen fixation has been shown a significant source of biological nitrogen for survival of oceanic living organisms. A different range of diazotrophs has been reported in low-temperature ice sea environments^{42,43}.

Microbial communities which are present in the shallow marine sediments have a significant role in the oxidation of complex organic compounds and regeneration of the nutrients. Recent search suggests that percentage of nitrogen fixing bacteria increases in deep anoxic bottom water. Similarly in South Pacific Ocean more nitrogen fixation was observed in dark suboxic zone^{44, 23}.

Classification

The organisms which fix nitrogen can be categorized broadly into two types one is agricultural and another is natural systems which further divided into different groups. Based on habitat, diazotrophic bacteria can be classified into four groups: free-living, associative, endophytic and symbiotic nodule formers (Table: 1).

Nitrogen fixation by free living Diazotrophs

Most of the heterotrophic soil bacteria participating in nitrogen fixation without direct interaction with other microorganisms for example *Azotobacter*, *Clostridium*, *Bacillus*, *Klebsiella*, *Burkholderia*, *Desulfovibrio*, *Enterobacter*, and *Serratia*^{45,46,47}. *Klebsiella pneumoniae* has applied as a model system for studying the regulation of nitrogen fixation by *nif* genes. The by-products of the nitrogen control genes *ntrA*, *ntrB*, and *ntrC* control the expression of the 17 *nif* genes in *Klebsiella* in response to the nitrogen source as shown in Fig: 1. The bacteria belonged to free living group are grown on decomposed organic matter in soil. Free living microorganisms use organic molecules in oxidized form that are synthesized by other organisms or from decomposition as their source of energy. Some of them are using chemo-lithotrophic method for utilization of inorganic compounds as source of energy. Since, oxygen could inhibit the activity of nitrogenase, free living diazotrophs works under anaerobic or micro-aerophilic condition for nitrogen fixation reactions.

In natural conditions suitable carbon and energy sources are very low to fix nitrogen by diazotrophs. Researchers are using various approaches to improve the agronomy conditions for biological nitrogen fixation as well as nitrogen use efficiency. A similar type of study is supported in Australia on intensive wheat rotation farming system showed that free living diazotrophs contributed 20 kg/hectare per year to the long term demands of nitrogen of this cropping system. In this system, wheat stubble is maintained to provide optimum carbon and nitrogen conditions, optimizing the activity of free-living organisms⁴⁸.

Nitrogen fixation by associative Diazotrophs

The rhizospheric bacteria belonging to associative group proliferate on the surface of roots and nourished on root exudates⁴⁹. The most studied examples of this class are *Azospirillum*, *Acetobacter*, *Burkholderia*, *Azoarcus* and *Herbaspirillum* which have ability to make close association with *Poaceae* members such as rice, corn, oats, wheat, barley, sugarcane and other non-leguminous plants^{50,51}. In addition to this, occurrence of microsites associated with plant residues decomposition, in mixtures containing decomposable particulate organic matter and in termite habitats were also

shown. This class of bacteria is able to perform suitable amount of nitrogen fixation within the rhizosphere of the host plants. An amount of nitrogen fixation depends on several factors such as soil temperature (*Azospirillum* survive in more temperate environment), area of host plant for rhizosphere, low oxygen pressure in environment, competitiveness among bacteria and efficacy of nitrogenase enzyme⁵². *Azospirillum* can convert atmospheric N_2 into NH_3^+ under low aerobic conditions at low nitrogen levels using nitrogenase enzyme complex. This enzyme is comprised of two components: the dinitrogenase protein (MoFe protein, NifDK), site of nitrogen reduction containing molybdenum-iron cofactor and dinitrogenase reductase protein (Fe protein, NifH) which transfers electrons from an electron donor to nitrogenase⁵³.

Nitrogen fixation by endophytic Diazotrophs

Similar to rhizospheric bacteria, endophytic bacteria belong to distinct class of bacteria that can colonize within plant tissues and offer a variety of benefits to the plant⁵⁴. This group of diazotrophs invade and multiply internally in plant parts without causing any plant disease and responsible to promote plant growth. Endophytic bacteria include *Gluconacetobacter*, *Herbaspirillum*, and *Burkholderia* act as plant growth promoters for grasses such as sugarcane and maize. Both endophytic and associative groups of bacteria may involve in promoting plant growth by establishing intimate and reciprocal associations with plants. The advantages of these diazotrophic bacteria include role in biological nitrogen fixation (BNF), production of plant hormones and solubilization of phosphate^{55,56}.

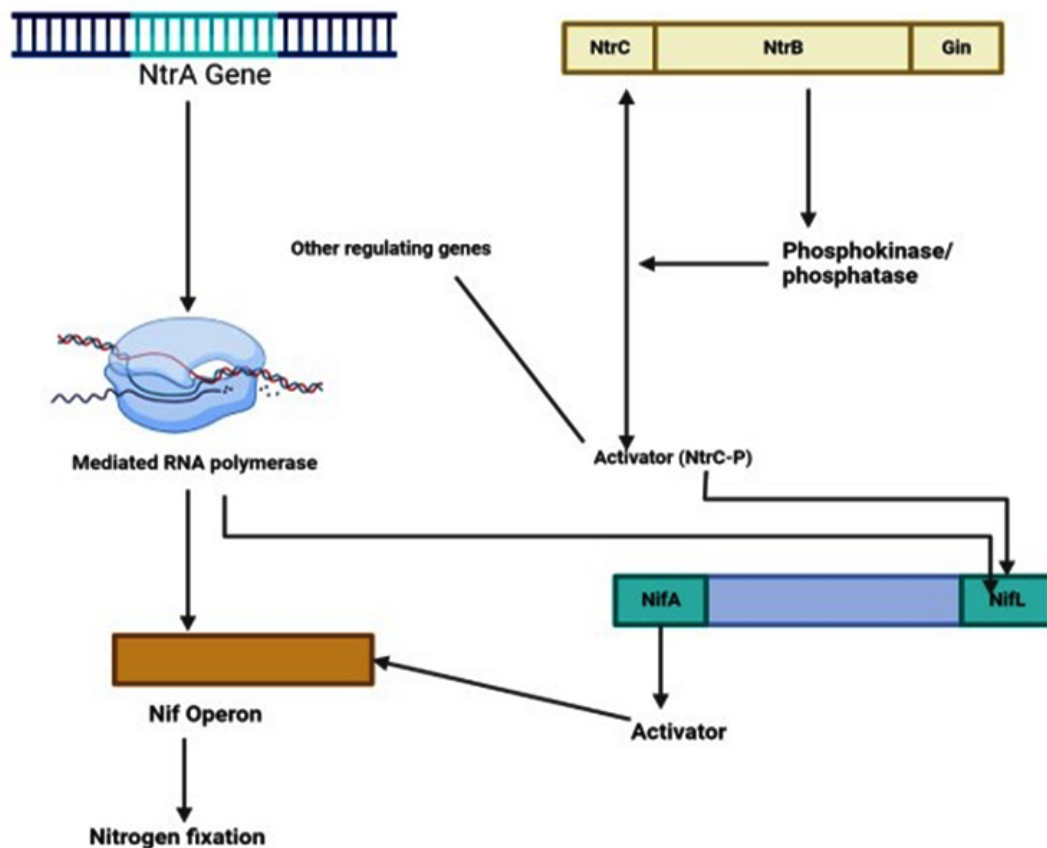


Fig. 1. Showing regulation of *nif* genes in *Klebsiella pneumoniae*

Nitrogen fixation by nodule formation

The diazotroph belonging to symbiotic group form nodules and established symbiotic relation with several shrubs and woody species. The important examples of this group are collectively known as rhizobia which form symbiotic relation with plants of *leguminosae* family. The other examples are nodule forming bacteria *Actinobacter* of Frankia genus with Casuarina and Allocasuarina species. Rhizobium are gram negative, motile bacteria (thin cell wall), easily grown on culture media while Frankia, a gram-positive-bacteria, growing by elongating branched hyphae, fused and producing vesicles and sporangia which release spores for dispersal⁵⁷. The nodule-forming bacteria, especially rhizobia’s symbiotic association with legumes are particularly the most efficient approach in exploiting nitrogen fixation in agriculture. The symbiotic and associative nitrogen fixers can be found in rhizosphere of legumes and non-legume plants^{29,58}. Diazotrophs can be classified into two groups based on their associations: free-living and symbiotic.

The most important example of nodule formation is legume nodule formation^{59,60}. Species of *Bradyrhizobium* or *Rhizobium* bacteria invade

root system of host plant and cause the formation of root nodules to reside and begin nitrogen fixation. The process of nodulation shows an ordered association between host and bacteria⁶¹. The process starts with an attraction of *Rhizobia* to flavonoid produced by roots of host legumes leads to an attachment of bacteria to root hairs. This process completes in two steps first attachment of bacteria using Ca⁺² binding protein (rhicadhesin) and then accumulation to root hair surface. A strong attachment involves lectins (produced by host plant) and fimbriae (produced by bacteria). The host plant then attracts towards chemicals released by rhizobia known as Nod factors (lipochitin oligosaccharides) which is responsible for colonization of root hairs to form curls known as shepherd’s crook. After, penetration of rhizobia into root hair, a tubular structure known as infection thread will be formed. After reaching to roots, bacteria stimulate cortical cell division which leads to nodule formation. The bacteria lose their cell wall and undergo various morphological changes to form large, irregular shaped branching cells known as bacteroids after reaching inside and entirely dependent on host legume for their energy requirements and fix nitrogen for plant⁶².

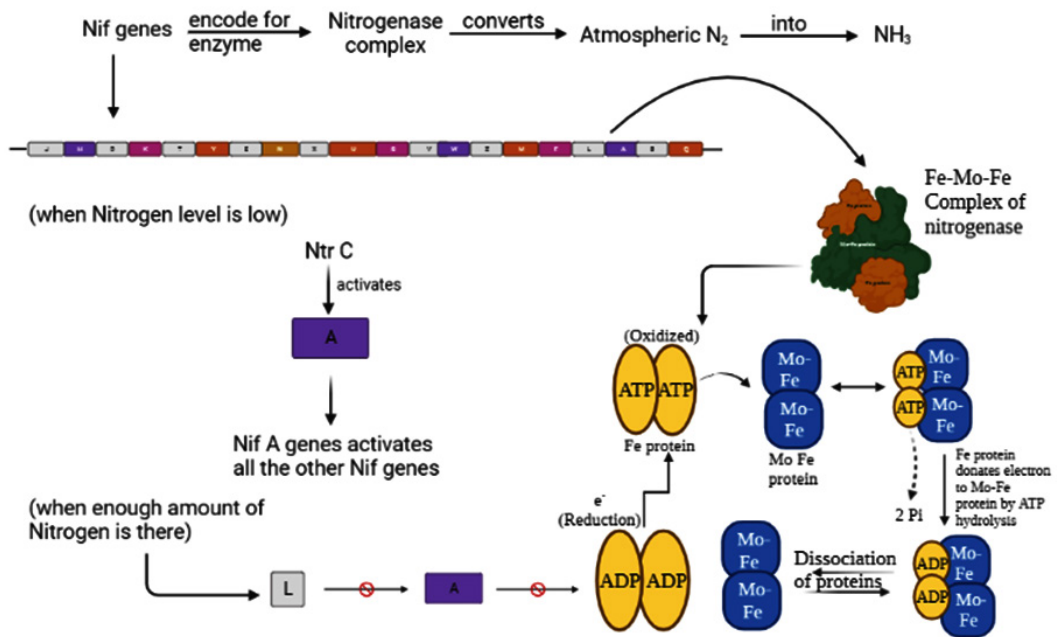


Fig. 2. Important roles of *nif* genes in biological nitrogen fixation and functioning of nitrogenase under low level and high level of nitrogen

Table 1. Classification of nitrogen fixing bacteria in different crop system

S. No	Types of nitrogen fixation	Description	Name of bacterial strains	Plants / Crops involved	References
1	Symbiotic nitrogen fixation	Mutual interaction between host plant and bacteria to fix nitrogen.	<i>Rhizobia</i> , <i>Frankia</i> , some of <i>cyanobacteria</i> etc.	Pea, chickpea and other legumes	[57, 58, 61, 62]
2	Free living nitrogen fixation	There is no symbiosis between host and microbes and bacteria are freely present in soil to fix nitrogen.	<i>Azotobacter</i> , <i>Beijerinckia</i> , <i>Clostridium</i> , <i>cyanobacteria</i>	Corn, wheat, rice and other cereals.	[90, 95,97]
3	Endophytic nitrogen fixation	Bacteria present within the plant tissues, fix nitrogen symbiotically.	<i>R. leguminosarum</i> , <i>G. diazotrophicus</i> , <i>Enterobacter</i> sp., <i>Bacillus</i> sp. <i>Burkholderia</i> etc.	Sugarcane, corn, canola etc.	[55, 56]
4	Associative nitrogen fixation	Nitrogen is fixed by some associative bacteria present in common association with plants (not symbiosis).	<i>Azoarcus</i> , <i>Pseudomonas stutzeri</i> , <i>Herbaspirillum</i> , <i>Paenobacillus</i> etc.	Rice, sugarcane and other non-legumes	[90]

Many diazotrophs perform nitrogen fixation by making symbiotic relation with host plant. The plants produced carbohydrates through photosynthesis which are utilized by nitrogen fixing diazotrophs as their energy source. The diazotrophs provide nitrogen in a usable state to host plants to promote growth and development. An example of this association is *Azolla* (water fern) with cyanobacterium *Anabaena azollae*. A significant amount of nitrogen is fixed by Cyanobacteria in specialized cells known as heterocysts. This symbiotic association has been utilized as a biofertilizer in wetland paddies in Southeast Asia from many years. It was reported that during growing season, rice paddies are typically covered with *Azolla* known as bloom which fix nitrogen upto 600kg N ha⁻¹ Yr⁻¹¹⁶³.

Although, symbiotic association plays a vital role in nitrogen fixation ecology worldwide but the most significant symbiotic associations are those among legumes (alfalfa, beans, clover, cowpeas, peanuts, soybean and lupines) and *Rhizobium*. In agricultural production, among all legumes, soybeans are grown on 50% of world's agricultural land and represent 68% of total legume production globally³. It is also known that the methanotrophic bacteria and covered deadwood are capable for nitrogen inputs in boreal forest⁶⁴.

The symbiotic association between bacteria and host legume⁶⁵ is specific such as specific *Rhizobium* or *Bradyrhizobium* will only nodulate a limited number of plant genus. For example, *Rhizobium melilotii* could be able to nodulate alfalfa while *Rhizobium leguminosarum biovar trifolii* only able to nodulate clover (*Trifolium*). The term host specificity refers to cross inoculation group cell signaling between legume host and bacteria which is mainly due to Nod factors^{65,66}. The major cross inoculation groups are mentioned in Table 2.

Regulation of N₂ fixation

Nitrogenase is an essential metalloenzyme that has specific features to convert atmospheric nitrogen into biological nitrogen. Three different nitrogenase system (molybdenum (Mo) nitrogenase, vanadium (V) nitrogenase, and iron (Fe) only) were known among the nitrogen-fixing bacteria Table: 3. It includes a catalytic protein (dinitrogenase) and an electron-transferring reductase protein (Fe protein) that transfers

Table 2. Nodule forming bacteria in specific host

S.No.	Host plant	Nodule forming bacteria
1	Sesbania	<i>Azorhizobium caulinodans</i>
2	Soybean	<i>Bradyrhizobium japonicum</i> , <i>Bradyrhizobium elkanii</i> , <i>Rhizobium fredii</i>
3	Lotus	<i>Mesorhizobium loti</i>
4	Peas	<i>Rhizobium leguminosarum</i> biovar <i>viceae</i>
5	Alfalfa	<i>Sinorhizobium melilotii</i>
6	Beans	<i>Rhizobium leguminosarum</i> biovar <i>phaseoli</i> and <i>Rhizobium tropici</i>
7	Clover	<i>Rhizobium leguminosarum</i> biovar <i>trifolii</i>

Table 3. Group of Nitrogenase *nifH* genes

S.No.	Groups	Genes
1	Cluster I	Mo-containing <i>nifH</i> and <i>vnfH</i>
2	Cluster II	<i>anfH</i> , <i>nifH</i>
3	Cluster III	A diverse group, many that are strictly anaerobes
4	Cluster IV	A divergent, loosely coherent group with <i>nifH</i> -like sequences

the electrons necessary for dinitrogen fixation. Both enzymes are highly sensitive to oxygen or inactivate nitrogenase. Furthermore, some specific genes such as *nif* genes control the expression of the nitrogen fixation process within plant system. Basically, *nif* gene regulon is a set of seven different operon which includes total 17 *nif* genes. Some of the important *nif* genes are *Nif A, D, L, K, F, H S, U, Y, W, Z*. *Nif A* is one of the essential gene which regulates the expression of further *nif* genes Fig 2. When plants have no sufficient biological nitrogen, *Ntr C* trigger *nif A* and immediately *nif A* activates all the required *nif* genes for biological nitrogen fixation. In reverse conditions (sufficient biological nitrogen) another protein encoded by *nif L* is activated which inhibit *nif A* for further processing. *NifK, D* and *H* are basically codes for subunits of metal ions within nitrogenase enzyme. Dinitrogenase is a hetero-dimeric (alpha/beta) protein which is encoded by *nifK* and *nifD* gene⁶⁶. It is studied that wide diversity of diazotroph found in marine due to *nifH* sequence variation as it contains four different groups (Table: 3)^{67,68}. Similarly, the expression of V-nitrogenase enzyme is regulated by some special sets of genes (*vnfH/vnfDGK* and *anfHDGK*)^{69,70}.

The activity of nitrogenase enzyme is inhibited under aerobic condition (presence of oxygen)^{71,72,73}. To protect the nitrogenase in

marine, cyanobacteria have special arrangement of non-photosynthetic cell known as heterocyst⁷⁴ which separates the sensitive nitrogenase from oxygen producing photosynthetic reaction. There is another process such as temporal separation of photosynthesis in day and N-fixation at night is also reported in hetrocyst lacking cyanobacteria spp^{75,76}. Similarly, *Trichodesmium* having a different way to protect the nitrogenase. For protection of nitrogenase, they have some special cells where no photosynthesis process is allowed²⁵. An alternative way to protect the nitrogenase is well studied in aerobic soil bacterium (*Azotobacter vinelandii*) is to maintain a low intracellular oxygen concentration through a high respiration rate^{61,77}.

Different approach to identify the diazotrophs

The most often method used in 19th century for determination of total amount of nitrogen is Kjeldahl method. It involves acid digestion of the total tissue which converts most nitrogen-containing materials to ammonia, distillation of the ammonia into dilute acid, followed by either a colorimetric or a titrimetric determination^{78,79}. Although it measures nitrogen but it does not calculate accurate nitrogen at different stages. With time various advanced analytical techniques have been developed for nitrogen determination. A simple instrument such as manometer is utilized to measure the disappearance of N₂ from

a sealed system⁷⁰. However, till late 1948, only few bacteria were recognized as N₂-fixer such as *Clostridium*, *Azotobacter*, *Nostoc* and *Rhizobium*. Furthermore, a new assay for detection of nitrogen fixing bacteria based on N isotope (¹⁵N) nitrogen enrichment was introduced^{80,81,82}. This assay is about 1000-times more sensitive than the Kjeldahl method. Simultaneously, an acetylene reductase assay is discovered for nitrogen content measurement in bacteria. This assay detects the ethylene production using gas chromatography and flame-ionization detectors^{83,84,85}. ARA is a simple, rapid, more accurate and cost-effective technique than ammonia production and ¹⁵N₂ tracer method, microdiffusion and distillation methods⁵⁶. Recently, an innovative method has been used in soybeans to increase *Bradyrhizobium diazoefficiens* nodulation by continuously inducing ROS using manganese ferrite nanoparticles⁸⁶. However, this study could be applied using various nitrogen fixing bacteria to see the effect of them on cereals to enhance the BNF.

CONCLUSION AND FUTURE PROSPECTS

As per research, biological nitrogen fixation is the most significant approach for nitrogen input. It is a low-cost procedure and can replace the use of synthetic nitrogen fertilizer which has harmful effects on environment as well as on human health^{87,88}. Diazotrophs is a biological source to fix the atmospheric nitrogen in terrestrial as well as in marine water⁸⁹. Nitrogen fixation by diazotrophic bacteria is a remarkable source of fixed nitrogen into an open ocean and hence controls carbon flux and primary production. It is now established that the total global nitrogen deposition has been increased by anthropogenic activities results in destabilization of biogeochemical cycles such as nitrogen cycle. Hence, there is an urge for detailed understanding of biological nitrogen fixation process to accurately address the consequences of anthropogenic activities on nitrogen cycle. Based on the available research, it has been concluded that biological nitrogen fixation is a global phenomenon across ecosystems which significantly contributed to the nitrogen inputs in various ecosystems^{90,91}. According to previous knowledge gaps, there is an urgent need to dissolve uncertainties between rates of nitrogen fixation

per unit area with spatial distribution of nitrogen fixing bacterial species⁹². A framework should be proposed for better understanding of free-living nitrogen fixation and ecology of free nitrogen fixing bacterial species. We should deepen our knowledge related to different factors such as biotic and abiotic factors which have impact on diversity and abundance of nitrogen fixing bacterial species present in rhizosphere which in turn influence the rate of nitrogen fixation⁹³.

We conclude that biological nitrogen fixation proved to be the best technology in fixation of total nitrogen globally because of its economic and environmental benefits. Thus, in agriculture, the use and benefits of BNF can be enhanced by addition of legumes in the cropping systems⁹⁴. Legumes have more than 3000 species with several opportunities to develop agricultural cropping systems that are both ecological sound and profitable. A study concluded that the use of diazotrophs to legumes and non-legumes can substitute 30-50% of the nitrogen demand of crops and save farmer input (by saving synthetic fertilizer) thus, it also improves overall yield (by decreasing pollution and increasing sustainability). There are some problems such as inadequate availability, non-uniformity of results, old technology and climate issues hamper the use of BNF technology by farmers on large scale. To overcome these problems and maximum use of BNF technology, nano-hybrid formulations should be developed which will improve stability, efficacy, applicability, shelf life and uniformity of product. Isolation and characterization of nitrogen fixing bacteria with pesticide degradation efficiency would be beneficial approach for improvement of agriculture⁹⁷. Government should also encourage private sector and farmers to come forward regarding nano-hybrid formulations and evaluations to harness the maximum implementation of BNF technology. For maximum utilization of benefits of BNF for ecosystem, a comprehensive approach and better implementation of agricultural policies, legislation, better market consideration and investment of private sector will support.

ACKNOWLEDGMENT

Authors are acknowledge to Department of Biosciences and Technology, Maharishi

Markandeshwar (Deemed to be University), Mullana for providing us full support and a collaborating team for this publication.

Conflict of Interest

There is no conflict of interest among the authors.

Funding Sources

For this manuscript authors have not used any financial support from any grant agency.

Authors' Contribution

Soniya Goyal- Written the manuscript; Amit Kumar- Written the manuscript; Pooja Sharma- The author has designed the concept of manuscript; Anita Rani Gill- The author has written the manuscript; Anil Kumar Sharma- The author has reviewed the manuscript; Ashwanti Devi- The author has written the oceanic diazotrophs; Chahat Sharma- The author has written the manuscript

Data Availability Statement

Authors have no issue for providing any information related to manuscript with prior request on corresponding author email.

Ethics Approval Statement

None.

REFERENCES

- Rosenblueth, M., Ormeño-Orrillo, E., López-López, A., Rogel, M. A., Reyes-Hernández, B. J., Martínez-Romero, J. C., ... & Martínez-Romero, E. Nitrogen fixation in cereals. *Frontiers in Microbiology* 2018, 9, 1794.
- Cleveland, C.C., Reis, C.R., Perakis, S.S., Dynarski, K.A., Batterman, S.A., Crews, T.E., Gei, M., Gundale, M.J., Menge, D.N., Peoples, M.B. and Reed, S.C. Exploring the role of cryptic nitrogen fixers in terrestrial ecosystems: a frontier in nitrogen cycling research. *Ecosystem* 2022 25(8), pp.1653-1669.
- Vance, C. Symbiotic nitrogen fixation and phosphorus acquisition. Plant nutrition in a world of declining renewable resources. *Plant Physiology* 2001, 127, 391-397.
- LeBauer, D. S. and Treseder, K. K.: Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed, *Ecology* 2008, 89, 371-379.
- Greed, S. Unveiling the final nitrogenase. *Nature Reviews Chemistry* 2023, pp.1-1.
- Beijerinck, M. W. Über oligonitrophile Mikroben. *Zbl. Bakt.* 7, 561-582 (1901).
- Pi, H.W., Lin, J.J., Chen, C.A., Wang, P.H., Chiang, Y.R., Huang, C.C., Young, C.C. and Li, W.H. Origin and evolution of nitrogen fixation in prokaryotes. *Molecular Biology and Evolution* 2022, 39(9), p.msac181.
- Zehr, J. P., & Capone, D. G. Problems and promises of assaying the genetic potential for nitrogen fixation in the marine environment. *Microbial ecology* 1996, 32, 263-281.
- Zehr, J. P., Wyman, M., Miller, V., Duguay, L., & Capone, D. G. Modification of the Fe protein of nitrogenase in natural populations of *Trichodesmium thiebautii*. *Applied and Environmental Microbiology*, 1993, 59(3), 669-676.
- Vaishampayan, A., R.P. Sinha, D.P. Hader, T. Dey, A.K. Gupta, U. Bhan, and A.L. Rao. Cyanobacterial biofertilizers in rice agriculture. *Bot. Rev.* 2001, 67:453-516.
- Liu, X. and Rousk, K. The moss traits that rule cyanobacterial colonization. *Annals of Botany* 2022, 129(2), pp.147-160.
- Zhu C, Friman VP, Li L, Xu Q, Guo J, Guo S, Shen Q, Ling N. Meta analysis of diazotrophic signatures across terrestrial ecosystems at the continental scale. *Environmental Microbiology* 2022, 24(4):2013-28.
- Young, J.P.W. Phylogenetic classification of nitrogenfixing organisms, pp. 43-86. In G. Stacey, R. H. Burris, and H. J. Evans (ed.), *Biological Nitrogen Fixation* 1992, Chapman and Hall, New York, NY
- Shu, W., G.P. Pablo, Y. Jun, and H. Danfeng. Abundance and diversity of nitrogen-fixing bacteria in rhizosphere and bulk paddy soil under different duration of organic management. *World J. Microbiol. Biotechnol* 2011, 28:493-503.
- Hu, W., Wang, X., Wang, X., Xu, Y., Li, R., Zhao, L., Ren, W. and Teng, Y. Enhancement of nitrogen fixation and diazotrophs by long-term polychlorinated biphenyl contamination in paddy soil. *Journal of Hazardous Materials* 2023, 446, p.130697.
- Fischer, D., B. Pfitzner, M. Schmid, J. L. Simoes-Araujo, V. M. Reis, W. Pereira, E. Ormeno-Orrillo, B. Hai, et al. Molecular characterisation of the diazotrophic bacterial community in uninoculated and inoculated field-grown sugarcane (*Saccharum* sp.). *Plant Soil* 2011, 356:83-99.
- Barua, S., S. Tripathi, A. Chakraborty, S. Ghosh, and K. Chakrabarti. Characterization and crop production efficiency of diazotrophic bacterial isolates from coastal saline soils. *Microbiol. Res.* 2012, 167:95-102.
- Bahulikar, R.A., I. Torres-Jerez, E. Worley, K. Craven, and M.K. Udvardi. Diversity of nitrogen-fixing bacteria associated with switchgrass in the

- native tall grass prairie of northern Oklahoma. *Appl. Environ. Microbiol* 2014, 80: 5636-43.
19. Dobbelaere, S., J. Vanderleyden, and Y. Okon. Plant growth-promoting effects of diazotrophs in the rhizosphere. *Crit. Rev. Plant Sci.* 2003, 22:107-149.
 20. Hutchins, D.A. and Capone, D.G. The marine nitrogen cycle: new developments and global change. *Nature Reviews Microbiology* 2022, 20(7), pp.401-414.
 21. Carpenter, E. J., & Romans, K. Major role of the cyanobacterium *Trichodesmium* in nutrient cycling in the North Atlantic Ocean. *Science* 1991, 254(5036), 1356-1358.
 22. Gruber, N., & Sarmiento, J. L. Global patterns of marine nitrogen fixation and denitrification. *Global biogeochemical cycles* 1997, 11(2), 235-266.
 23. Karl, D., Letelier, R., Tupas, L., Dore, J., Christian, J., & Hebel, D. The role of nitrogen fixation in biogeochemical cycling in the subtropical North Pacific Ocean. *Nature* 1997, 388(6642), 533-538.
 24. Capone, D. G., Subramaniam, A., Montoya, J. P., Voss, M., Humborg, C., Johansen, A. M., ... & Carpenter, E. J. An extensive bloom of the N₂-fixing cyanobacterium *Trichodesmium erythraeum* in the central Arabian Sea. *Marine Ecology Progress Series* 1998, 172, 281-292.
 25. Gruber, N., & Galloway, J. N. An Earth-system perspective of the global nitrogen cycle. *Nature* 2008, 451(7176), 293-296.
 26. Lesser, M. P., Morrow, K. M., Pankey, S. M., & Noonan, S. H. Diazotroph diversity and nitrogen fixation in the coral *Stylophora pistillata* from the Great Barrier Reef. *The ISME Journal* 2018, 12(3), 813-824.
 27. Berman-Frank, I., Cullen, J. T., Shaked, Y., Sherrell, R. M., & Falkowski, P. G. Iron availability, cellular iron quotas, and nitrogen fixation in *Trichodesmium*. *Limnology and Oceanography* 2001, 46(6), 1249-1260.
 28. Karl, D., Michaels, A., Bergman, B., Capone, D., Carpenter, E., Letelier, R., ... & Stal, L. Dinitrogen fixation in the world's oceans. *The nitrogen cycle at regional to global scales* 2002, 47-98.
 29. Deutsch, C., Sarmiento, J. L., Sigman, D. M., Gruber, N., & Dunne, J. P. Spatial coupling of nitrogen inputs and losses in the ocean. *Nature* 2007, 445(7124), 163-167.
 30. Hamersley, M. R., Turk, K. A., Leinweber, A., Gruber, N., Zehr, J. P., Gunderson, T., & Capone, D. G. Nitrogen fixation within the water column associated with two hypoxic basins in the Southern California Bight. *Aquatic Microbial Ecology* 2011, 63(2), 193-205.
 31. Galloway, J.N., Dentener, F.J., Capone, D.G., Boyer, E.W., Howarth, R.W., Seitzinger, S.P., Asner, G.P., Cleveland, C.C., Green, P.A., Holland, E.A. and Karl, D.M. Nitrogen cycles: past, present, and future. *Biogeochemistry*, 2004, 70, 153-226.
 32. Wilson, E.W., Rowntree, S.C., Suhre, J.J., Weidenbenner, N.H., Conley, S.P., Davis, V.M., Diers, B.W., Esker, P.D., Naeve, S.L., Specht, J.E. and Casteel, S.N. Genetic gain× management interactions in soybean: II. Nitrogen utilization. *Crop Science* 2014, 54(1), pp.340-348.
 33. Walsby, A. E. The properties and buoyancy-providing role of gas vacuoles in *Trichodesmium Ehrenberg*. *British Phycological Journal* 1978, 13(2), 103-116.
 34. Lipschultz, F., Owens, N.J.P. An assessment of nitrogen fixation as a source of nitrogen to the North Atlantic Ocean. *Biogeochemistry* 1996, 35, 261-274.
 35. Hansell, D. A., & Feely, R. A. Atmospheric intertropical convergence impacts surface ocean carbon and nitrogen biogeochemistry in the western tropical Pacific. *Geophysical Research Letters* 2000, 27(7), 1013-1016.
 36. Sellner, K. G. Physiology, ecology, and toxic properties of marine cyanobacteria blooms. *Limnology and oceanography* 1997, 42(5part2), 1089-1104.
 37. Nevison, C., Hess, P., Goodale, C., Zhu, Q., & Vira, J. Nitrification, denitrification, and competition for soil N: Evaluation of two Earth System Models against observations. *Ecological Applications* 2022, 32(4), e2528.
 38. Montoya, J. P., Carpenter, E. J., & Capone, D. G. Nitrogen fixation and nitrogen isotope abundances in zooplankton of the oligotrophic North Atlantic. *Limnology and Oceanography* 2002, 47(6), 1617-1628.
 39. Chen, Y. B., Zehr, J. P., & Mellon, M. Growth and nitrogen fixation of the diazotrophic filamentous nonheterocystous cyanobacterium *Trichodesmium* sp. Ims 101 in defined media: evidence for a circadian rhythm 1. *Journal of Phycology* 1996, 32(6), 916-923.
 40. Kapellos, George E., Hermann J. Eberl, Nicolas Kalogerakis, Patrick S. Doyle, and Christakis A. Paraskeva. Impact of microbial uptake on the nutrient plume around marine organic particles: High-resolution numerical analysis. *Microorganisms* 2022, 10(10), 2020.
 41. Fernández-Méndez, M., Turk-Kubo, K. A., Buttigieg, P. L., Rapp, J. Z., Krumpfen, T., Zehr, J. P., & Boetius, A. Diazotroph diversity in the sea ice, melt ponds, and surface waters

- of the Eurasian Basin of the Central Arctic Ocean. *Frontiers in Microbiology* 2016, 7, 1884.
42. Von Friesen, L. W., & Riemann, L. Nitrogen fixation in a changing Arctic Ocean: An overlooked source of nitrogen?. *Frontiers in microbiology* 2020, 11, 596426.
 43. Fernandez, C., Farías, L., & Ulloa, O. Nitrogen fixation in denitrified marine waters. *PLoS one* 2011, 6(6), e20539.
 44. Baldani JI, Baldani VLD. History on the biological nitrogen fixation research in graminaceous plants: special emphasis on the Brazilian experience. *An Acad Bras Ciênc* 2005, 77:549–579
 45. Silva K, Nóbrega RSA, Lima AS, Barberi A, Moreira FMS. Density and diversity of diazotrophic bacteria isolated from Amazonian soils using N-free semi-solid media. *Sci Agric* 2011, 68:518–525
 46. Norman JS, Friesen ML. Complex N acquisition by soil diazotrophs: how the ability to release exoenzymes affects N fixation by terrestrial free-living diazotrophs. *The ISME journal* 2017, 11(2):315-26.
 47. Vadakattu, G. & Paterson, J. Free-living bacteria lift soil nitrogen supply. *Farming Ahead* 2006, 169, 40.
 48. Carvalho, T.L.G., Balsemão-Pires, E., Saraiva, R.M., Ferreira, P.C.G. and Hemerly, A.S. Nitrogen signalling in plant interactions with associative and endophytic diazotrophic bacteria. *Journal of experimental botany* 2014, 65(19), pp.5631-5642.
 49. Gupta, V.V., Zhang, B., Penton, C.R., Yu, J. and Tiedje, J.M. Diazotroph diversity and nitrogen fixation in summer active perennial grasses in a mediterranean region agricultural soil. *Frontiers in molecular biosciences* 2019, 6, p.115.
 50. Guo, W., Zhang, K., Liang, Z., Zou, R., & Xu, Q. Electrochemical nitrogen fixation and utilization: theories, advanced catalyst materials and system design. *Chemical Society Reviews* 2019, 48(24), 5658-5716.
 51. Cruz-Hernández, María Antonia, Alberto Mendoza-Herrera, Virgilio Bocanegra-García, and Gildardo Rivera. *Azospirillum* spp. from plant growth-promoting bacteria to their use in bioremediation. *Microorganisms* 2022, 10(5) 1057.
 52. Guo, Kaiyan, Jun Yang, Nan Yu, Li Luo, and Ertao Wang. Biological nitrogen fixation in cereal crops: Progress, strategies, and perspectives. *Plant Communications* 2023, 4(2).
 53. Puri A, Padda KP, Chanway CP. Evidence of endophytic diazotrophic bacteria in lodgepole pine and hybrid white spruce trees growing in soils with different nutrient statuses in the West Chilcotin region of British Columbia, Canada. *Forest Ecology and Management* 2018, 430: 558–565.
 54. Bashan Y, de-Bashan LE. How the plant growth-promoting bacterium *Azospirillum* promotes plant growth – A critical assessment. *Adv Agron* 2010, 108:77–136
 55. Hungria M, Nogueira MA, Araújo RS. Inoculation of *Brachiaria* spp. with the plant growth-promoting bacterium *Azospirillum brasilense*: An environment-friendly component in the reclamation of degraded pastures in the tropics. *Agric Ecosyst Environ* 2016, 221:125–131
 56. Giller KE. Nitrogen fixation in tropical cropping systems. CABI Publishers, Wallingford, 2001.
 57. Santi, C.; Bogusz, D.; Franche, C. Biological nitrogen fixation in non-legume plants. *Ann. Bot.* 2013, 111, 743–767.
 58. Sullivan, B. W., Smith, W. K., Townsend, A. R., Nasto, M. K., Reed, S. C., Chazdon, R. L., and Cleveland, C. C.: Spatially robust estimates of biological nitrogen (N) fixation imply substantial human alteration of the tropical N cycle. *P. Natl. Acad. Sci. USA* 2014, 111, 8101–8106.
 59. Vitousek, P. M., Menge, D. N., Reed, S. C., and Cleveland, C. C.: Biological nitrogen fixation: rates, patterns and ecological controls in terrestrial ecosystems. *Philos. T. R. Soc. B* 2013, 368, 20130119.
 60. Hawkins, Justin P., and Ivan J. Oresnik. The rhizobium-legume symbiosis: Co-opting successful stress management. *Frontiers in Plant Science* 2022, 12, 796045.
 61. Herridge, D. F., Peoples, M. B., & Boddey, R. M. Global inputs of biological nitrogen fixation in agricultural systems. *Plant Soil* 2008, 311, 1-18.
 62. Fattah, Q. A. Plant Resources for Human Development.” Third International Botanical Conference 2005. Bangladesh Botanical Society, Dhaka, Bangladesh, 2005.
 63. Singh, P., Singh, R. K., Kumar, D., & Tiwari, S. P. Ecology of the Diazotrophic Microbiome. *The Plant Microbiome in Sustainable Agriculture* 2020, 81-99.
 64. Yang, Zhimin, Yunlei Han, Yao Ma, Qinghua Chen, Yuhua Zhan, Wei Lu, Li Cai et al. Global investigation of an engineered nitrogen-fixing *Escherichia coli* strain reveals regulatory coupling between host and heterologous nitrogen-fixation genes. *Scientific reports* 2018, 8(1) 10928.
 65. Benoist, A., Houle, D., Bradley, R.L. and Bellenger, J.P. Evaluation of biological nitrogen fixation in coarse woody debris from Eastern Canadian boreal forests. *Soil Biology and Biochemistry* 2022, 165, p.108531.

66. Soto, M.J., Staehelin, C., Gourion, B., Cardenas, L. and Vinardell, J.M. Early signaling in the rhizobium-legume symbiosis. *Frontiers in Plant Science* 2022, 13, p.1056830.
67. Daims, Holger, Elena V. Lebedeva, Petra Pjevac, Ping Han, Craig Herbold, Mads Albertsen, Nico Jehmlich et al. Complete nitrification by Nitrospira bacteria. *Nature* 2015, 528(7583), 504-509.
68. Dolkhani, F., Bijanzadeh, E., Boostani, H. R., & Hardie, A. G. Effect of Nitrogen-Fixing Bacteria Application on Biochemical Properties, Yield, and Nutrients of Barley. *Journal of Soil Science and Plant Nutrition* 2022, 1-15.
69. Kraepiel, A. M. L., Bellenger, J. P., Wichard, T., & Morel, F. M. Multiple roles of siderophores in free-living nitrogen-fixing bacteria. *Biometals* 2009, 22, 573-581.
70. Fredriksson, C., & Bergman, B. Ultrastructural characterisation of cells specialised for nitrogen fixation in a non-heterocystous cyanobacterium, *Trichodesmium* spp. *Protoplasma* 1997, 197, 76-85.
71. Guerinot, M. L., West, P. A., Lee, J. V., & Colwell, R. R. *Vibrio diazotrophicus* sp. nov., a marine nitrogen-fixing bacterium. *International Journal of Systematic and Evolutionary Microbiology* 1982, 32(3), 350-357.
72. Zhao, Wenbin, Xing Chen, Ronghua Liu, Peng Tian, Wentao Niu, Xiao-Hua Zhang, Jiwen Liu, and Xiaolei Wang. Distinct coral environments shape the dynamic of planktonic *Vibrio* spp. *Environmental Microbiome* 2023, 18(1), 77.
73. Foster, R. A., Kuypers, M. M., Vagner, T., Paerl, R. W., Musat, N., & Zehr, J. P. Nitrogen fixation and transfer in open ocean diatom-cyanobacterial symbioses. *The ISME journal* 2011, 5(9), 1484-1493.
74. Church, M. J., Mahaffey, C., Letelier, R. M., Lukas, R., Zehr, J. P., & Karl, D. M. Physical forcing of nitrogen fixation and diazotroph community structure in the North Pacific subtropical gyre. *Global Biogeochemical Cycles* 2009, 23(2).
75. Bergman, B., Sandh, G., Lin, S., Larsson, J., & Carpenter, E. J. *Trichodesmium*—a widespread marine cyanobacterium with unusual nitrogen fixation properties. *FEMS microbiology reviews*, 2013, 37(3), 286-302.
76. Heimann, Kirsten, and Samuel Cirés. N₂-fixing cyanobacteria: ecology and biotechnological applications. In *Handbook of Marine Microalgae* 2015, 501-515.
77. Sáez-Plaza, Purificación, María José Navas, S³awomir Wybraniec, Tadeusz Micha³owski, and Agustín García Asuero. An overview of the Kjeldahl method of nitrogen determination. Part II. Sample preparation, working scale, instrumental finish, and quality control. *Critical Reviews in Analytical Chemistry* 2013, 43(4), 224-272.
78. Leylasi Marand, M. and Sarikhani, M. Evaluation of Biological Nitrogen Fixation by *Azotobacter* Isolates in Solid and Liquid LG Medium by Kjeldahl method. *Water and Soil Science* 2018, 28 (2), 207-218.
79. Wilson, P.W. The comparative biochemistry of nitrogen fixation. *Advances in Enzymology and Related Areas of Molecular Biology* 1952, 13, pp.345-375
80. Burris, R.H. and Miller, C.E. Application of N₁₅ to the study of biological nitrogen fixation. *Science* 1941, 93(2405), pp.114-115.
81. Shaaban M, Hatano R, Martínez-Espinosa RM and Wu Y. Editorial: Nitrogen in the Environment. *Front. Environ. Sci.* 2022, 9:829104.
82. Yu, L., Zheng, T., Hao, Y. and Zheng, X. Determination of the nitrogen isotope enrichment factor associated with ammonification and nitrification in unsaturated soil at different temperatures. *Environmental Research* 2021, 202, p.111670.
83. Alexander, Lucille, and Don Grierson. Ethylene biosynthesis and action in tomato: a model for climacteric fruit ripening. *Journal of experimental botany* 2002, 53(377), 2039-2055.
84. Zaidi, N.A., Tahir, M.W., Vinayaka, P.P., Lucklum, F., Vellekoop, M. and Lang, W.J.P.E. Detection of ethylene using gas chromatographic system. *Procedia engineering* 2016, 168, pp.380-383.
85. Primrose SB and Dilwortht MJ. Ethylene Production by Bacteria. *Journal of General Microbiology* 1976, 93, 177-181
86. Ma, J., Zhou, Y., Li, J., Song, Z. and Han, H. Novel approach to enhance Bradyrhizobium diazoefficiens nodulation through continuous induction of ROS by manganese ferrite nanomaterials in soybean. *Journal of Nanobiotechnology* 2022, 20(1), pp.1-18.
87. Chaudhary, M, Mukherjee T.M., Singh R., Gupta M., Goyal S., Singhal P., Kumar R., Bhusal N., and Sharma P. CRISPR/Cas technology for improving nutritional values in the agricultural sector: an update. *Molecular Biology Reports*, 2022 49(7), 7101-7110.
88. Chaudhary, M., Sharma, P. and Mukherjee, T.K. Applications of CRISPR/Cas technology against drug-resistant lung cancers: an update. *Molecular Biology Reports*, 2022, 49(12), 11491-11502.
89. Zehr, J. P., & Capone, D. G. Changing

- perspectives in marine nitrogen fixation. *Science* 2020, 368(6492), eaay9514.
90. Khan, S., Nadir, S., Iqbal, S., Xu, J., Gui, H., Khan, A., & Ye, L. Towards a comprehensive understanding of free-living nitrogen fixation. *Circular Agricultural Systems* 2021, 1(1), 1-11.
91. Galloway JN, Townsend AR, Erisman JW, Bekunda M, Cai Z, et al. Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. *Science* 2008, 320:889-92
92. Bellés-Sancho, P., Beukes, C., James, E. K., & Pessi, G. (2023). Nitrogen-fixing symbiotic Paraburkholderia species: current knowledge and future perspectives. *Nitrogen*, 4(1), 135-158.
93. Imran, A., Hakim, S., Tariq, M., Nawaz, M. S., Laraib, I., Gulzar, U., ... & Ahmad, M. Diazotrophs for lowering nitrogen pollution crises: looking deep into the roots. *Frontiers in Microbiology* 2021, 12, 637815.
94. Goyal, R.K., Mattoo, A.K. and Schmidt, M.A. Rhizobial–host interactions and symbiotic nitrogen fixation in legume crops toward agriculture sustainability. *Frontiers in Microbiology* 2021, 12, p.669404.
95. Ladha, J.K., Peoples, M.B., Reddy, P.M., Biswas, J.C., Bennett, A., Jat, M.L. and Krupnik, T.J. Biological nitrogen fixation and prospects for ecological intensification in cereal-based cropping systems. *Field Crops Research* 2022, 283, p.108541.
96. Yao, W., Kvale, K.F., Koeve, W., Landolfi, A., Achterberg, E., Bertrand, E.M. and Oschlies, A. Simulated future trends in marine nitrogen fixation are sensitive to model iron implementation. *Global Biogeochemical Cycles* 2022, 36(3), p.e2020GB006851.
97. Sharma, C., Sharma, P., Kumar, A., Walia, Y., Kumar, R., Umar, A., Ibrahim, A.A., Akhtar, M.S., Alkhanjaf, A.A.M. and Baskoutas, S., 2023. A review on ecology implications and pesticide degradation using nitrogen fixing bacteria under biotic and abiotic stress conditions. *Chemistry and Ecology*, 39(7), pp.753-774.