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Review Article

# Archaea, bacteria and termite, nitrogen fixation and sustainable plants production

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## Abstract

Certain bacteria and archaea are responsible for biological nitrogen fixation. Metabolic pathways usually are common between archaea and bacteria. 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*. Three types of nitrogenase are iron and molybdenum (Fe/Mo), iron and vanadium (Fe/V) or iron only (Fe). The Mo-nitrogenase have a higher specific activity which is expressed better when Molybdenum is available. The best hosts for *Rhizobium legumiosarum* are *Pisum*, *Vicia*, *Lathyrus* and *Lens*; Trifolium for *Rhizobium trifolii*; *Phaseolus vulgaris*, *Prunus angustifolia* for *Rhizobium phaseoli*; Medicago, Melilotus and Trigonella for *Rhizobium meliloti*; Lupinus and Ornithopus for Lupini, and *Glycine max* for *Rhizobium japonicum*. Termites have significant key role in soil ecology, transporting and mixing soil. Termite gut microbes supply the enzymes required to degrade plant polymers, synthesize amino acids, recycle nitrogenous waste and fix atmospheric nitrogen. The positive effects of *Arbuscular mycorrhizal* (AM) fungi such as growth promotion, increased root length, leaf area, stem diameter, transplant performance and tolerance to stresses have been reported previously.

***Keywords:*** Archaea; Azotobacter; bacteria; nitrogenase; nitrogen fixation; termite

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## Introduction

Nitrogen is a key factor in protein and nucleic acids and all organisms need nitrogen (Benavides *et al.*, 2013). Moreover, nitrogen is the major limiting nutrient for plant biomass production in environment (Nardi *et al.*, 2002). It is the most important parameters for the synthesis of amino acids, DNA, RNA and proteins (Fowler *et al.*, 2013; Rago *et al.*, 2019). Several processes such as nitrogen fixation, dissimilatory nitrate reduction to ammonia, nitrification, anammox, and denitrification carried out by microbes. Biological nitrogen fixation is called the most critical biological process in the world (Kizilkaya, 2009). 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

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rhizosphere with numerous interactions with the plant host (Odelade and Babalola, 2019). So, this review explores various unique beneficial microbes, especially archaea, bacteria, and their especial roles in the environment according to acquisition of nutrients for plant growth and improve productivity.

### Nitrogen

Nitrogen (N) is a vital parameter for crop productivity (Egamberdieva and Kucharova, 2008; Broumad*et al.*, 2010; Soleymani *et al.*, 2011 a,b). Nitrogen is most important limiting nutrient for crop production and plant productivity in many parts 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 (Olson and Kurtz, 1982; Hammad and Ali, 2014; Kumar *et al.*, 2017; Mahato and Kafle, 2018). One of the most important nutrient cycles in ecosystem is biological nitrogen cycle which includes four main processes, namely, nitrogen fixation, mineralization (decay), nitrification and denitrification (Mao *et al.*, 2011; Xiao *et al.*, 2019). The most important functional genes are *nifH*, *amoA*, and *nosZ* genes which are participate in encoding key enzymes in nitrogen fixation, ammonia oxidation and complete denitrification, respectively (Ruiz-Rueda *et al.*, 2009; Bru *et al.*, 2011; Orr *et al.*, 2011). Mao *et al.* (2011) indicated that utilization of nitrogen fertilizer many have both short-term environmental problems, and long-term impact on the global biogeochemical cycles via altering the soil microbial community structure and abundance. The global nitrogen cycle represents the transformation of nitrogen gases and nitrogen-containing compound which consists of microbial-driven processes, such as assimilation, ammonification, nitrification, denitrification, nitrogen fixation and anaerobic oxidation (You *et al.*, 2009).

### Nitrogen fixation

Nitrogen fixation has also significant role in biochemical pathways which play an important role in controlling oceanic nitrogen inventory (Ohkuma *et al.*, 1996; LaRoche and Breitbarth, 2005; Sylvia *et al.*, 2005; 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 (Raymond *et al.*, 2004; 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 (Kargi and Ozmihci, 2002; 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). In the Ocean, dissolved N2 would have been converted into $NO_3^-$ and $NO_2^-$ (Mancinelli and Mckay, 1998). 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 (Smith-Moore and Grunden, 2018; 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; Hugenholtz, 2002; 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). 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, 2012). Nitrogenase is an ATP-hydrolyzing, redox-active complex of two component proteins, the dinitrogenase reductase $\gamma_2$ homodimer (NifH protein), and the dinitroenase $\alpha_2\beta_2$ heterotetramer, where $\alpha$ is NifD, and $\beta$ is NifK proteins (Raymond *et al.*, 2004). The molybdenum nitrogenase is an oxygen sensitive complex dinitrogenase (NifDKheterotetramer), and dinitrogenase reductase (NifH homodimer) (Ortiz-Marquez *et al.*, 2014). $MoFe_7S_9$ metal cluster is the active site for dinitrogen reduction for $\alpha$, 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) (Joerger and Bishop, 1988; Miller and Eady, 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). 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). Keshri *et al.* (2013) also reported that the key functional genes namely cbbL, nifH, amoA, and apsAinvolced 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 (Zehr *et al.*, 2003; 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 (Che *et al.*, 2018; 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 (Sullivan *et al.*, 2002; 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 (Hageman and Burris, 1978), and Nif (nitrogen fixing) proteins NifH ($\gamma_2$homodimericazoerredoxin), and NifD/K ($\alpha_2\beta_2$heterotetramericmolybdoferredoxin) 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 (Eady, 2003; 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 (Barns *et al.*, 1996; Sabra *et al.*, 2000; Kneip *et al.*, 2007; Boyd and Peters, 2013; Offre *et al.*, 2013; Ulyshen, 2015). Other notable nitrogenases are vanadium- and iron-dependent nitrogenases encoded by the *vnfHDGK* and *anfHDGK* genes, respectively (Rehder, 2000; Herridge *et al.*, 2008; Seefeldt *et al.*, 2009; Hartmann and Barnum, 2010). There are different ways of nitrogen fixing from unavailable gaseous forms in the atmosphere to usable forms for plants and other organisms.

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 (Chain *et al.*, 2003; Papineau *et al.*, 2005; Kneip *et al.*, 2007; Philippot*et al.*, 2007; Shridhar, 2012; 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-oxidizing bacteria. In bacteria, ammonia is oxidized to nitrite via the intermediate hydroxylamine and the enzyme hydroxylamine oxidoreductase (HAO). Functional characterization of upregulated and downregulated selected proteins during low temperature N depletion is shown in Table 1. Homologs of the nifH is indicted in Table 2.

**Table 1.** 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 kDachperonin | 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 |
| *leu*$C_2$ | 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 |

**Table 2.** 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*. 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. |
| 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 (Souillard *et al.*, 1988; Fujia *et al.*, 1992; 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 (Koskey*et al.*, 2017; Wang *et al.*, 2017; Xiao *et al.*, 2020). The estimate areas of biological nitrogen fixation and related factors controlling BNF is done by diazotroph distribution (Ratten *et al.*, 2015; 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* (Knoth*et al.*, 2013; 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 (Hemmina and Duarte, 2000; Welsh, 2000; 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 Gresshoff, 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 growth factors in cereals and other nonlegumes (Evans, 1983; Gillis *et al.*, 1989; Fuentes-Ramirez *et al.*, 1993; Sevilla *et al.*, 2001; 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 (Dixon and Kahn, 2004; 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 *Richeliaintracellularis* are important marine diazotrophs (Capone *et al.*, 1997; 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. Microbial domains comparisons are indicated in Table 3.

**Table 3.** 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 |

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 (Kenndey *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 sucroserich 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). Biology and potential role of some diazotrophs are shown in Table 4.

**Table 4.** Biology and potential role of some diazotrophs promoting crop production (Kenndey *et al.*, 2004)

| Diazotrophs | Condition for BNF | Habitat | Energy source | Mechanism of effect |
|---|---|---|---|---|
| *A. chroococcum* | Aerobic | Rhizosphere | Organics in soil | BNF |
| *Clostridium spp.* | Anaerobic | Soil saprophyte | Organics in soil | BNF |
| *Azospirillum spp.* | Microaerobic | Rhizosphere, mildly endophytic in roots, stems and leaves | Organics in soil, root exudates and plant tissue | BNF, PGP |
| *H. seropedicae* | Microaerobic | Endophytic, rhizosphere | Root exudates | BNF, PGP |
| *Azoarcus sp.* | Microaerobic | Endophytic | Root exudates | BNF |
| *A. vietnamiensis* | - | Rhizosphere, endophytic | Organics in soil and root exudates | BNF, PGP |
| *R. leguminosarum bv. Trifolii* | - | Endophytic in roots | Root exudates | PGP |
| *R. etlibv. phaseoli* | - | Endophytic in roots | Root exudates | PGP |
| *A. caulinodans* | Microaerobic | Endophytic in roots | Root exudates | PGP |
| *A. diazotrophicus* | Microaerobic | Endophytic in roots, stems and leaves | Root exudates and plant tissue | BNF |

BNF, Biological nitrogen fixation; PGP, plant growth promotion.

### Termites

Termites are insects belonging to the order *Isoptera* (Gomathi *et al.*, 2018). Termites often divided into two broad classes, a) those that nest in and feed on a single source of dead plant material such as felled dead wood for the whole lifespan of the colony, and those that forage outside the nest (Higashi *et al.*, 1992; Tokuda *et al.*, 2012; Sapountzis *et al.*, 2016). Soil-feeding species are able to feed on nitrogenous soil components such as peptides, proteins and amino acid (Kappler and Brune, 2000; Brune, 2001), while wood-feeding termites can thrive on nutrient-poor materials (Tayasu *et al.*, 1994). It has been reported that the soil organic matter in the termitoshpere is significantly more stable and protected from the intense mineralization compared to the control soil (Brauman, 2000). Unlike reported results, Majeed *et al.* (2012) found that wood-feeding termites were able to take up atomospheric $N_2O$. Symbiotic nitrogen ($N_2$) fixation occurs in a wide variety of trees, and the endosymbionts in legume trees and in the non-legume genus *Parasponia* (Ulmaceae) are rhizobia (Sprent and Parsons, 2000). Wood-eating termites feed on a diet highly deficient in nitrogen (Frohlich *et al.*, 2007). Curtis and Waller (1998) deduced that termites nitrogenase activity was highest in autumn and spring. Ulyshen (2015) found that by accelerating the release of nutrients immobilized in fungal tissues and promoting $N_2$ fixation by free-living and endosymbiotic prokaryotes, saproxylic insects have potential to influence N dynamic in forests. Termites have been used as a biological pointer to evaluate both quality and fertility of soil, because of their important role in nitrogen fixation, methanogensis, soil transportation, nutrient circulation and acetogenesis (Dawes, 2010; Brauman *et al.*, 2015; Enagbonma and Babalola, 2019). They have been also known as gold mine of bacterial communities (Benndorf *et al.*, 2018; Devi and Thakur, 2018; Kumar *et al.*, 2018; Enagbonma and Babalola, 2019). $N_2$-fixing activity has been showed in the termite gut, because wood-feeding termites must supplement their food with nitrogen (Breznak*et al.*, 1973; French *et al.*, 1976; Bentley, 1984). It could be accomplished with the aid of nitrogen-fixing bacterial isolates, such as *Enterobacter*, *Desulfovibrio*, and *Treponema* species (Breznak *et al.*, 1973; Kuhnigk*et al.*, 1996; Lilburn *et al.*, 2001).

**Table 5.** Examples of timber producing nitrogen fixing legume trees (Allen and Allen, 1981)

| Species | Some uses |
|---|---|
| Caesalpiniodeae | |
| *Melanoxylon brauna* | Construction; tannin |
| *Erythrophleum suaveolens* | Construction; charcoal |
| *Campsiandra laurifolia* | Construction; starch (seeds); medicinal |
| Mimosoideae | |
| *Acacia senegal* | Tools; charcoal; gum Arabic; fodder |
| *Albizia lebbeck* | Construction; shade; fodder |
| *Anadenanthera colubrine* | Construction; gum; hallucinogenic drugs |
| *Enterolobium cyclocarpum* | Construction; tannin (pods); soap; drugs |
| Papilionoideae | |
| *Andira inermis* | Construction; ornament; shade; drugs |
| *Hymenolobium excelsum* | Construction |
| *Robinia pseudoacacia* | Construction; toxins; reclamation |
| *Swartzia madagascariensis* | Construction; fodder |
| *Xanthocercis madagascariensis* | Construction; edible fruit |

Termite gut microbes supply the enzymes required to degrade plant polymers, synthesize amino acids, recycle nitrogenous waste and fix atmospheric nitrogen ($N_2$) (Bignell, 2000; Brune and Ohkuma, 2010; Sapountzis *et al.*, 2016). It has been reported that termites depend on a range of microflora in their guts to promote digestion of the plant material (Gomathi *et al.*, 2018). Sprent and Parsons (2000) found that the success depends on both their ability to fix $N_2$ symbiotically, but also on a range of other adaptations as well as flooding and drought tolerance, mycorrhizal formation, cluster root production and herbivore defenses. Fall *et al.* (2001) showed that termite mound soil has nearly more than two times calcium and phosphorus, and approximately five times carbon and nitrogen, as well as 50 times ammonia and organic matter than other soil experiments. *Microctermes*, *Nasutitermes*, and *Macrotermes* are main termites of forest vegetation (Gomathi *et al.*, 2018). Some examples of timber producing nitrogen fixing legume trees are shown in Table 5. Examples of non-nodulated legumes used for timber are shown in Table 6. $N_2$ ($C_2H_2$) fixation in termites is presented in Table 7. $N_2$ ($C_2H_2$) fixation in other insects is indicated in Table 8.

**Table 6.** Examples of non-nodulated legumes used for timber (Sprent and Parsons, 2000)

| Species | Some uses |
|---|---|
| Caesalpinioideae | |
| *Caesalpinia echinata* | Construction; dyes |
| *Gleditsia triacanthos* | Construction (local); fodder, drugs |
| *Parkinsonia aculeata* | Carving; fodder; ornamental |
| | |
| Mimosoideae | |
| *Adenanthera pavonina* | Construction; jewelry (seeds); drugs |
| *Parkia biglobosa* | Construction; food; fodder |
| *Tetrapleura tetraptera* | Construction; food; drugs |
| | |
| Papilionoideae | |
| *Dipteryx odorata* | Construction; food; gum |
| *Vataria guianensis* | Construction (local); drugs |
| *Zollernia falcata* | Construction; drugs; tannins |

**Table 7.** $N_2$ ($C_2H_2$) fixation in termites (Mertins, 1973)

| Termite | Caste | Diet |
|---|---|---|
| *Coptotermes formosanus* | Worker | Wood (colony) |
| | Soldier | Wood (colony) |
| *Reticulitermes flavipes* | Worker | Wood (colony) |
| | Soldier | Wood (colony) |
| *Zootermopsis sp.* | Reproductive nymphs and workers | Wood (colony) |
| *Cryptotermes brevis* | Reproductive nymphs | Moist filter paper (12h) |

**Table 8.** $N_2$ ($C_2H_2$) fixation in other insects (Mertins, 1973)

| Insect | Common name |
|---|---|
| *Acyrthosiphon pisum* | Pea aphid |
| *Attagenus megatoma* | Black carpet beetle |
| *Blattella germanica* | German cockroach |
| *Camponotus sp.* | Carpenter ant |
| *Dermestes maculates* | Hide beetle |
| *Drosophila melanogaster* | Fruit fly |
| *Lasioderma serricorne* | Cigarette beetle |
| *Mezium americanum* (adults and larvae) | Spider beetle |
| *Musca domestica* | House fly |
| *Oncopeltus fasciatus* | Milkweed bug |
| *Periplaneta americana* | American cockroach |
| *Rhyzopetha dominica* | Lesser grain borer |
| *Stegobium paniceum* | Drugstore beetle |
| *Supella supellectilium* | Brown-banded cockroach |
| *Tenebrio molitor* (Larvae) | Yellow mealworm |
| *Tribolium confusum* | Confused flour beetle |
| *Trogoderma inclusum* | Large cabinet beetle |

$N_2$ fixing bacteria, especially members of *Clostridia*, *Spirochaetes* and gram-negative *protoeobacteria* including members of genera *Desulfovibrio*, *Enterobacter* and *Rhizobia*have a large phylogenic diversity of nitrogenase reductase (*nif*H) genes in xylophagous termite guts (Breznak, 2002; Frohlich *et al.*, 2007; Ngugi and Brune, 2011). The termite gut is ideal for denitrification activities such as $N_2O$ to $N_2$ (Braker, 2011), because its gut constitutes a specific microhabitat with both physical and chemical conditions like an alkaline pH with oxygen and hydrogen gradients (Brune *et al.*, 1995). Garba *et al.* (2011) found that the soil amended with termite mound soils resulted in better plant height, as well as an increase in leave number, fruits, and dry matter than those plants grown on unamended soil in fields under the cultivation of *Solanum lycopersicum*. Miyagawa *et al.* (2011) concluded that termite improved the growth of *Oryza sativa* L., and *Phaseolus vulgaris* L. Batalha *et al.* (1995) noted that combined use of 200 g of termite mound material with NPK led to a substantial increase in *Solatium melongena* production. Watson (1977) reported that *Lolium perenne* gave higher dry-matter yields with substrates derived from termite mounds than the comparable soil. Bama and Ravindran (2018) concluded that combined use of termite mound materials and inorganic fertilization significantly increase the *Zea mays* growth and yield. Kisa *et al.* (2006) showed that termite mound materials consisting of *Pseudomonas monteilii* species enhanced the *ectomycorrhizal* development between *Acacia holosericea* and *Scleroderma dictyosporum*. Suzuku *et al.* (2007) observed that combining sandy soil with termite mound materials at a proportion of 120 Mg/ha improved porosity and transformed the pore size distribution, thus causing a stepping up in the obtainable water content for the crop growth.

### Archaea

Soils in all terrestrial ecosystems are habitat of broad diversity of bacteria, archaea, fungi, annelids, insects as well as plants and algae. Archea is the smallest independently living, single-celled organisms on the earth, and it requires carbon to provide the building blocks for cell materials, as archeae are distributed in many environments such as soil (Aisalbie and Deslippe, 2013). Archea was considered as extremophile bacteria until it was introduced as the third domain of life by Woese and Fox (1977). Archaea plays an important impact in the global geochemical cycles in the world, because it constitutes a principle proportion of the microbial biomass (Offre*et al.*, 2013). The methanogenic Archaea bring a broadened viewpoint to the field of nitrogen fixation, and at least which also found in diazotrophic methanogens present in Bacteria (*nifH*, *D*, *K*, *E*, *N* and *X*), besides, most nitrogenase in methanogens are belong to the molybdenum type (Leigh, 2000). The Archea differences with bacteria are in having isoprene lipids conjugated by ether bonds to glycerol-1-phosphate in their membranes, they lack peptidoglycan in their cell walls, and also their informational proteins are more similar to eukaryotes than to bacteria (Spang *et al.*, 2017). Zhao *et al.* (2020) concluded that the functional genes of the archaeal community were mostly involved in nitrogen cycles, and it has principal role in biological soil cruts. The most important soil microbes' roles in soil ecosystems are physical support, raw materials, growth medium for plants, buffering water flows, nutrient cycling, recycling of wastes and detoxification, filtering of contaminants, habitat for biodiversity, biological control of pests, weeds and pathogens, carbon storage and regulation of green house gas emissions (Dominati*et al.*, 2010). The reduction of atmospheric nitrogen gas to ammonium just happened by existence of bacteria and archaea (Aislabie and Deslippe, 2013). Three kingdoms of Archaeaon the basis of phylogenetically divisions are presented in Table 9. Nitrogen fixing species in the Archaea is shown in Table 10. General characteristics of methanogenic *Archaea* are shown in Table 11. Distribution and phylogenetic affiliation of nonextremophilic*Archaea* is shown in Table 12.

**Table 9.** Three kingdoms of Archaea on the basis of phylogenetically divisions (Barns *et al.*, 1996; Luo and Wasserfallen, 2001; Reysenbach *et al.*, 2000)

| | |
|---|---|
| 1- | The *Crenarchaeota* which comprise most of the hyperthermophiles including the hyperthermophilic genera *Thermoproteus* and *Pyrodictium* as well as the thermoacidophilic genera *Sulfolobus*, *Acidianus* and *Desulfurococcus*. |
| 2- | The Euryarchaeota, a phenotypically more diverse collection of microorganisms including the hyperthermophilic genera *Thermoplasma*, *Thermococcus* and the sulfate-reducing *Archaeoglobus*, the extreme halophiles, the strictly anaerobic methanogens. |
| 3- | The *Korarchaeota*, which are momentarily represented by several, as yet uncultured species. |

**Table 10.** Nitrogen fixing species in the Archaea (Leigh, 2000)

| | |
|---|---|
| *Methanococcales* | *Methanococcus thermolithotrophicus* |
| | *Methanococcus maripaludis* |
| *Methanomicrobiales* | *Methanosarcina barkeri* |
| | *Methanospirilum hungatei* |
| *Methanobacteriales* | *Methanobacterium bryantii* |

**Table 11.** General characteristics of methanogenic *Archaea*

| Order Genus | Morphology | Substrates | Temperature (°C) |
|---|---|---|---|
| **Methanobacteriales** | | | |
| *Methanobacterium* | Long rods | $H_2$+$CO_2$, formate | 35-40 |
| *Methanobrevibacter* | Short rods | $H_2$+$CO_2$, formate | 30-38 |
| *Methanosphaera* | Cocci | Methanol+$H_2$ (both needed) | 36-40 |
| *Methanothermus* | Rods | $H_2$+ $CO_2$ | 83-88 |
| **Methanococcales** | | | |
| *Methanococcus* (*medsophilic* sp.) | Irregular cocci | $H_2$+$CO_2$, pyruvate+$CO_2$, formate | 35-40 |
| *Methanococcus* (*thermophilic* sp.) | Irregular cocci | $H_2$+$CO_2$ | 88 |
| **Methanomicrobiales** | | | |
| *Methanomicrobium* | Short rods | $H_2$+$CO_2$, formate | 40 |
| *Methanogenium* | Irregular cocci | $H_2$+$CO_2$, formate | 30-57 |
| *Methanospirillum* | Spirilla | $H_2$+$CO_2$, formate | 30-40 |
| *Methanoplanus* | Plate-shaped cells | $H_2$+$CO_2$, formate | 32-40 |
| *Methanoculleus* | Coccus | $H_2$+$CO_2$, formate | 37-60 |
| **Methanosarcinales** | | | |
| *Methanosarcina* | Large irregular cocci in packets | $H_2$+$CO_2$, methanol, methylamines, acetate | 35-50 |
| *Methanolobus* | Irregular cocci in aggregates | Methanol, methylamines | 30-40 |
| *Methanohalobium* | Irregular cocci | Methanol, methylamines | 50 |
| *Methanococcoides* | Irregular cocci | Methanol, methylamines | 23-35 |
| *Methanohalophilus* | Irregular cocci | Methanol, methylamines, methyl sulfides | 26-36 |
| *Methanothrix* | Long rods to filaments | Acetate | 35-60 |
| **Methanopyrales** | | | |
| *Methanopyrus* | Rods in chains | $H_2$+$CO_2$ | 100 |

**Table 12.** Distribution and phylogenetic affiliation of nonextremophilic*Archaea*

| Distribution | Phylogenetic affiliation |
|---|---|
| **Marine Habitats** | |
| Surface and deep waters (up to 3000 m) | Crenarchaeota, Euryarchaeota |
| Temperate coastal sediments (12 m) | Crenarchaeota, Euryarchaeota |
| Low-temperature deep-sea sediments (1500 to 4500 m) | Crenarchaeota, Euryarchaeota |
| Temperate microbial mats at deep-sea hydrothermal vent | Crenarchaeota, Euryarchaeota |
| Antarctic low-temperature surface waters | Crenarchaeota, Euryarchaeota |
| Salt marsh | Euryarchaeota |
| | |
| Associated with Marine Metazoans | |
| Gut of abyssal holothurians *Oneirophantamutabilis* (4870 m) | *Crenarchaeota* |
| Digestive tract of fish | Crenarchaeota, Euryarchaeota |
| Tissues of sponge Axinella Mexicana (10-20 m) | Crenarchaeon*, Crenarchaeota symbiosum* |
| | |
| **Freshwater Habitats** | |
| Lake sediments | Crenarchaeota, Euryarchaeota |
| | |
| **Terrestrial Habitats** | |
| Soils | Crenarchaeota, Euryarchaeota |
| Subsurface paleosol (188 m) | Crenarchaeota |
| Contaminated aquifer | Crenarchaeota, Euryarchaeota |
| Rice roots | Crenarchaeota, Euryarchaeota |

### Fungi

The legumes-*Rhizobium* symbiosis is the most efficacious system for nitrogen fixation, the bacteria will interact with leguminous plant in the host specific way and form nitrogen fixing root bacteria (Volpin and Kapunik, 1994; de Faria*et al.*, 2010). The positive effects of Arbuscular mycorrhizal (AM) fungi such as growth promotion, increased root length, leaf area, stem diameter, transplant performance and tolerance to stresses have been reported previously (Gohre and Pazkowski, 2006; Guether *et al.*, 2009; Kafkas and Ortas, 2009; Sharma *et al.*, 2009: Kiers *et al.*, 2011; Sharma *et al.*, 2011; Sharma *et al.*, 2012). AM fungi are vital in ecological agriculture, and they generally characterized by short life cycles of arbuscules and also frequent and rapid colonization of new roots and the emergence of vesicles in the oldest colonizing units (Alexander *et al.*, 1988; Smith and Read, 1997; Azcon-Aguilar *et al.*, 2002; Singh and Adholeya, 2004). Volpin and Kalpunik (1994) described that *Glycine max*, *Pisum sativum*, *Medicago sativa*, *Cicer arietinum*, *Psophocarpus tetragonolobus* inoculated with *Azospirillum* and *Rhizobium* showed increase in early nodulation, enhancement nodule on main root, total nodule number, nodule weight and nodule specific activity in nitrogen fixation process. Ingraffia *et al.* (2019) proved the role of *Arbuscular mycorrhizal* fungi (AMF) in driving biological interactions amongst neighboring plants as they are obligate soil biotrophs (Guisande-Collazo *et al.*, 2016). Two main endosymbioses for legume plants are a) with soil fungi, forming phosphorus acquiring arbuscular mycorrhiza, and b) with nitrogen-fixing bacteria leading to the formation of nitrogen-fixing root nodules (Manchanda and Garg, 2007).One of the most characteristic of mycorrhizal symbiosis is improving root nodulation and $N_2$ fixation by boosting the uptake of main nutrients or affecting legume-Rhizobium symbiosis (Barea *et al.*, 2005; Saia *et al.*, 2014), and of course its significant influence on biological nitrogen fixation (Puschel *et al.*, 2017). Xie *et al.* (2019) showed that *P. liquidambaris* promotes peanut nodulation and nitrogen fixation which is useful in building a sustainable agricultural system. Veselaj *et al.* (2018) concluded that the combination of application of *Rhizobium leguminosarum* and *arbuscular mycorrhizae* fungi is a sustain way to provide a significantly higher yield for non-saline plants. Bauer *et al.* (2012) found the importance of soil microbial communities because of impact of AMF and $N_2$-fixers on both community structure and crop productivity.

### Azotobacter

The Azotobacteriaceae consists of two genera which is *Azomonas sp.* and *Azotobacter sp.* Azotobacter has significant roles in availability of some nutritions like Nitrogen, Phosphorus, Sulphur and carbon via boosting mineralization of organic residues (Fekete *et al.*, 1989; Levai*et al.*, 2008; Rojas-Tapias *et al.*, 2013).*Azotobacter*genus belongs to the γ-subclass of the *Proeobacteria* (Tchan and New, 1984; Becking, 2004) which constitutes seven species namely, *A. chroococcum*, *A. vinelandii*, *A. beijerinckii*, *A. paspali*, *A. armeniacus*, *A. nigricans*, and *A. salinestri* (Jimenez *et al.*, 2011). A. chroococcum is the most inhabitingvarious soils (Balandreau, 1986; Tchan and New, 1984; Dobereiner, 1995; Martyniuk and Martyniuk, 2003). These free-living aerobic bacteria found in soils dominantly, and its populations influenced by soil physic-chemical such as organic matter, pH, temperature, soil moisture and depth and also microbiological interactions (Kizilkaya, 2009; Chowdhury-Paul *et al.*, 2018; Nag *et al.*, 2018). It is able to fix at least 10 mg N per gram of carbohydrate, and although, it is an obligate aerobic bacterium, is can grow under low $O_2$ (Tejera*et al.*, 2005). It has been reported that *Azotobacteria* are much available in the rhizosphere of plants than in surrounding soil, and this abundance mostly depends of crop species (Sariv and Ragoviv, 1963; Garg *et al.*, 2001; Aquilanti*et al.*, 2004; Jnawali*et al.*, 2015; Inomura*et al.*, 2017; Rodrigues *et al.*, 2018). Several studies have shown the microbial secretion of stimulating hormones, like auxins, cytokinins and gibberellins (Azcon and Barea, 1975; Martinez Toledo *et al.*, 1989, Salmeron *et al.*, 1990; Gonzales-Lopez *et al.*, 1991). Azotobacter also benefit soil fertility by reducing the toxic level of soil accumulated phenolic acids (Gauri *et al.*, 2012). Like *A. paspali* which can be found just in the rhizosphere of a grass (*Paspalum notantum*), the occurrence of other Azotobacter species in more restricted in the environment (Tchan and New, 1984; Dobereiner, 1995). It was first described by Dobereiner and Pedrosa (1975). The increase in final yield of maize by *Azotobacter* inoculation because of nitrogen fixation has been reported (Mrkovacki and Milic, 2001; Wani*et al.*, 2013). Azotobacteriaceae classification is presented in Table 13.

**Table 13.** Azotobacteriaceae classification

| Azotobacteriaceae | |
|---|---|
| | |
| *Azomonas sp.* (non-cyst forming) | *Azomona sagilis* |
| | *Azomonas insignis* |
| | *Azomonas macrocytogenes* |
| | |
| *Azobacter sp.* (cyst forming) | *Azotobacter chroococcum* |
| | *Azotobacter vinelandii* |
| | *Azotobacter beijerinckii* |
| | *Azotobacter nigricans* |
| | *Azotobacter armeniacus* |
| | *Azotobacter paspali* |

*Azotobacter vinelandii* is a gram-negative bacterium, capable of fixing nitrogen in various environments (Sadoff, 1975), and it used to consider as a model for different studies such as biochemical physiology and genetics (Dixon and Kahn, 2004; Yu and Ullrich, 2018). It is known to produce alginate under aerobic environment (Jarman, 1979; Horan *et al.*, 1981; Annisonand Couperwhite, 1986; Clementi, 1997; McRose*et al.*, 2019). *A. vinelandii* showed nearly constant respiration rates and insignifiacant decreases in nitrogen activity, even when $O_2$ concentrations ranging from 30 to 100% air saturation (Post *et al.*, 1983; Boiardi, 1994; Liu *et al.*, 2004). Sabra *et al.* (2000) suggested that the production of alginate, mainly capsule on the cell surface, forms a productive barrier for $O_2$ transfer into the cell. Several studies have showed that *Azotobacter* strains could increase seed germination and primarily seedling growth (Shino Suzuki *et al.*, 2002; Gholami*et al.*, 2009). *Azotobacter* sp. produces chemical substances which have been similar Gibberellic acid ($GA_3$), and 3-indole acetic acid (IAA) (Sivaskthi *et al.*, 2017). Martinez-Toledo *et al.* (1985) reported that in natural habitats, where corn plants are not influenced by nitrogen stress, *A. chroococcum* could be of the main importance in production of stimulatory factors. Kizilkaya (2008) introduced A. chroococcum strains as the key way to achieve sustainable agricultural production of spring wheat. The important and effects of *A. chroococcum* strains and AM fungi on host plants have been reported in previous researches (Savenkova*et al.*, 1999; Kumar *et al.*, 2006; Sharma and Kumar, 2008; Kumar and Sharma, 2009; Sharma *et al.*, 2012; Dutt *et al.*, 2013a,b; Khudhur and Askar, 2013; Sharma *et al.*, 2014; Velmourougane*et al.*, 2019). It has been proved that *A. chroococcum* has been found to be the most efficacious in nitrogen fixing and also in phytohormone production (IAA) (Ravikumar *et al.*, 2004). Romero-Perdomo *et al.* (2017) proved that *Azotobacter chroococcum* AC1 and AC10 showed a viable alternative to improve final cotton yield as well as lowering the nitrogen fertilizer dose and let to reduce the environmental deterioration connected with nitrogen pollution. Din *et al.* (2019) found that *Aspergillus niger* (*A. niger*) and *Azotobacter* may replace expensive and harmful chemical fertilizers with both eco-friendly and cost-effective biofertilizers. Mittal *et al.* (2011) reported that great benefits of *A. chroococcum* in nitrogen fixing for cotton crops in arid and semi-arid tropical regions. Aminpanah and Firouzi (2019) also found that inoculation of seeds with a combination of *A. lipoferum* and *A. chroococcum*, increase rice grain yield and a thousand grain weight. Application of *Azospirillum spp.* and *Azotobacter spp.* increased the growth and final yield of strawberry under hydroponic condition compare to the control treatment (Rueda *et al.*, 2016). Azotobacter can be an appropriate option for nitrogen deman and best alternative to nitrogen fertilizer in wheat cultivation which may lead to higher productivity (Mahato and Kafle, 2018).

### Bacteria

Nitrogen fixation by microbes also found in root sheaths of cereals and tropical grasses (Kirchhof*et al.*, 1997; Gutierrez-Zamora and Martinez-Romero, 2001; Rosenblueth and Martinez-Romero, 2004; Patra *et al.*, 2006; Chowdhury *et al.*, 2007; Montanez *et al.*, 2008 Bergmann *et al.*,2009; Davis *et al.*, 2010; Burbano*et al.*, 2011; Sessitsh*et al.*, 2012; Vitousek*et al.*, 2013; Ritchie and Raina, 2016). All grasses, especially C4 grasses show the highest potential for barboringdiaotrophs (James, 2000; Reis *et al.*, 2001; Davis *et al.*, 2010; Treseder*et al.*, 2012). Biological nitrogen fixation in depends on different types of species (Marques *et al.*, 2017; Barazetti*et al.*, 2019). Muangthong*et al.* (2015) found that Novosphingobiumsediminicola and Ochrobactrum intermedium which were isolated form the leaves of chewing sugarcane plants are capable of to get significant nitrogen concentrations when growing in nitrogen free sand. Legume Nodulation Bacteria (LNB) divided into six general which are *Agrobacterium*, *Rhizobium*, *Bradyrhizobium*, *Sinorhizobium*, *Burkholderia* and *Herbaspirillum* (Mwangi *et al.*, 2011). The genus Azospirillum, which belong to the family Rhodospirillaceae of the class Alphaproteobacteria, is known as a representative nitrogen fixing bacterium containing plant-growth promoting properties (Bashan and de-Bashan, 2010), and almost all of them dwell in aquatic environments (Kwak and Shin,2016). Zhang *et al.* (2020) observed that nitrogen fixing bacteria can improve the quality of compost and may result in meaningful reduction in N- and C- losses. Bahulikar*et al.* (2014) reported that the most important nitrogen-fixing bacteria related to Switchgrass were *Rhizobium* and *Methylobacterium* species of the alphaproteobacteria, *Burkholderia* and *Azoarcus* species of the betaproteobacteria and *Desulfuromona* and *Geobacter* species of the deltaproteobacteria. Hara *et al.* (2019) found that the main important nitrogen fixing bacteria in sorghum roots are bradyrhizobia which resemble photosynthetic *B. oligotrophicum* S58$^T$ and non-nodulating*Bradyrhizobium* sp. *S23321.* The unique role of Bradyrhizobium members have been reported in nitrogen fixation of sugarcane (Thaweenut*et al.*, 2011; Fischer *et al.*, 2012; Rosenblueth*et al.*, 2018), and sweet potato (Terakado-Tonooka*et al.*, 2008). Zhang *et al.* (2020)

noted that the higher C/N promote the relative abundance of nitrogen fixing bacteria like *Thermoactinomyces*, *Planifilum*, *Flavobacterium*, *Bacillaceae*, *Pseudomonas*, *Sphingobacterium*, *Paenibacillus*, *Bacillus* and *Thermobifida*. Meaningful influence of both arbuscular mycorrhizal fungi (AMF) and $N_2$-fixer on crop production and community structure which has proved the essential role of soil microbial communities (Xiao *et al.*, 2010; Bauer *et al.*, 2012; Goss and de Varennes, 2012; Abd-Alla*et al.*, 2014; Mbarki*et al.*, 2017; Hu *et al.*, 2019; Thioub*et al.*, 2019; Xu *et al.*, 2019; Massa *et al.*, 2020). AMF biofertilizer usage can increase continuous cropping of American ginseng growth by boosting the AMF inoculation rate, promoting plant-uptake of essential elements such as nitrogen and phosphorus and by increasing soil-borne pathogens (Johnson, 2010; Smith *et al.*, 2011; Bucking and Kafle, 2015; Liu *et al.*, 2020). Arbuscular mycorrhizal fungi is also beneficial to ameliorate the negative effects of a stressful environment (Levy *et al.*, 1983; Goicoechea *et al.*, 2005; Jebara*et al.*, 2010; Ruiz-Lozano *et al.*, 2012; Garg and Pandey, 2016; Li *et al.*, 2016; Verzeaux *et al.*, 2017; Zhang *et al.*, 2019). It has been proved that AMF can influence plant competition interaction between co-occurring plant species which may represent the vital role of mycorrhizal symbionts for sustainable crop management strategies (Raimam *et al.*, 2007; Nafady*et al.*, 2018; Bahadur *et al.*, 2019). The most important nitrogen fixing bacteria is shown in Table 14. Association of cereals and nitrogen-fixing PGPR are shown in Table 15.

**Table 14.** The most important nitrogen fixing bacteria (La Rue,1977)

| Family | Genera |
|---|---|
| Thiorhodaceae (Chromatiaceae) | *Thiocapsa, Chromatium* |
| Athiorhodaceae (Rhodospirillaceae) | *Rhodospirillum, Rhodopseumonas* |
| Hyphomicrobiaceae | *Rhodomicrobium* |
| Chlorobacteriaceae | *Chlorobium* |
| Spirillaceae | *Desulfovibrio, Desulfotomaculum* |
| Azotobacteriaceae | *Azotobacter, Beijerinckii, Derxii* |
| Entrobacteriaceae | *Klebsiella, Escherichia, Enterobacter* |
| Corynebacteriaceae | *Corynebacterium* |
| Bacillaceae | *Bacillus, Clostridium* |

**Table 15.** Association of cereals and nitrogen-fixing PGPR (Santi *et al.*, 2013)

| Cereals | Diazotroph inoculants |
|---|---|
| Wheat | *H. seropedicae* |
| | *Azospirillum sp.* |
| | *Azotobacter sp.* |
| | |
| Maize | *Burkholderia sp.* |
| | *Azospirillumbrasilense* |
| | |
| Rice | *Azoarcus* |
| | *Burkholderia* |
| | *A. vietnamiensis* |
| | *Gluconacetobacterdiazotrophicus* |
| | *Herbaspirillumseropedicae* |
| | *Serratia marcescens* |

## Conclusions

Nitrogen availability often restricts biological productivity in ecosystems. Nitrogen is the most important element for all forms of life, because it is found in nucleic acids, proteins and chlorophyll, and all forms of live bacteria, fungi, green plants and animals of all kinds can not grow and work unless they get nitrogen in an acceptable form. The sole usage of chemical fertilizer, especially nitrogen may have many unfavorable impacts on not only human and environmental health, but also on green house gasses and negative influence on the ozone layer. Nitrogen gas is abundant, but unreachable to majority of organisms. 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*. Within Bacteria, nitrogen fixation is much more extensively distributed and has been seed in phyla *Actinobacteria*, *Bacteroidetes*, *Cyanobacteria*, *Chlorobi*, *Chloroflexi*, *Firmicutes*, and *Proteobacteria*. *nif*H is the gene that encodes the nitrogenase which is the major structural protein that catalyzes the $N_2$ fixation reaction. The most important requirements for nitrogen fixation is a nitrogenase enzyme system, a source of adenosive triphosphate (ATP), a source of reducing power, a protective system for the enzyme from oxygen inactivation, and the rapid removal of nitrogen fixed from the site of nitrogen-fixation to prevent inhibition of the nitrogenase. The key element to improve environmental sustainability is recognizing the importance to improve the biophysical systems which support both short and long-term quality of all aspects of life on the earth with complete understanding of both health and diversity of natural ecosystems. Termite and its bacteria may lead to increase the soil fertility, improved plant growth, increased final crop yield and promote both better health and healthy environment. While, application of chemical fertilizer may lead to soil degradation and reduced crop yield which are the main reason of starvation. In all tropical and subtropical biomes, termites have a leading role in nitrogen mineralization. Two kinds of termites namely soil-feeding and humus-feeding termites normally thrive on nitrogen in soil substances, while, grass- and weed-feeding termites depend on the symbiotic bacterial communities in their gut for biological nitrogen fixation. The utilize of *Azotobacter* sp. presents a sustainable way to replace chemical fertilizer and even pesticides, which may lead to lower production cost, increase the productivity, reducing environmental pollution in the agricultural systems which heavily depend on chemical fertilizer and pesticides. This important bacterium is an obligate aerobic which can also grow in low $O_2$ situation. The most important ecological parameters which affect the spreading of termites are mean annual rainfall, mean number of rain days, atmospheric humidity and temperature, altitude, vegetation, soil type natural enemies and other related organisms. To have sustainable agriculture, replacing expensive chemical nitrogen fertilizers with environmentally friendly ways is the most accepted practice.

## Authors' Contributions

All authors read and approved the final manuscript.

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## Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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