Chapter 13

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Biological Dinitrogen Fixation: Introduction and Nonsymbiotic

David A. Zuberer

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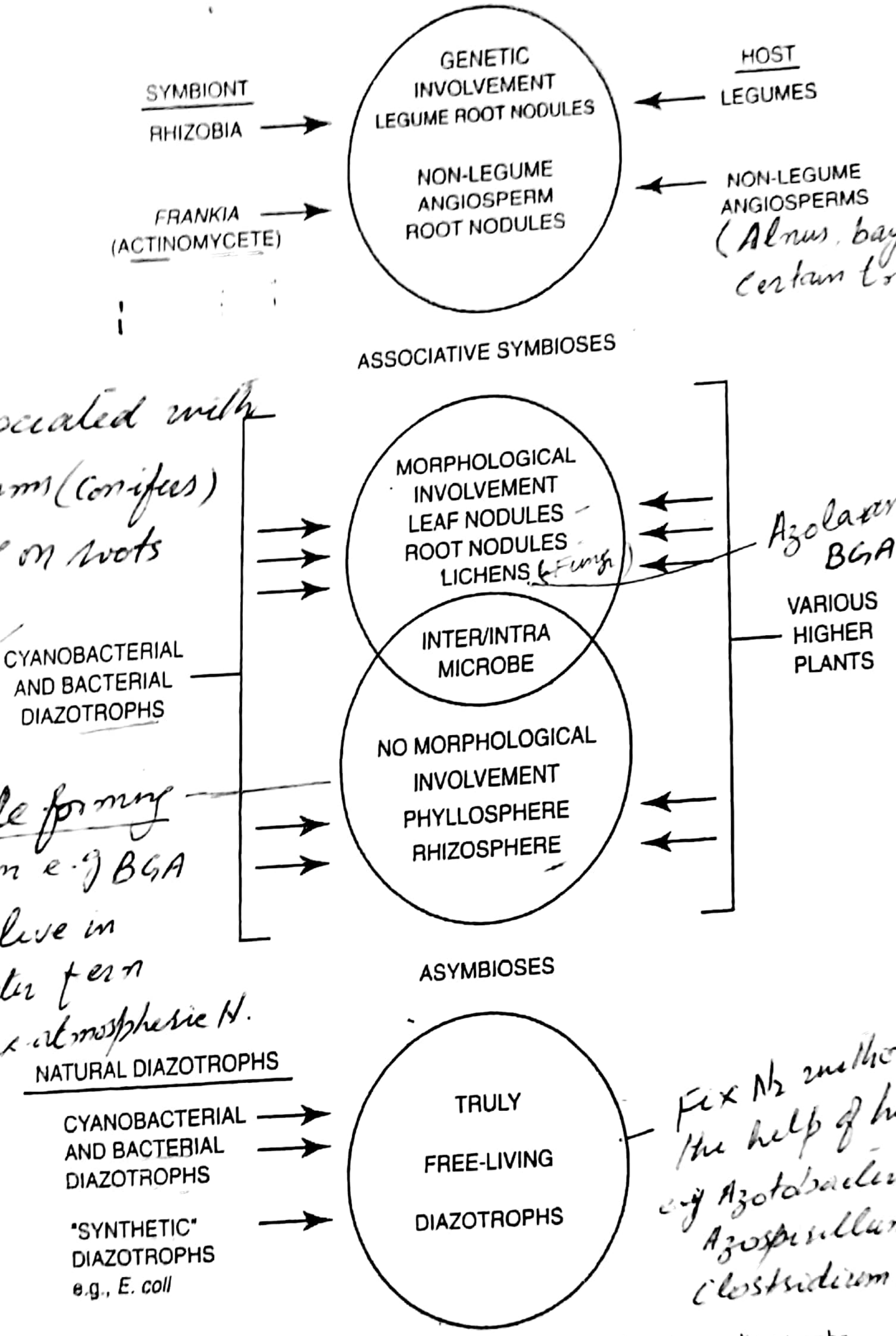
> *In nature there is an enormous reserve of organic matter poor in nitrogen. One should ask how all this organic carbon could be circulated in nature without the existence of organisms capable of fixing free nitrogen. These organisms . . . could be nothing else but microbes.*
>
> Sergei Winogradsky

After photosynthesis, biological dinitrogen fixation, the reduction of atmospheric dinitrogen (N_2) to two molecules of ammonia, is the second most important biological process on earth. In the absence of modern fertilizers or animal wastes, natural ecosystems rely on the biological conversion of atmospheric dinitrogen to forms available for plant and microbial growth by a variety of prokaryotic microbes. Dinitrogen fixation is mediated exclusively by prokaryotes, including many genera of bacteria, cyanobacteria, and the actinomycete *Frankia*. The N_2-fixing microbes can exist as independent, free-living organisms or in associations of differing degrees of complexity with other microbes, plants, and animals. These range from loose associations, such as **associative symbioses,** to complex **symbiotic** associations (Fig. 13–1) in which the bacterium and host plant communicate on an exquisite molecular level and share physiological functions (Chapter 14 details information on symbiotic N_2-fixing plant/microbe associations). Central to all of these systems is an N_2-fixing prokaryote containing the enzyme complex **nitrogenase,** which is responsible for the conversion of dinitrogen to ammonia. Only certain prokaryotes fix dinitrogen. Thus, when one sees statements about "N_2-fixing" plants, keep in mind that they do so by virtue of their prokaryotic partner, not on their own. Organisms that can use atmospheric dinitrogen as their sole source of nitrogen for growth are called **diazotrophs** ("diazo" for dinitrogen).



(Alnus, bay bessy) Certain tropical grasses))

BGA associated with Gymnosperms (conifers) nodulating on roots surfaces

(Fungi)

Azola and Fungi (lichens) BGA + Fungi = Lichens.

Non-Nodule forming association e.g. BGA (Anabaena) live in leaves of water fern Azolla and fix atmospheric N.

Fix N_2 without the help of host e.g Azotobacter Azospirillum (clostridium)

Figure 13–1 Biological N_2 fixation is mediated exclusively by free-living diazotrophs and a wide variety of plant-microbe associations of varying complexities. The most elaborate of these are the root-nodule symbioses between rhizobia and legumes and between the actinomycete *Frankia* and a variety of nonleguminous trees and shrubs. Between the free-living diazotrophs and the root-nodule symbioses lie a broad array of associations between plants and diazotrophs. These are the so-called "associative symbioses" characterized by a loose association between the plant and diazotroph. *Modified from Burns and Hardy (1975) Used with permission.*

Historical Background

The agricultural importance of legumes was recognized very early in the history of humans, reaching a milestone in 1888 with the isolation and description of the first organism, the root-nodule bacterium *Rhizobium*, determined (though much later) to have the ability to fix dinitrogen. *Rhizobium* and its relatives fix only small amounts of dinitrogen in pure culture, and significant rates of N_2 fixation only occur when the bacteria are in root nodules. In 1893, Winogradsky isolated the first free-living bacterium, an anaerobe, capable of significant N_2 fixation. He named this organism *Clostridium pasteurianum* in recognition of Louis Pasteur; Winogradsky, though a Russian, did much of his later scientific work at the Pasteur Institute in Paris. A few years later, in 1901, the Dutch microbiologist Martinus Beijerinck isolated an aerobic free-living, N_2-fixing bacterium, which he named *Azotobacter chroococcum* (derived from the French word "azote," meaning "lifeless" in recognition of the inertness of nitrogen).

bessy)
(mud grasses)

unga (lichens)
ingi - Lichens

Thus by the late nineteenth century, the foundation was laid for the extensive studies of N_2 fixation that would follow. These early discoveries began what has become more than 100 years of research on the fascinating and important subject of biological N_2 fixation.

The Significance of Biological Dinitrogen Fixation

A fundamental principle of agriculture and plant physiology is that plants require relatively high levels of nitrogen to produce abundant biomass, or yield. All forms of life require nitrogen to synthesize proteins and other important biochemicals, and nitrogen is often the limiting nutrient for plant and microbial growth in soils. In natural systems, nitrogen for plant growth comes from the soil, from rainfall or other atmospheric deposition, or through biological N_2 fixation.

Industrial N_2 fixation amounts to about 85 million metric tons per year (Hauck, 1985; Waggoner, 1994) and requires substantial inputs of energy, usually in the form of natural gas (Box 13–1). However, this output is less than the contribution of fixed nitrogen acquired through biological N_2 fixation. Accurate estimates of the magnitude of biological N_2 fixation are hard to derive, but reported values range from 100 to 180 million metric tons per year. Biological processes contribute 65% of the nitrogen used in agriculture (Burris and Roberts, 1993). While much of this is through symbiotic N_2 fixation, nonsymbiotic and associative fixation are of some significance in crops, such as sugar cane and sorghum, which have a C_4 photosynthetic pathway, and in specific ecosystems where nitrogen for plant growth is a limiting factor. Because of its culture in flooded soils, rice derives significant benefit from the activities of free-living diazotrophic bacteria and cyanobacteria.

Biological N_2 fixation offers an alternative to the use of expensive ammonium-based fertilizer nitrogen. However, the high-yielding agricultural systems of the United States and elsewhere are difficult to sustain solely on biological N_2 fixation. Keeping up with an expanding population will probably always require the judicious use of fertilizers. Yet, legumes and perhaps other N_2-fixing systems (e.g., the *Azolla-Anabaena* symbiosis) have an important place in sustainable agricultural production. Dinitrogen-fixing plants are also of high value in restoring disturbed or impoverished

The Energy Costs of N_2 Fixation. Dinitrogen is described as the most stable diatomic molecule known. The two atoms of nitrogen in the diatomic molecule are joined by a very stable triple bond. This triple bond requires a lot of energy (945 kJ, or 226 kcal, per mole) to break and therein lies one of the major challenges of fixing dinitrogen chemically or biologically. Dinitrogen fixation is "energy expensive" because it requires much energy to break the triple bond of dinitrogen and also to provide the hydrogen necessary to reduce dinitrogen to two ammonia molecules. The chemical fixation of dinitrogen is accomplished most widely through the high pressure catalytic method called the Haber-Bosch process, named after the German scientists who developed the process in 1914. The process served as a source of ammonium and nitrates for manufacture of explosives during World War I. The reaction for chemical fixation of dinitrogen can be written as follows:

$$N_2 + 3H_2 \xrightarrow[K_2O,\ Al_2O_3]{} 2\ NH_3\ (aq.)\quad \Delta G = -53\text{kJ}\ (12.7\text{kcal})$$

To obtain high yields from this reaction in reasonable time, high pressures (approximately 200 atm. or 20 MPa) are applied to the reaction vessel and the temperature is raised to 400–500°C. Natural gas (methane) is usually employed as the feedstock for the hydrogen needed to reduce the dinitrogen and is also required to heat the reaction vessel. To put these energy requirements in terms more easily understood, it takes about 875 cubic meters (31,000 cubic feet) of natural gas, 5.5 barrels of oil, or 2 metric tons of coal to fix 1 metric ton (2,200 lb) of ammonia (Dixon and Wheeler, 1986). About 40% of this fuel is used to provide the necessary heat, and the remainder provides the hydrogen needed for the reaction. Because we rely on fossil fuel (e.g., natural gas) in the production of ammonium-based fertilizers, costs are high; at present, those costs are somewhat inextricably linked to fossil fuel prices and the whims of that market. For example, an oil embargo against the United States in the early 1970s brought about long lines at gas pumps and quadrupled fertilizer prices. The high fertilizer prices also were due, in part, to short supplies at a time of peak demand.

Just as the chemical fixation of dinitrogen is energy intensive, so too is its fixation in biological systems. The principal differences lie in the sources of reductant and energy and the fact that biological N_2 fixation takes place at ambient pressures and temperatures. That is quite a feat when we consider the rigors of the industrial process. Energy for biological N_2 fixation comes from the oxidation of organic carbon sources, such as glucose, or from light in the case of photosynthetic diazotrophs.

soils. They serve as excellent cover crops, green manures, and forage crops for livestock production. Use of N_2-fixing crops also has the potential to reduce the contamination of groundwater with nitrate. Further aspects of the utility of symbiotic N_2 fixation in agriculture and other ecosystems are discussed in Chapter 14.