

# Plants that live on air

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*Use nitrogen from the air to synthesize its essential biomolecules? Plants achieve this feat by establishing a mutually beneficial association with soil bacteria. After a specific recognition process, the partners establish a symbiosis. Bacteria enter the root and the cortex cells divide. A specific organ is then formed, the nodule, in which atmospheric nitrogen from the air is used for the synthesis of amino acids that are then used to form proteins. This association has major economic and environmental advantages. This makes the use of nitrogen fertilizers virtually unnecessary and reduces air and groundwater pollution. These plants that live on the air of the times also have important nutritional qualities and are therefore particularly interesting.*

## **1. Nitrogen, a plant growth limiting factor**

Nitrogen (N), like carbon (C), hydrogen (H) and oxygen (O), is an essential component of life and ecosystems. It is essential to life because it is involved in the constitution of many biomolecules such as proteins, nucleic acids, nucleotides or chlorophyll. Reactive nitrogen, which can be used by plants, is present in the soil mainly in the form of nitrate ( $\text{NO}_3^-$ ) and ammonia ( $\text{NH}_3$ ).

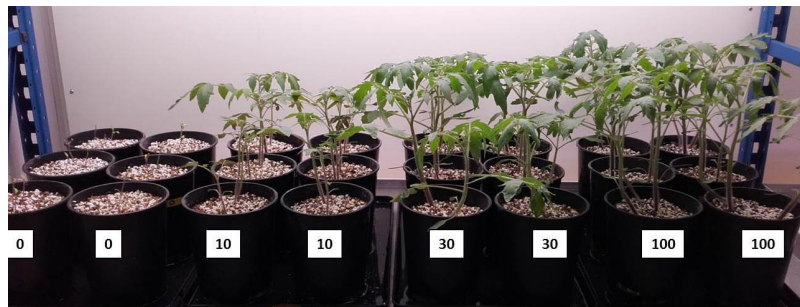


Figure 1. Effects of nitrogen deficiency on plant growth. 4-week-old tomato plants grown in the presence of 0, 10, 30 and 100% of the plant's nitrogen requirements [Source: © Renaud Brouquisse].

The distribution of plants on the surface of the globe is uneven: it is denser in temperate and tropical regions, and more sparse in polar and desert regions. This is due to the fact that plant development is limited by many environmental factors. After water availability, reactive nitrogen availability\* is the second limiting factor for plant growth (Figure 1). This is why world agriculture uses large amounts of nitrogen fertilizers.

Plants obtain the nitrogen they need by absorbing it from the roots in the form of  $\text{NO}_3^-$  or  $\text{NH}_3$ . But some species are also able to establish a relationship with marine or terrestrial bacteria that can use nitrogen from the air: this is the biological nitrogen fixation.

## 2. The plant - nitrogen-fixing bacteria relationship

### 2.1. The variety of nitrogen-fixing organisms



Figure 2. Biological nitrogen fixers. A, Marine filamentous cyanobacteria; B, Sea Buckthorn, in symbiosis with actinomycete *Frankia*. [Source: A, © Jean-Claude Druart, INRA Media Library; B, Vmenkov (GFDL or CC BY-SA 4.0), via Wikimedia Commons]

Biological fixation of atmospheric nitrogen is only achieved by bacteria. Three categories of nitrogen-fixing bacteria can be distinguished:

**Marine cyanobacteria**, including filamentous cyanobacteria living in colonies (*Trichodesmium*, ...), and unicellular cyanobacteria living either free or in symbiosis with phytoplankton (*Nostoc*, *Anabaena*,...; Figure 2). They are responsible for 40 to 50% of biological nitrogen fixation.

**Free soil bacteria**, some of which are aerobic (*Azomonas*, *Azotobacter*,...) and others anaerobic (*Desulfovibrio*, *Clostridium*,...). Some are called phototrophic because they derive their energy source from light (*Chromatium*, *Chlorobium*,...), others are called chemotrophic because they use the energy of the oxidation of mineral compounds (chemo-lithotrophic: *Thiobacillus*, *Methanococcus*,...) or organic (chemo-organotrophic: *Methylomonas*, *Azotobacter*,...). They are said to be responsible for 5 to 10% of biological nitrogen fixation.

The third category, the one of interest here, includes **soil bacteria that live in symbiosis\*** with the root system of plants (see [Symbiosis & parasitism](#)). These are the actinomycete *Frankia*, which establishes symbioses with various angiosperms species (alder, sea buckthorn, Casuarinaceae; Figure 2) and *Rhizobium*-type bacteria that enter into symbiosis with plants of the legume family (Fabaceae) [1]. This family, which includes about 18,000 species (soybean, alfalfa, bean, lentil, groundnut, licorice, clover, wisteria, mimosa,...), is characterized by papilionaceous flowers (butterfly-shaped), a pod (fruit from the flower's ovary) containing seeds and, for the majority of its members, the ability to use atmospheric nitrogen to produce its own nitrogen

## 2.2. How do the two partners recognize each other?

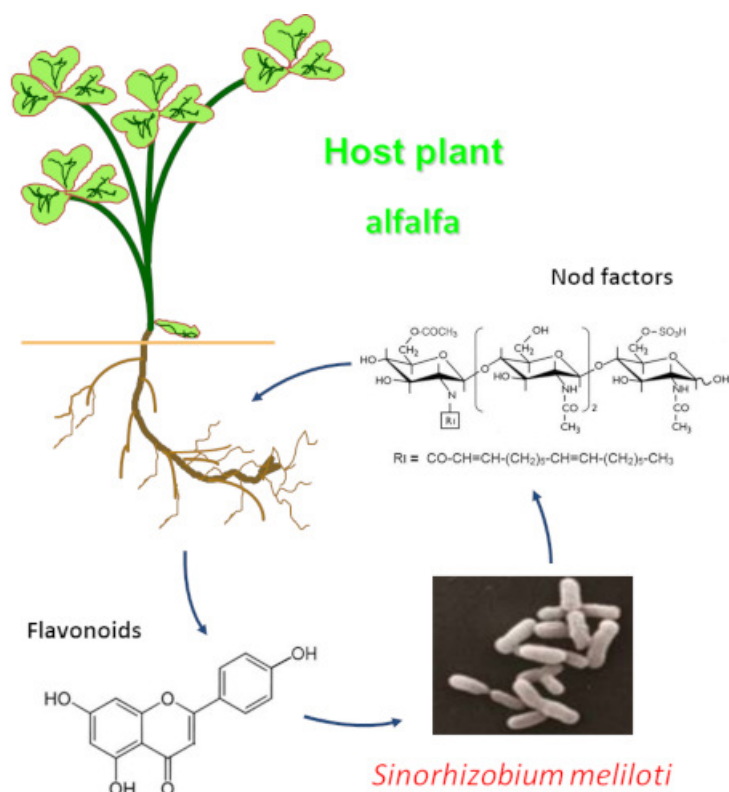


Figure 3. Mutual recognition process between a legume (alfalfa) and a bacterium of the *Rhizobium* type (*Sinorhizobium meliloti*). [Source: © Renaud Brouquisse & Alain Puppo]

It is through the **mutual recognition of the two partners (the plant and the bacteria)** that the symbiotic process begins. In response to the secretion of flavonoids\* by the root (Figure 3), bacteria are attracted to it and synthesize lipo-chito-oligosaccharides\*, called nod factors (for nodulation). This process is characterized by a high specificity: *Rhizobia* recognized by soybean will not be recognized by alfalfa and vice versa.

## 2.3. Nodules formation

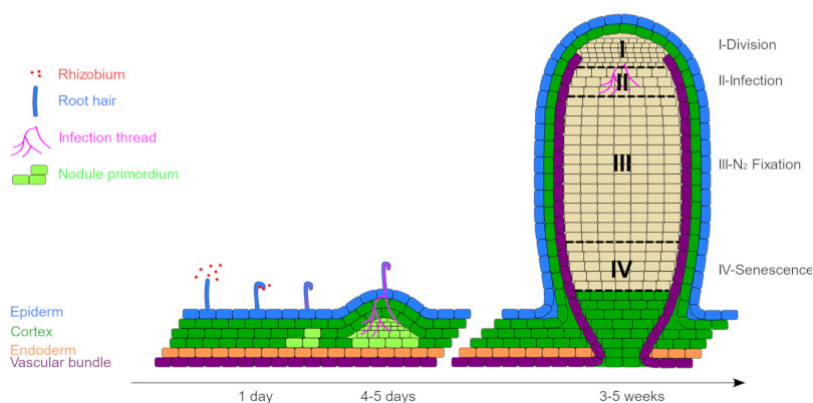


Figure 4. Diagram of the establishment, formation and structure of an indeterminate growing nitrogen fixing nodule of the alfalfa type. [Source: © Marc Bosseno, INRA Media Library]

At the root surface, under the influence of nod factors, bacteria attach themselves to the root hair whose end curves into a “shepherd’s cross” in which microsymbiotes\* gather (Figure 4). Root infection is initiated by invagination of the plasma membrane of the cell constituting the root hair. A tubular structure, called an infection thread, contains bacteria that progress to the root cortex\*.

Nod factors also trigger the de-differentiation (divisional entry) of cells from the root cortex, leading to the creation of a meristem\* at the origin of a new organ: the nodule (Figure 4). This is gradually invaded by symbiotic bacteria, now called bacteroids. However, they are not free in the plant cells; they are surrounded by a membrane of plant origin, the peribacteroid membrane, which will regulate exchanges between the two partners (Figure 5).

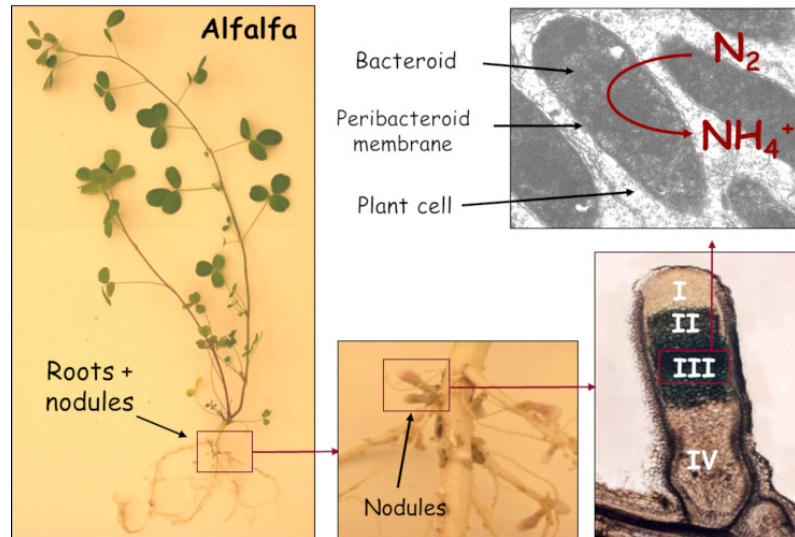


Figure 5. Exploded view and detail of an alfalfa nodule (*Medicago sativa*), photo credit INRA. Refer to Figure 5 and text for the numbers of zones I to IV. [Source: © Renaud Brouquisse & Alain Puppo]

Some nodules, with undeterminate growth, retain a meristem and their structure can be divided into several zones (Figures 4 and 5) [2]; the division zone (I) where the cells divide and nodule increases; the infection zone (II) where bacteria enter the plant cells and transform into bacteroids; the fixation zone (III) where atmospheric nitrogen ( $N_2$ ) is reduced to ammonia ( $NH_3$ ) by bacterial nitrogenase; the senescence zone (IV) where the bacteroids, then the plant cells die.

## 2.4. Nodule: a haven for bacteroids

Within the nodule, the oxygen concentration is strongly reduced compared to the atmospheric content. These microaerophilic\* conditions allow the enzyme responsible for nitrogen fixation, bacterial nitrogenase, to be active. This enzyme is inactivated by oxygen. These conditions are allowed by the combination of two processes:

On the one hand, a gas diffusion barrier is established in the nodule cortex thanks to layers of cells without intercellular space;

On the other hand, infected plant cells contain a haemoprotein with a high affinity for oxygen: the leghaemoglobin. This protein, which is red in colour and has a structure comparable to animal myoglobin, provides oxygen to bacteroids at concentrations low enough not to inactivate nitrogenase.

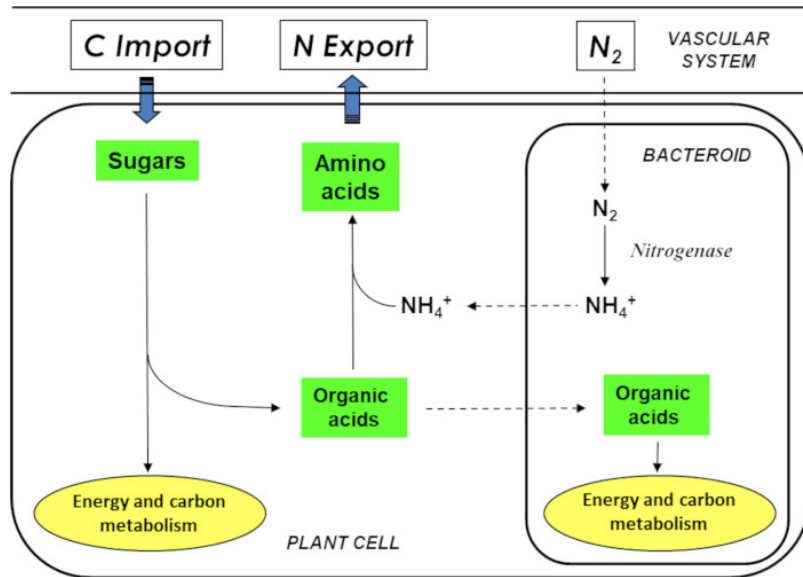


Figure 6. Carbon-nitrogen exchanges between the plant cell and the bacteroid in a nodule. [Source: © Renaud Brouquisse & Alain Puppo]

In the functioning of symbiosis, the plant supplies the microbial partners with carbon-containing nutrients (organic acids), to feed their energy metabolism; in exchange, the bacteroids provide the plant with ammonia ( $\text{NH}_3/\text{NH}_4^+$ ) which is incorporated into the plant proteins (Figure 6). The expression "living from the air of the times" thus takes on its full meaning, since the plant is able to draw from the atmosphere the carbon (via photosynthesis) and nitrogen (via biological fixation) it needs for the synthesis of its biomolecules.

It is important to note that when the legume grows on soil naturally rich in nitrate or ammonia, it uses the latter as a source of nitrogen. The symbiotic process is then inhibited and symbiosis does not take place.

### 3. Symbiotic association, a major advantage for ecosystems and agronomy

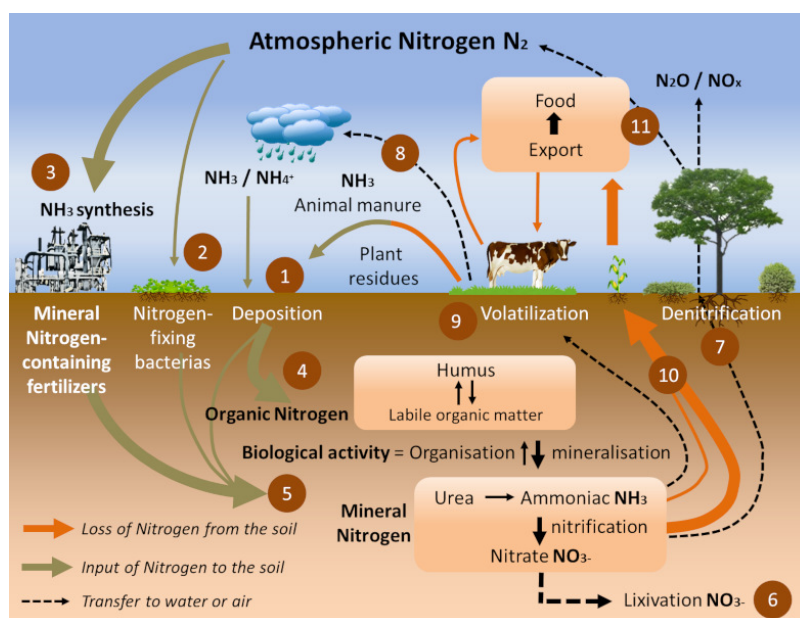


Figure 7. Nitrogen cycle diagram.

Nitrogen permanently exits and enters the soil through the nitrogen cycle (Figure 7). There are three main ways in:

**Recycling of decomposing organic matter** (Step 4) [3]. Humus, derived from dead plants, crop residues and livestock manure,



supplies soil and aquatic environments with organic matter (Step 1). In well oxygenated soils and aquatic environments, bacteria convert ammonia into nitrite ( $\text{NO}_2^-$ ) and then nitrate ( $\text{NO}_3^-$ ) during the nitrification process (Step 5).

The **spreading of fertilizers and nitrogen fertilizers** (Step 5). These have been produced for more than 80 years by the Haber-Bosch chemical process [4] (Figure 8), which is used to synthesize ammonia by hydrogenation of atmospheric gaseous dinitrogen by gaseous hydrogen ( $\text{H}_2$ ) in the presence of a catalyst (Step 3). They now represent the main contribution of nitrogen to agriculture in industrialized countries in North and South America, Europe and Australia, and their use has increased significantly in recent decades (Figure 8).

The **biological nitrogen fixation** we have described, in particular via the symbiosis between Rhizobia and legumes (Step 2). The latter, cultivated for at least 12,000 years around the Mediterranean basin, have for centuries played an essential role in the regeneration of agricultural land for nitrogen through the three-year crop rotation (a fallow year, a legume year, a cereal year). Today, biological nitrogen fixation is estimated at between 150 and 250 million tonnes per year, including about 50 million tonnes from legumes in symbiosis; by comparison, industrial production of nitrogen fertilizers by the Haber-Bosch process represents about 100 million tonnes per year [5].

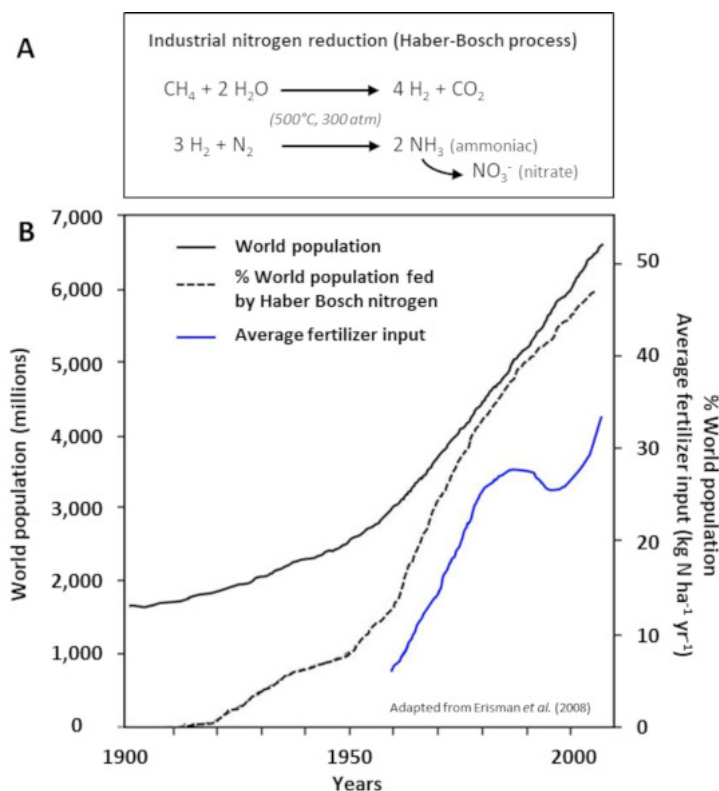


Figure 8. Haber-Bosch process for the manufacture of chemical fertilizers (A) and the contribution of chemical fertilizers to food production and human population since the beginning of the 20<sup>th</sup> century (B). [Source: © Renaud Brouquisse & Alain Puppo]

The amount of nitrogen contained in a soil and bound to carbon (97 to 99% of total nitrogen) varies from 2 to 10 tonnes per hectare. Nitric and ammoniacal fractions, available and used for plant growth, represent only 1 to 3% of total nitrogen. **Nitrogen is removed from the soil** in three types of processes:

Through their roots, **plants absorb nitrate and ammonia from the soil** and incorporate them into amino acids, proteins and all nitrogen molecules necessary for their growth (Step 10).

Plants are then a **primary source of available nitrogen for herbivorous animals, including humans**, to consume (Steps 9 & 11).

In poorly oxygenated soils or aquatic environments, **so-called “denitrifying” bacteria transform ammonia and nitrate into  $\text{N}_2$**  which can be returned to the atmosphere via a denitrification process (nitrate,  $\text{NO}_3^-$ , to dinitrogen,  $\text{N}_2$ , reduction process) (Step 7).

However, not all the **nitrogen in the soil** is assimilated or transformed into nitrogen. A significant part, which varies according

to the nature and content of the soil in nitrogen compounds, is:

**either carried to groundwater** by runoff (referred to as leaching) (Step 6),

**or volatilized into the atmosphere** as ammonia or nitrogen oxides (NO<sub>x</sub>: mainly NO and NO<sub>2</sub>) (Step 8).

These losses constitute a nitrogen depletion of the soil and a source of pollution of the atmosphere and groundwater (see [Nitrates in the environment](#)).

Over the past 100 years or so, through the increase in industrial activities and the massive use of nitrogen fertilizers, human activities have significantly altered the nitrogen cycle. Today, the risk of saturation of the planet with reactive nitrogen is real and its implications for ecosystems are still poorly assessed.

## 4. Interest of legumes for food and the environment

### 4.1. A food source and a nutritional asset



*Figure 9. Food legumes. From left to right and top to bottom: green bean (*Phaseolus vulgaris*), lupin (*Lupinus albus*), faba bean (*Vicia faba*), brown lentil (*Lens culinaris*), faba bean (*Vicia faba*), red bean (*Phaseolus vulgaris*), coral lentil (*Lens culinaris*), pea (*Pisum sativum*), chickpea (*Cicer arietinum*). [Source: © Jean-Marie Bossennec, © Roland Bruneau, © Chantal Nicolas, © Christophe Maitre, © Gérard Duc, © Renaud Brouquisse, © Jean Weber, all from the INRA Media Library]*

**Legumes have important qualities for human and animal nutrition** [6]. Protein-rich fodder legumes are used to feed animals either by grazing or after fodder storage (hay, silage, dehydration). Seed legumes are used for animal and human food (Figure 9). They are particularly rich in protein, which accounts for 20 to 40% of their dry weight. In comparison, proteins represent only 6 to 13% of the dry weight of cereals. In addition, legume proteins contain essential amino acids that humans are not able to synthesize and must find in their diet. They are rich in lysine, but low in sulphur-containing amino acids (methionine, cysteine), while cereal proteins are rich in methionine and cysteine, but low in lysine. Legumes are therefore an excellent complement to cereals for a balanced diet of plant proteins [7].

In most edible legume species, the **fat content of the seeds is low** (1-10% of dry weight), but there are **exceptions** and in some species referred to as **oilseeds**, the seeds may contain 20% (soybean) and up to 50% (peanut) of their dry weight in fat.

While carbohydrate levels vary widely among legumes (20 to 65% of dry weight), their glycemic index\* is generally low; moreover, they essentially contain a form of starch (amylose) that the body digests slowly and causes **little variation in blood sugar**. In addition, soluble and insoluble **fibres** present in seeds have a **beneficial effect** on digestive tract **health** and digestion; on the other hand, some **fermentable fibres** (a-galactosides) can cause risks of **flatulence**.

Legume seeds are also **rich in vitamins** (B1, B2, B3, E) and minerals (potassium, phosphorus, magnesium, zinc, iron, manganese, calcium, etc.). However, in dry seeds, the bioavailability of these compounds is reduced by the presence of anti-nutritional factors, such as protease and amylase inhibitors, or secondary metabolites such as phytates or tannins [8]. Most of



these **anti-nutritional factors are destroyed** or inactivated by hydrothermal treatments (seed soaking and **cooking**), which increases the bioavailability of the various seed components.

Because of their **good digestibility** (80 to 90% after soaking and cooking), legume proteins can be compared to animal proteins and provide an alternative to meat consumption [2]. In terms of health and well-being, the nutritional intakes of legumes therefore have their rightful place in a varied and balanced diet. Their consumption makes it possible to better prevent cardiovascular diseases, as well as the risks of type 2 diabetes, obesity and certain cancers (colorectal, breast) [4]. A minor drawback, however, is that soybeans and especially groundnuts are highly **allergenic** legumes whose consumption can sometimes lead to food allergies.

## 4.2. A source of nitrogen and an environmental asset

Because of the symbiosis with nitrogen-fixing bacteria, legumes are therefore a **natural fertilizer**. They were among the first plant species domesticated in the fertile crescent more than 12,000 years ago. At the death of the plant, during the degradation of the root and aerial parts, the mineralization of organic matter releases nitrogen in forms (nitrate, ammonia, amino acids) that are easily assimilated by neighbouring plant species or subsequent crops. Today, legume cultivation accounts for about 25% of the nitrogen input to cultivated areas (about 20% in European and North American countries, and more than 50% in Asia, Africa and South America), with industrial fertilizers accounting for 63% and combustion processes for 13% [9]. The cultivation of legumes has a major environmental impact because:

Because they **require little or no fertilization**, they allow a significant saving in fertilizers whose manufacturing cost is high (it takes the equivalent of 2 tons of oil to produce 1 ton of nitrogen fertilizer by the Haber-Bosch process).

Reducing the use of fertilizers **reduces** groundwater **pollution** by reducing leaching\*, which in turn reduces eutrophication\*;

They have a **positive effect on the balance of greenhouse gas (GHG) emissions**, including CO<sub>2</sub>, N<sub>2</sub>O or ammonia, which are mainly derived from mineral fertilizers and animal waste (symbiotic fixation produces only a small amount of N<sub>2</sub>O).

To these advantages, directly linked to the existence of symbiosis, are added those inherent to legumes, whether or not they establish a symbiosis with *Rhizobium*:

They **improve**, by also being able to establish a mycorrhizal symbiosis, the **removal of phosphorus from the soil** (another factor limiting plant growth), and make it more available to other plant species.

They have a **positive impact on the soil** (stabilization, limitation of runoff and erosion through the development of their root system) and a positive effect on crop rotations (reduction in fertilizer use, reduction in plant protection treatments).

Finally, as a natural fertilizer, they have a **positive effect on biodiversity** by increasing the productivity of plant species, promoting pollinators (bees) and providing refuges for crop auxiliaries and macrofauna (birds, mammals).

## 5. Crop associations, a solution for agro-ecology



Figure 10. Associated cultures. A, left: Alternate cereal-vetch cultivation; B, center: Organic mixed cereal (triticale; barley; oats) and legumes (peas) cultivation; C, right: Simple agroforestry system combining one perennial woody species in row (olive tree) and two species in culture, under the row, cover or nitrogen fixing plant (alfalfa) and inter row, food plant (durum wheat). [Source: A, Michel Gosselin INRA Media Library; B and C, Christophe Maître, INRA Media Library]

In large-scale farming regions, French agriculture is strongly influenced by the simplification of crop rotation and the increased use of inputs (fertilizers). Diversified agro-ecosystems are increasingly recognized as a crucial lever for sustainable development. The increase in diversity cultivated within the plot was specifically tested through cereal - legume associations, sown and harvested together (Figure 10). It appears that these associations make it possible to meet the challenges of production, reduction

of inputs and environmental impacts of crops and stability in the face of biotic and abiotic hazards in both organic and conventional agriculture [10]. The selection of varieties well adapted to these crop associations is becoming a major challenge in this context.

Associated crops\* cereal - legume improve resource utilization compared to a single crop. This leads in particular to:

a better grain/seed yield\* than the average yield of each single crop,

a reduction in weed biomass compared to growing legumes alone,

and a higher protein concentration in the grains of the cereal compared to the cultivation of the cereal alone.

The advantages of the associated crop are all the more marked when the conditions are unfavourable: low yield of each crop or both grown separately or low protein content of the grain of the cereal grown alone. Associated crops are particularly beneficial when nitrogen is limiting for crops and helps to stabilize yields in organic agriculture.

The achievement of these benefits is closely linked to the proportion of legumes within the association. Thus, in grasslands, the optimal operating conditions are about 30 to 40% fodder legumes for grazed associations and 50 to 70% for mowing associations. In any case, legumes can thus be a driving force for reorienting crops towards sustainable agriculture that respects the ecological, economic and social standards that ensure that agricultural production is maintained over time.

## 6. Messages to remember

Some bacteria are able to use atmospheric nitrogen for the synthesis of their biomolecules (marine cyanobacteria, soil bacteria that are free or in symbiosis with the roots of certain plants): this is the biological fixation of nitrogen.

In the case of legumes, symbiotic bacteria penetrate the root system and cause the formation of a new organ: the nodosity. This organ is the place where atmospheric nitrogen is reduced to ammonia; it contains a protein comparable to animal myoglobin: leghaemoglobin.

Legumes are high in protein and are an excellent complement to cereal protein for a balanced vegetable protein diet.

The benefits of biological nitrogen fixation are multiple: economic (nitrogen fertilizer savings) and environmental (reduced air and groundwater pollution, crop associations for sustainable development).

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## References and notes

**Cover image.** [Source: © Marie-Christine Lhopital, INRA Media Library]

[1] Vegetables and legumes should not be confused. Vegetable comes from the Latin legumen which referred to plants whose fruit is a pod (pod of "legumes"). Subsequently, legumen has designated all vegetable plant species and, in its current use, a vegetable is a cultivated plant whose consumption depends on the species, leaves, roots, tubers, fruits, seeds; vegetable also refers to the consumed part of this plant. Note that in French, legumes are called "légumineuses".

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[3] The numbers in brackets refer to the nitrogen cycle diagram (Figure 7).

[4] The Haber-Bosch process makes it possible to economically fix atmospheric nitrogen in the form of ammonia, which in turn allows the synthesis of different explosives and nitrogen fertilizers. As such, from a demographic point of view, it is probably the most important industrial process ever developed during the 20<sup>th</sup> century.

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