Chapter 1
The Role of Legume-*Rhizobium* Symbiosis in Sustainable Agriculture

Takuji Ohyama

Abstract The world's population is increasing rapidly, and world food production needs to be commensurate with the demands of human consumption. To increase cultivated acreage would be very difficult, so we need to promote crop production, or the efficient use of existing croplands. The use of chemical nitrogen (N) fertilizers in the twentieth century promoted crop production by 4–10 times, and has supported food production over the past 100 years. However, the cost of chemical N fertilizers is high for farmers in developing countries, and their production requires a lot of fossil fuel. In addition, the inappropriate or excess application of chemical N fertilizers causes environmental problems, such as contamination of ground water by nitrates, and air pollution and global warming due to nitrous oxide. On the other hand, most leguminous crops, such as soybeans, beans, chickpeas, and groundnuts, and legume forage crops such as alfalfa and clover can fix atmospheric dinitrogen (N_2) by symbiosis with soil microorganisms (collectively termed rhizobia). The supply of N by symbiotic N_2 fixation via legume-rhizobium symbiosis is the most important source of N in agro-ecosystems. This renewable and environmentally sustainable N source also ensures soil restorative agents for maintaining soil fertility and sustainable crop production. Legume crops provide an important source of protein, oil and carbohydrate for human diets and livestock feeds. The production of legumes depends on symbiotic N_2 fixation, and this process is affected by various environmental conditions, as well as the supply of water and mineral nutrients, especially the availability phosphorous—the main theme of this book.

Keywords Chemical N fertilizer • Global population increase • Legume-*Rhizobium* symbiosis • N_2 fixation • Root nodules • Sustainable agriculture

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1.1 Current Situation of Global Population and Food Security

1.1.1 Estimate of Global Population Change and Food Production

The world's population was only about 1 billion in 1800, doubled in 100 years to 2 billion in 1900, then tripled in the next 100 years to 6 billion in 2000. The total human population of the world reached 7.3 billion in 2015, and is still increasing. In 2015, about 60% of the global population (4363 million) lived in Asia, 16% (1186 million) in Africa, 10% (738 million) in Europe, 9% (634 million) in Latin America and the Caribbean, 5% (358 million) in Northern America, and 0.5% (39 million) in Oceania (Table 1.1) (United Nations Department of Economic and Social Affairs/Population Division 2015a). China (1376 million) and India (1311 million) are the two largest countries in the world in terms of population, followed by the USA (322 million), Indonesia (258 million), Brazil (208 million), Pakistan (189 million), Nigeria (182 million), Bangladesh (161 million), the Russian Federation (143 million) and Mexico (127 million) in 2015 (Table 1.2).

The United Nations estimates that the world population will reach 8.5 billion in 2030, 9.7 billion in 2050, and 11.2 billion in 2100 (Table 1.1) (United Nations 2015b). The population in Asia after 2030 will be relatively stable at around 5 billion; however, the population in Africa is estimated to increase markedly from 2050 to 2100 (4.4 billion), and to become comparable to the Asian population by 2100 (4.9 billion) (Table 1.1). The population in Europe will decrease slightly from 2050 (707 million) to 2100 (646 million). By 2050, the population in India is estimated to reach 1.71 billion, overtaking that in China (1.35 billion), followed by Nigeria (399 million), the USA (389 million), Indonesia (321 million), Pakistan (310 million), Brazil (238 million), Bangladesh (202 million), the Democratic Republic of the Congo (195 million), and Ethiopia (188 million) in 2050 (Table 1.2). From 2015 to 2050, the total increase will be about 2.38 billion, with 394 million in India, 216

Table 1.1 Population of the world and major areas, 2015, 2030, 2050, 2100

		Population	(million)	
Major area	2015	2030	2050	2100
World	7349	8501	9725	11,213
Africa	1186	1679	2478	4387
Asia	4363	4923	5267	4889
Europe	738	734	707	646
Latin America and the Caribbean	634	721	784	721
Northern America	358	396	433	500
Oceania	39	47	57	71

Data from United Nations Department of Economic and Social Affairs/Populaion Division (2015a)

Table 1.2 Ten countries with the largest populations, 2015 and 2050

Rank	Country	2015 Population (million)	Country	2050 Population (million)
1	China	1376	India	1705
2	India	1311	China	1348
3	USA	322	Nigeria	399
4	Indonesia	258	USA	389
5	Brazil	208	Indonesia	321
6	Pakistan	189	Pakistan	310
7	Nigeria	182	Brazil	238
8	Bangladesh	161	Bangladesh	202
9	Russian Federation	143	Democratic Republic of the Congo	195
10	Mexico	127	Ethiopia	188

Data from United Nations (2015b)

million in Nigeria, 121 million in Pakistan, 118 million in the Democratic Republic of the Congo, 89 million in Ethiopia, 84 million in Tanzania, 67 million in the USA, 65 million in Indonesia, and 63 million in Uganda.

The main point to note in these population changes in the world as estimated by the United Nations is that the most rapid increase will occur in the poorest area (Africa) and countries (African and Asian countries). Stable food supply by self-production in these areas will be very difficult due to the severe weather conditions and poor soils in these countries.

From the Technical Summary of Status of the World's Soil Resources (FAO, ITPS 2015), between 1961 and 2000, global population grew by 98%, but food production rose by 146% and food production per person increased by 24%. Crop yields have more than doubled and, quite remarkably, the area of arable land in use increased by only 8%. Arable land per person reduced substantially from 0.45 ha to 0.25 ha during this period. To achieve any increase in cultivated acreage would be very difficult, thus we need to promote crop production, or the efficient use of croplands.

1.1.2 Human Nutrition

All human beings need to eat a certain amount of food, from either plant or animal products, to support life. Most higher plants are "photoautotrophs", i.e., organisms capable of synthesizing their own food materials themselves from simple inorganic substances such as carbon dioxide, water, and minerals, using light energy. However, all animals, including humans, are "heterotrophs", i.e., organisms that are dependent for nutrition on organic substances derived from other organisms. Humans are omnivorous and eat many kinds of plant and animal products, such as crops, vegetables, fruits, meat, milk, fish, eggs, etc., unless these are dangerous due to natural toxins or harmful microbial contamination, or just taste bad. There are a wide variety of foods all over the world depending on the region, culture, religion, history and natural habitat of traditional crops and animal products. Asian people traditionally depend on rice, soybean, vegetables and fish, and European people generally depend on wheat, meat and dairy products. However, the fundamental requirements of human nutrition are common to all.

There are three macronutrients (carbohydrates, proteins, lipids) and two micronutrients (minerals and vitamins). Sometimes water is included in the list of human nutrients (Vorster 2009), although water does not contain any organic substances or energy. Carbohydrates, such as starch and sugars, and lipid or oil provide energy and carbon materials for maintaining metabolism and growth in humans. On the other hand, protein in food is digested to amino acids and used for protein synthesis in the body.

Human nutrition is complex and the quantity and quality of essential nutrients in foods are important for the maintenance of life (Voster 2009). Optimal, balanced nutrition is a major determinant of health, and can promote health and well-being, as well as prevent ill-health and treat disease (Vorster 2009). However, hundreds of millions of food- and nutrition-insecure people still exist globally, leading to the coexistence of both under-nutrition and inappropriate nutritional behavior such as overconsumption in the world today.

The amount of food necessary for humans differs depending on their age, sex, body weight and the area in which they live (Ministry of Health, Labor and Welfare, Japan 2016). It was recommended that Japanese men and women aged 40 years old need food containing about 2300 kcal and 1750 kcal per day, respectively. On the other hand, world average food consumption in 2005/2007 was about 2772 kcal per person per day; this is expected to increase to 3070 kcal in 2050, and to 3200 kcal in 2080 (Table 1.3; Alexandratos and Bruinsma 2012). It is calculated that 7.3 billion

Table 1.3 Projections of food requirement in 2050 and 2080

Year	2005/2007	2050	2080
Energy (kcal person^{-1} day^{-1})	2772	3070	3200
Cereals, food (kg person^{-1} year^{-1})	158	160	161
Cereals, all uses (kg person^{-1} year^{-1})	314	330	339
Meat, food (kg person^{-1} year^{-1})	38.7	49.4	55.4
Oilcrops,[a] food (kg person^{-1} year^{-1})	12.1	16.2	16.9
Oilcrops,[a] all uses (kg person^{-1} year^{-1})	21.9	30.5	33.8
Cereals, production (million tons/year)	2068	3009	3182
Meat, production (million tons/year)	258	455	524
Paddy rice yield (tons/ha)	3.32	4.30	4.83
Arable land area (million ha)	1592	1661	1630

(Modified from Alexandratos and Bruinsma. World Agriculture Towards 2030/2050; The 2012 Revision) (FAO 2012)

[a] Oil equivalent

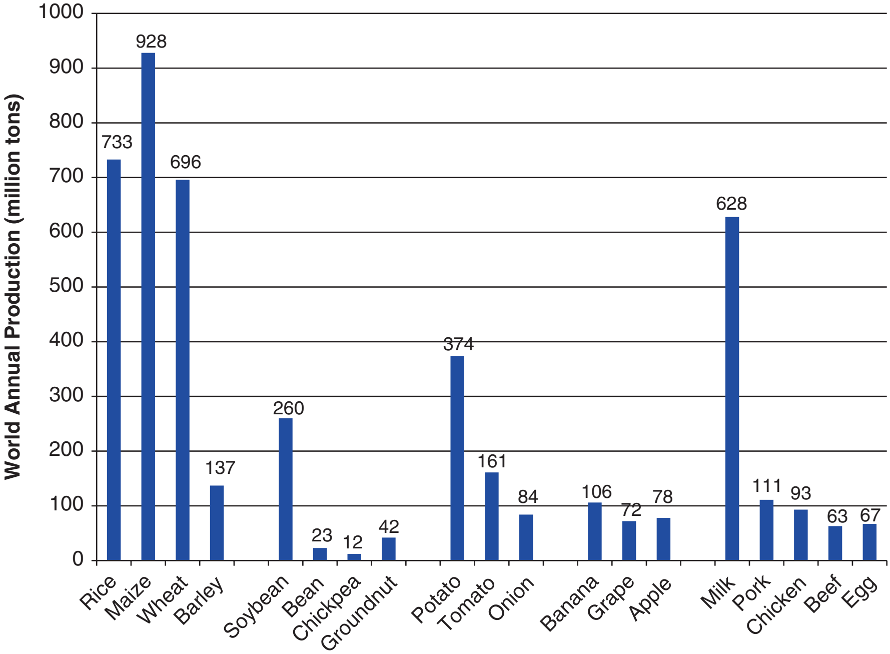

Fig. 1.1 World annual production of major foods (Average 2011–2013) (Data from MAFF, Japan based on FAOSTAT (2015.3))

people in 2015 and 9.7 billion people in 2050 will need 7.4×10^{12} and 10.9×10^{12} M cal per year, respectively.

As shown in Fig. 1.1, maize (928 million tons/year averaged over 2011–2013), rice (733), wheat (696), and barley (137) are major cereals, which provide mainly carbohydrates and starch, as does potato (374). Soybean (260) provides protein and oil, and beans (23), chickpeas (12) and groundnuts (42) are the major legume crops. Vegetables, such as tomato and onion, and fruits, such as banana, grape, and apple, are major foods. Regarding animal-derived foodstuffs, milk (628) production is the highest, followed by pork (111), chicken (93), egg (67) and beef (63). Although the production and consumption of dairy foods and meats are increasing rapidly, the world total production of cereals still far exceeds that of animal products. This is partly because relatively poor people in Asia and Africa depend mostly on plant products rather than animal products, and also because the requirement of cereals for animal feed is also increasing.

Proteins in our body are in a state of homeostasis, which means proteins are broken down constantly, and almost the same amount of protein is newly synthesized. Therefore, although the total protein content in our body is relatively constant, we need to take in a certain level of protein daily. It was estimated that every Japanese person needs 0.65 g protein (kg body weight)$^{-1}$ day^{-1}, which is equivalent to 39 g protein for a 60 kg person each day, i.e., 14.2 kg per year. The world's population

Table 1.4 Comparison of nutrient contents in 100 g edible part of major crops, vegetables, fruits, dairy foods and meets

Food	Energy kcal	Water g	Protein g	Lipid g	Carbohydrate g	Minerals g
Rice	350	15.5	6.8	2.7	73.8	1.2
Maize	350	14.5	8.6	5.0	70.6	1.3
Wheat	337	12.5	10.6	3.1	72.2	1.6
Barley	341	14.0	10.9	2.1	72.1	0.9
Soybean	417	12.5	35.3	19.0	28.2	5.0
Bean	336	15.5	23.9	2.0	55.0	3.6
Chickpea	374	10.4	20.0	5.2	61.5	2.9
Groundnut	562	6.0	25.4	47.5	18.8	2.3
Potato	76	79.8	1.6	0.1	17.6	0.9
Tomato	19	94.0	0.7	0.1	4.7	0.5
Onion	37	89.7	1.0	0.1	8.8	0.4
Banana	86	75.4	1.1	0.2	22.5	0.8
Grape	59	83.5	0.4	0.1	15.7	0.3
Apple	54	84.9	0.2	0.1	14.6	0.2
Milk	67	87.4	3.3	3.8	4.8	0.7
Pork (loos)	291	58.0	18.3	22.6	0.2	0.9
Chicken (thigh)	253	62.9	17.3	19.1	0	0.7
Beef (chuck roll)	318	56.4	16.2	26.4	0.2	0.8
Egg	151	76.1	12.3	10.3	0.3	1.0

(Modified from All Guide, Standard Tables of food composition in Japan (2009))

thus requires about 100 million tons of protein annually, either from meat, fish, milk, egg, beans or cereals.

Table 1.4 compares the compositions of major foods per 100 g edible parts (All Guide, Standard Tables of Food Composition in Japan 2009). In rice grains, carbohydrate, mainly starch, is the major component, accounting for 74% of grain weight. The carbohydrate contents in grains are similar in maize, wheat, and barley. Unlike cereal grains, starch is usually not present in mature soybean seeds. The composition of soybean seeds produced in Japan is as follows (per 100 g): energy 417 kcal, water 12.5 g, protein 35.3 g, lipids 19.0 g, carbohydrate 28.2 g, and minerals 5 g. The composition, especially the higher protein content, is quite different from cereal grains. Other legume crops, such as bean, chickpea, and groundnut, also contain relatively high protein content (20–25 g) compared with cereals, although less than in soybean. The lipid content in groundnut (47.5 g) and soybean (19 g) is much higher than in cereals grains (2–5 g).

Soybean seed is one of the most important protein sources for both humans and livestock. The amino acid composition of soybean seeds is relatively well balanced from amino acid scores, although soybean seeds contain a relatively low amount of the sulfur-containing amino acids, methionine and cysteine (Fig. 1.2) (Ohyama et al. 2013a). On the other hand, rice protein contains higher levels of methionine

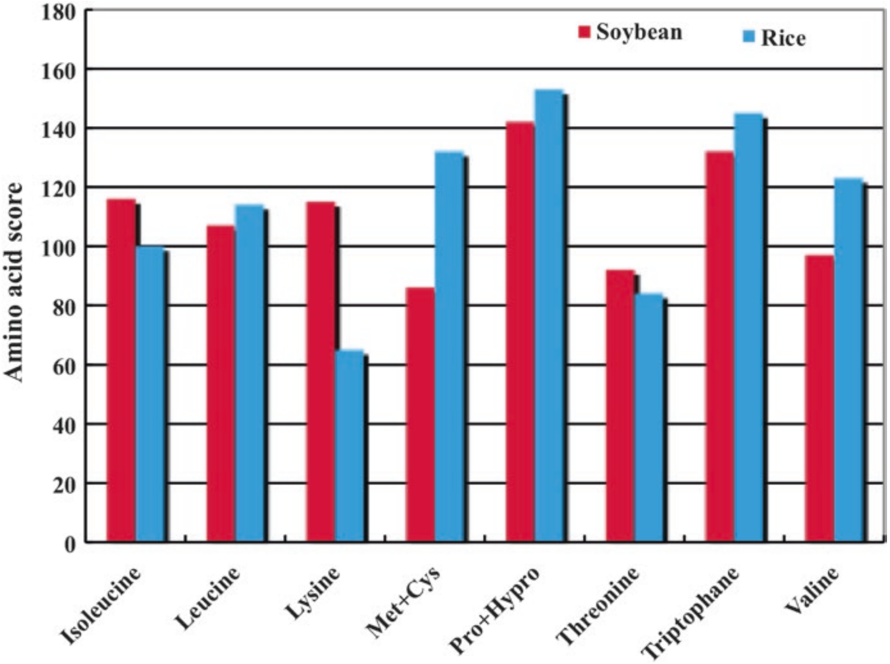

Fig. 1.2 Comparison of amino acid scores of essential amino acids in soybean seeds and rice grain (Figure from Ohyama et al. (2013a))

and cysteine, in spite of a low level of lysine. Therefore, by eating soybean and rice together, the balance of amino acid composition is compensated, and becomes very good.

1.1.3 Food Security

"Food security" is defined as "access for all, at all times, to a sustainable affordable supply of nutritionally adequate and safe food for normal physical and mental development and healthy, productive life" (Vorster 2009). Conversely, "food insecurity" is the state in which people live with hunger and fear starvation. To ensure food security, it is important to supply safe foods continuously. Being free from contamination of harmful microorganisms, toxins and other hazardous substances that cause disease is also important for food safety (Vorster 2009).

FAO estimates indicate that 12.5% of the world population (868 million people) is undernourished in terms of food energy intake, 26% of the world's children are stunted, 2 billion people suffer from micronutrient deficiencies, and 1.4 billion people are overweight or obese (FAO 2013).

Many complex and interacting factors contribute to malnutrition on different levels (Vorster 2009):

1. "Individual level" related to food and nutrient intake, health status, growth, and physical activity.
2. "Household level" related to family size and composition, gender equity, food distribution in household, income, availability of food, and access to food.
3. "National level" related to health, education, sanitation, agriculture, war, political instability, urbanization, population growth, natural disasters, and decreased resources.
4. "International level" related to social, economic and political structures, trade agreements, population size, population growth distribution, and environmental degradation.

World demand for crops is increasing due not only to global population growth, but also to increased biofuel production, and changing dietary preferences, with a move towards meat and animal products rather than plant foods (Cassidy et al. 2013). According to a 2011 analysis, 75% of all agricultural land, including crop and pasture land, is dedicated to animal production. Currently, 36% of the calories produced by the world's crops are being used for animal feed, and only 12% of those feed calories ultimately contribute to the human diet (Cassidy et al. 2013). The calorie conversion efficiency of milk, eggs, chicken meat, pork, and beef are 40%, 22%, 12% 10% and 3%, respectively, and the protein conversion efficiency of milk, eggs, chicken meat, pork, and beef are 43%, 35%, 40%, 10% and 5%, respectively (Cassidy et al. 2013).

A sufficient supply of sustainable food is essential in each country. Food balance sheets in 2003 showed that the self-sufficiency rates of grain [percentage of grain production per total amount of (production + import − export)] were highest in Australia (333%) and Argentina (249%), while Thailand (162%), Myanmar (131%), Vietnam (127%), Laos (123%), and Cambodia (122%) were relatively high in Asia. China (100%), India (98%), Indonesia (89%), and the Philippines (82%) are well balanced. However, Japan (27%) and Korea (28%) were in a critical situation (Ohyama et al. 2013a). Meat consumption increases rapidly in developing countries. However, meat production requires a large amount of crops. The production of 1 kg chicken, pork and beef requires about 2, 5 and 8 kg crops, respectively. Therefore, it is important that humans depend directly on protein from plant sources rather than from animal sources in order to provide sufficient food for the increasing population.

FAO and ITPS (Intergovernmental Technical Panel on Soils) reported the Status of the World's Soil Resources in 2015—the International Year of Soils (FAO and ITPS 2015). This is of particular relevance to the Sustainable Development Goals that the international community has pledged to achieve. They reported that 33% of land today is moderately-to-highly degraded due to erosion, salinization, compaction, acidification, and chemical pollution of soils (FAO and ITPS 2015). Sustainable soil management is a key foundational concept of this report, and is defined as follows: "Soil management is sustainable if the supporting, provisioning, regulating, and cultural services provided by soil are maintained or enhanced without signify impairing either the soil functions that enable those services or biodiversity". In the report, the following actions have the highest priority: (1) Sustainable soil management can increase the supply of healthy food for the most food insecure people among us; (2) the global stores of soil organic matter should be stabilized or increased, and (3) humanity is close to the global limits for total chemical nitrogen fertilizer and regional limits for phosphorous use. Therefore, we should act to stabilize or reduce global nitrogen (N) and phosphorus (P) fertilizer use, while simultaneously increase fertilizer use in regions of nutrient deficiency.

1.2 N Chemical Fertilizers

1.2.1 N as a Major Essential Element of Plants

Most higher plants are photoautotrophs, and can synthesize all the compounds they need from carbon dioxide, water, light energy and inorganic nutrients absorbed from the roots. Aside from nutrient requirements, plants also need essential elements, which are classified as macro-elements (N, K, Ca, Mg, P and S), and microelements (Cl, Fe, Mn, B, Zn, Cu, Mo and Ni).

N is a major essential element for all organisms, being a main constituent of proteins, nucleic acids, and other indispensable organic compounds. Despite the high concentration of N_2 (about 78% by volume) in air, its concentrations in soil, rocks and sea water are relatively low, and the availability of N is often a limiting factor for crop production and natural plant growth. Major forms of inorganic N in soil are ammonium and nitrate, which plants absorb from their roots. Some plants, e.g., legumes, can fix atmospheric N_2 symbiotically in association with N_2-fixing soil bacteria, rhizobia.

1.2.2 Global N Cycling

N is cycled globally on land, in the atmosphere and in the ocean (Matsushima et al. 2010). Although the concentration of N_2 in air is high, all "eukaryotes", including plants, animals, and fungi, cannot use it by themselves. Only a wide variety of "prokaryotes", which have enzymes of the "nitrogenase complex" can fix N_2 to ammonia, and thus use N for the synthesis of organic compounds. There are two types of "biological N_2 fixation" (BNF) in agricultural systems, crops, pastures and fodder, as well as natural ecosystems (Herridge et al. 2008): free-living BNF, and symbiotic or plant-associated BNF.

As shown in Fig. 1.3, the total amount of annual global BNF was estimated at about 122 T gN (= million tons of N), of which 50–70 T gN is estimated to be present in agricultural systems (Herridge et al. 2008). About 100 T gN is synthesized

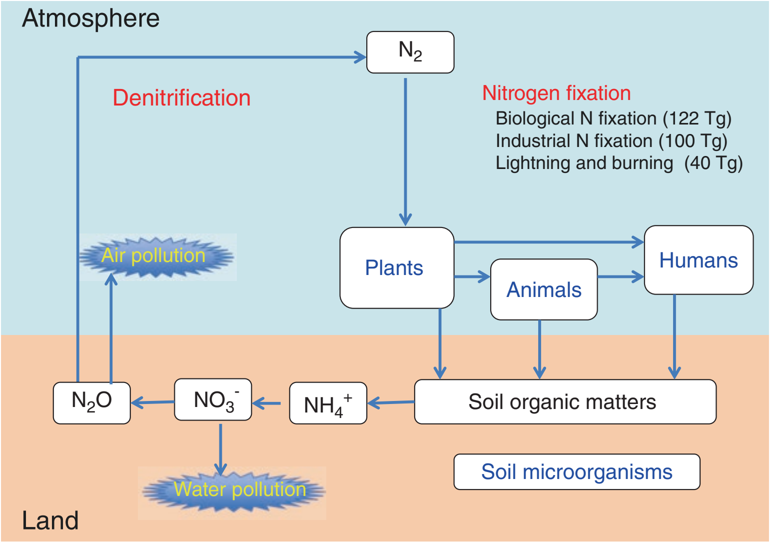

Fig. 1.3 Global nitrogen cycle

artificially by the Harbor-Bosch process, of which 80 T gN is used for N fertilizers. Due to lightning and burning of fuel, atmospheric N_2 is oxidized to NO_x; the total amount of N contributed from these processes is estimated to be 40 T gN. Plants can use the fixed N in the form of either ammonia or nitrate, and animals depend on the organic matter produced by plants. Humans eat foods originating from plant or animal products. The waste and dead bodies of organisms are broken down mainly by soil microorganisms, and the residual soil organic matter—humus—is deposited in the soil. Soil organic matter is eventually decomposed to minerals by soil microorganisms, and ammonia or ammonium (NH_4^+) is released through the mineralization process; NH_4^+ is readily oxidized to nitrate (NO_3^-) by nitrifying bacteria in nitrification processes in upland fields. And some part of NO_3^- is reduced to nitrous oxide (N_2O) by denitrifying bacteria, and finally reduced to N_2. The N cycle is related to environmental issues, e.g., NO_3^- elution to underground water, rivers and pond water, which may cause water pollution, and emission of N_2O—a potent global warming gas (Matsushima et al. 2010)—causes air pollution. Heavy use of N chemical fertilizers may aggravate these serious environmental problems.

1.2.3 *Chemical N Fertilizers*

Since human beings started agriculture about 10,000 years ago, maintenance of soil fertility has been of great concern to farmers and landowners, because sustainable crop production depends on soil fertility. Until the early 1800s, farmers used mainly livestock excreta or plant residues as fertilizers, or used crop rotation agriculture to recover or maintain soil fertility. In 1876, Liebig elucidated the "mineral element theory" in which he proposed that plants take up minerals for their food, and this theory became widely accepted (Marschner 1995). Liebig's achievements led to a rapid increase in the use of mineral fertilizers, such as Chili saltpeter and superphosphate, in the nineteenth century. Superphosphate was the first industrial fertilizer, and was produced by Lawes and Gilbert in 1843. Around 1910, the Haber-Bosch method for producing ammonia from N_2 and H_2 was established, and chemical N fertilizers could be produced inexhaustibly.

The 1960s saw a dramatic increase in agricultural production—the so-called "the Green Revolution". This success was due mainly to the adaptation of modern crop cultivars that had superior lodging resistance by semi-dwarf trait, and these responded to applied N fertilizers more intensively than did traditional cultivars (Matsushima et al. 2010). As a result of the Green Revolution, the yield of major cereal crops became several times higher than that of previous traditional farming to meet the food demands of an increasing world population.

However, people began to notice that there are both advantages and disadvantages to the use of chemical fertilizers compared with organic fertilizers or compost.

The advantages of chemical fertilizer are as follows (Gowariker et al. 2009):

1. Individual nutrients, such as N, P, or K, can be supplied separately by chemical fertilizers depending on crop requirements and nutrient availability in the soil. Organic fertilizers contain a wide range of nutrients.
2. The effect of chemical fertilizer is generally rapid after its application, because they are usually water-soluble chemical compounds. Organic fertilizers release nutrients by decomposition of organic compounds by soil microorganisms, thus nutrient availability is usually slow.
3. The concentrations of nutrients in chemical fertilizers are very high, ~100 times higher than in organic fertilizers. Thus, small amounts of chemical fertilizers can provide sufficient nutrients. Also, chemical fertilizers are easy to apply, either manually or by using agricultural machines. The cost of labor and transportation are lower than with organic compost.
4. Chemical fertilizers are relatively clean and odorless, and they do not contain toxic compounds such as heavy metals, pesticides, harmful pests, insects or weed seeds, which some organic composts may contain.
5. Chemical fertilizers are chemically stable, and can be stored for a long time if kept in plastic bags.

Disadvantages of chemical fertilizers are as follows:

1. Production of chemical fertilizers, especially N fertilizer, requires a large amount of energy consumption derived from fossil fuels. This causes the increase in CO_2 concentration in the air, resulting in global warming.
2. Some of chemical fertilizers originate from mineral resources, such as phosphate rock and potash ore, and the estimated amounts of affordable reserves are limited, especially for phosphate rock.

Table 1.5 The consumption of nitrogen fertilizer (T gN) in the world and Asian countries

		Consumption	(T gN)	
Major area	1961	1971	2002	2012[a]
World	11.6	33.5	86.5	119.7
Americas	3.58	8.97	17.5	23.6
Europe	5.49	15.43	13.1	14.1
Oceania	0.04	0.14	1.3	1.4
Africa	0.35	0.95	2.6	3.3
Asia	2.12	8.04	52.0	77.3
China	0.54	3.37	29.1	45.1
India	0.25	1.80	10.5	16.8
Japan	0.63	0.68	0.53	0.43

(Modified from Matsushima et al. Global nitrogen cycling and its availability from soil, nitrogen assimilation in plants 2010). 2012[a] (FAOSTAT 2016)

3. Dependence on chemical fertilizers may cause a decrease in the input of organic matter to agricultural fields. This may lead to a decrease in soil organic matter, soil fertility, and thus to a deterioration in the physical, chemical and biological properties of the soil.
4. Excess or inappropriate use of chemical fertilizers cause environmental problems, such as NO_3^- pollution in ground water, eutrophication of rivers, lakes and oceans, and air pollution by nitrous oxides such as N_2O, which also cause global warming.
5. Chemical fertilizers are expensive, especially for small farmers in poor developing countries.

As shown in Table 1.5, the total consumption of chemical N fertilizers increased rapidly from 11.6 T gN in 1961 to 120 T gN in 2012. In Europe and Japan, consumption became stable or decreased after 1971 due to efforts to decrease excess and inefficient use of chemical N fertilizers. However, most other areas increased their use of N fertilizers. The amount of consumption varies widely, and is much lower in Africa and Oceania compared with Asia, the Americas, and Europe. To buy N fertilizer is economically difficult for small farmers in poor areas experiencing population explosions, e.g. as in areas of Africa and Asia. The high price of N fertilizer depends on production costs, due mainly to fossil fuel required to generate the high temperature and high-pressure conditions needed to react N_2 and H_2, as well as producing H_2 gas from fossil oils. The cost also reflects packaging, storage, transportation, and profit margins for the producing and trading companies.

The price of N fertilizers remained relatively stable from 1975 to 2000, but increased dramatically (~3-fold) from about $ 200 per ton in 2000 to about $ 600 per ton in 2013 (Fig. 1.4) (Schnitkey 2014). Poor family farmers cannot afford to buy N fertilizers at such a high price.

To avoid the excess use of N fertilizer, one of the best ways to save on cost and avoid environmental problems without decreasing crop yield is to increase “N use efficiency”. Takahashi et al. reported that the recovery rate of the basal dressing of

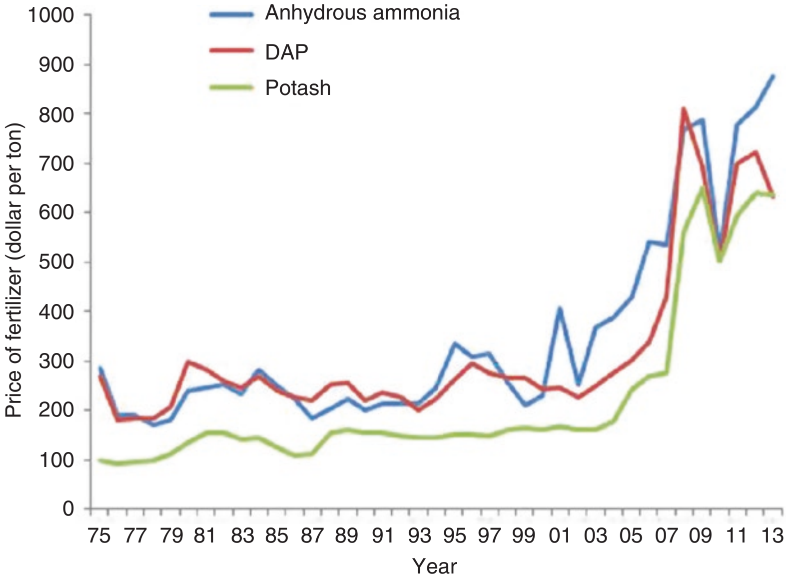

Fig. 1.4 Fertilizer prices, spring of year, 1970–2013 (Figure from Schnitkey (2014), Farmdoc daily Homepage)

chemical N fertilizer was only 10% in soybean cultivation using ^{15}N labeled ammonium sulfate, but that the basal deep placement of coated urea—a controlled release N fertilizer—was 62% (Takahashi et al. 1992). The recovery rate is widely affected by timing, placement, amount of application, and the form of N fertilizers. An optimum fertilizer treatment should be devised for each crop for each specific area and soil conditions. An alternative way of saving on chemical fertilizer is to use BNF. Symbiotic N_2 fixation by legume plants and rhizobia is the most potent BNF system available.

1.3 Legume Nodule Symbiosis

1.3.1 Legume Nodule Symbiosis: The Most Important N Source in Agroecosystems

As shown in Fig. 1.3, the total amount of BNF per a year is estimated at about 122 T gN, and is equal to, or greater than, the annual production of industrial N fixation (100 T gN). Therefore, BNF is still the most important N source in agroecosystems. The most important N_2-fixing agents in agricultural systems are the symbiotic associations between crop and forage/fodder legumes and rhizobia (Herridge et al. 2008). Herridge et al. calculated annual inputs of fixed N to be 18.5 T gN for oilseed legumes and 2.95 T gN for pulses. They calculated soybean to fix 16.4 T gN annually, representing 77% of the N fixed by crop legumes. The estimates of annual N_2 fixation inputs except legumes are 12–25 T gN by pasture and fodder legumes, 5 T gN by rice, 0.5 T gN for sugarcane, <4 T gN by non-legume crops, and <14 T gN by extensive savannas (Herridge et al. 2008).

The advantages and disadvantages of symbiotic N_2 fixation for agriculture are as follows (Zahran 1999):

Advantages (Peoples et al. 2009; Giller and Cadisch 1995):

1. Unlike expensive chemical fertilizers, symbiotic N_2 fixation is low cost, indeed usually free, for farmers. When efficient indigenous rhizobia already dominate in the soil, farmers do not need to use any agricultural materials to establish nodule formation and N_2 fixation. When legume crops are planted on the virgin land, the inoculation of compatible rhizobia may be necessary to establish the legume-rhizobia symbiosis. The rhizobia inoculant is available commercially in many countries as biofertilizers (FNCA biofertilizer manual 2006) at relatively low cost compared with chemical N fertilizers.
2. Nitrogen use efficiency is very high, at nearly 100% for plant growth. Most of the N fixed by root nodules is assimilated and transported to host plant shoots through xylem vessels to support vegetative and reproductive growth, and the N_2 fixation rate is controlled to meet plant N demands. A small part of the fixed N may be lost by nodule degradation, root exudation or plant senescence as plant residues, but this N increases soil fertility by remaining in the soil.
3. Because of the high N use efficiency of symbiotic N_2 fixation, nitrification of fixed N is very low and NO_3^- leaching and denitrification loss are negligible in agricultural land (Hungria and Mendes 2015). Therefore, symbiotic N_2 fixation is an ecologically friendly measure.

Disadvantages:

1. It is difficult to control the rhizobium strains, especially the occupancy of principal indigenous rhizobia that has already been established (Deaker et al. 2015). When a selected strain is inoculated onto seeds, it will produce some nodules around the basal part of the primary roots, but most nodules formed in the lateral roots are infected by indigenous strains already present in the soil. Furthermore, the newly inoculated strain is easily lost after a few years (Ohyama et al. 2013b).
2. Symbiotic N_2 fixation is a physiologically high cost system that depends on the high consumption of photoassimilates compared with N absorption from roots. Therefore, N_2 fixation alone will result in poor growth and low yield. Symbiotic N_2 fixation will also suffer severe damage under stress conditions, such as drought or water logging, compared with N absorption from roots.
3. Application of a large amount of chemical N fertilizer causes decreased nodule mass and N_2 fixation activity. So, unlike in cereal crops, no dramatic increase in legume yield can generally be expected upon the application of chemical N fertilizers.

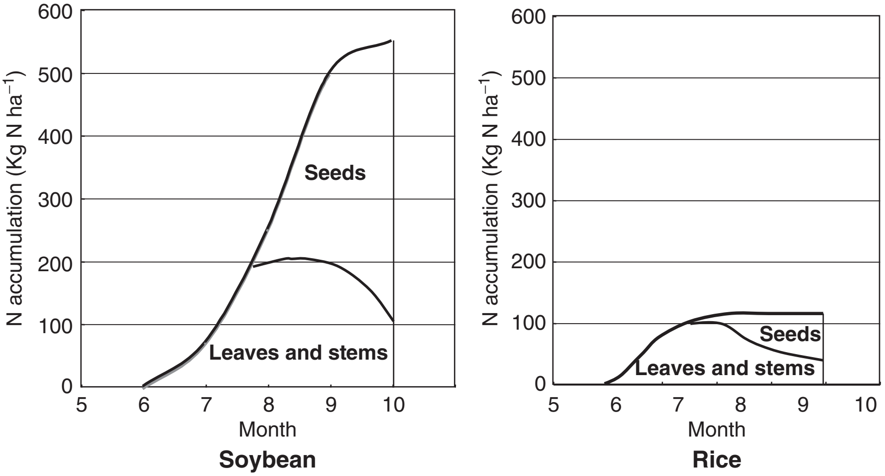

Fig. 1.5 Comparison of N accumulation in soybean and rice plant during vegetative and reproductive stages (Figure from Ohyama et al. (2013b))

1.3.2 N Metabolism and Seed Yield of Legume Crops

Legume crops such as soybean plants assimilate a large amount of N during both vegetative and reproductive stages of growth, and the total amount of N assimilated in a plant is highly correlated with soybean seed yield. One t of soybean seed requires about 70–90 kg N, which is about four times more than in the case of rice (Fig. 1.5) (Ohyama et al. 2013b).

Soybean plants assimilate N from three sources: (1) N derived from symbiotic N_2 fixation by root nodules (Ndfa), (2) N absorbed from soil mineralized N (Ndfs), and (3) N derived from applied fertilizer (Ndff) (Fig. 1.6) (Ohyama et al. 2009). For maximum seed yield of soybean, it is necessary to use both N_2 fixation and absorbed N from roots (Harper 1974, 1987). When only N_2 fixation is available to the plant ,vigorous vegetative growth does not occur, which results in reduced seed yield. On the other hand, a heavy supply of N often severely depresses nodule development and N_2 fixation activity and induces nodule senescence, which also results in reduced seed yield. In addition, a heavy supply of N from fertilizer or from the soil causes luxuriant shoot growth, which results in lodging and poor pod formation. Therefore, in Japan, for soybean cultivation, no, or only a small amount of, N fertilizer is applied as a “starter N” to promote initial growth.

The protein concentration in soybean seeds is about 4 times higher than in cereal seeds such as rice grains (Table 1.4). Soybean plants assimilate about 20% of total N until the initial flowering stage, and 80% of N during the reproductive stage (Fig. 1.5). On the other hand, rice assimilates about 80% of N until flowering. Therefore, the continuous assimilation of N after the initial flowering stage is essential for good growth and high seed yield in soybean cultivation (Ohyama et al. 2013b).

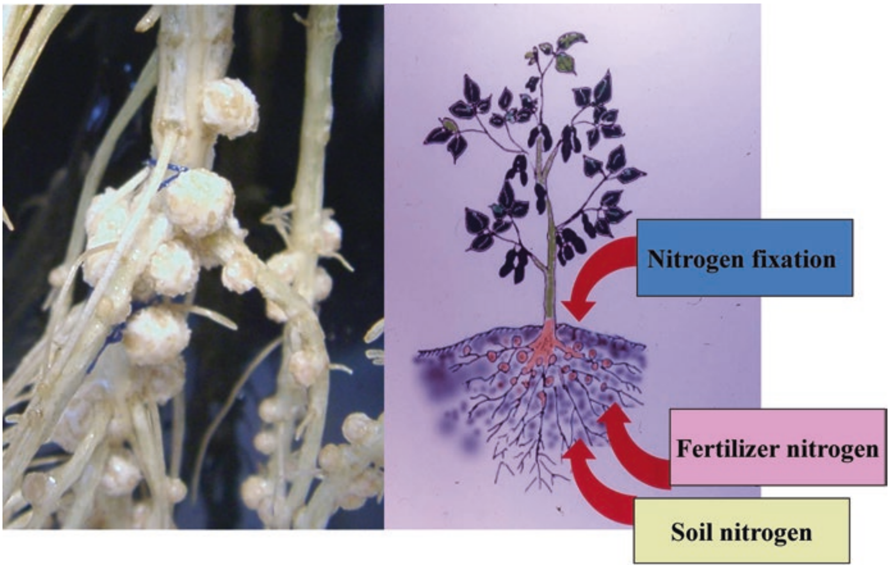

Fig. 1.6 Nodulated soybean roots cultivated with hydroponics, and three sources of N for soybean plants (Figure from Ohyama et al. (2009))

To obtain high seed yield of leguminous crops such as soybean, good nodulation and high (and long-lasting) N_2 fixation activity are essential. Nodule formation and nodule growth are influenced by various soil (water content, pH, mineral nutrition) and climatic (solar radiation, temperature, rainfall, etc.) conditions. In addition, legumes can absorb inorganic N, such as nitrate (NO_3^-) and ammonium (NH_4^+) from soil or fertilizers. Usually, high yield of soybean is obtained in fields with high soil fertility. By supplying a constant but low concentration of N either from soil or organic manure, good soybean growth will occur without depressing nodulation and N_2 fixation activity. However, it is well known that a high concentration of mineral N depresses nodule formation and N_2 fixation activity. Especially, NO_3^-, the most abundant inorganic N in upland soils, rapidly and reversibly inhibits nodulation and N_2 fixation of soybean, when nodulated roots are in direct contact with soil solution containing NO_3^- (Fujikake et al. 2003; Ohyama et al. 2011).

1.3.3 Symbiotic N_2 Fixation Related to Other Minerals

Seventeen elements are essential for the growth and reproduction of plants (Kovacevic et al. 2011). Whereas carbon (C) and oxygen (O) are derived from air, and hydrogen (H) from water, other elements are derived mainly from soils. The primary macronutrients are N, P and potassium (K), and secondary macronutrients are sulfur (S), calcium (Ca) and magnesium (Mg). Micronutrients are iron (Fe),

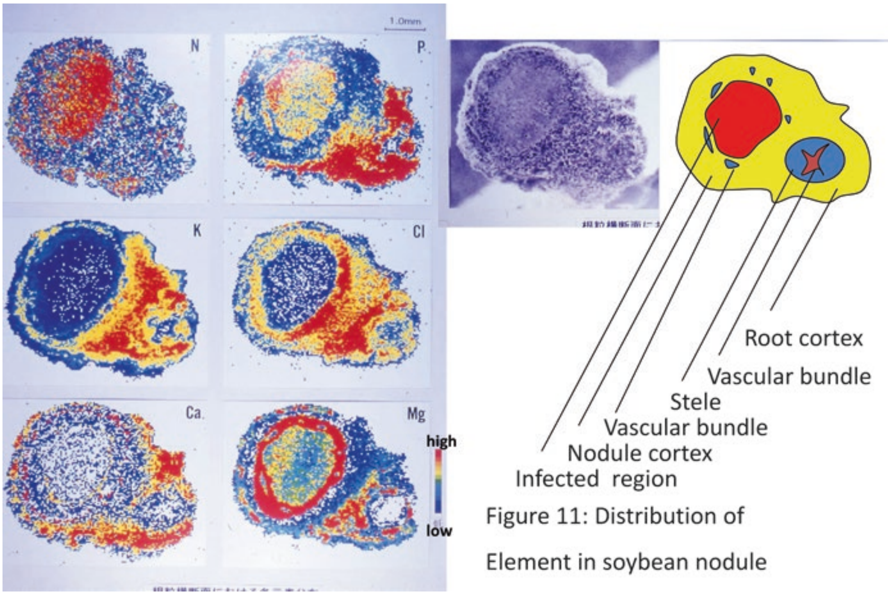

Fig. 1.7 Distribution of various minerals in nodulated roots of soybean (Figure from Ohyama et al. (2009))

manganese (Mn), boron (B), zinc (Zn), copper (Cu), molybdenum (Mo), nickel (Ni) and chlorine (Cl). Nutrient removal of one ton of soybean grain and corresponding biomass are ~100 kg N, 23–27 kg P_2O_5, 50–60 kg K_2O, 13–15 kg CaO, and 13–16 kg MgO (Kovacevic et al. 2011).

Soybean nodules are highly organized complex organs, as shown by the distribution of minerals examined by EPMA (Electron Probe X-ray Microanalysis) (Mizukoshi et al. 1995). Figure 1.7 shows the distribution of minerals in nodulated roots. The concentrations of N and P are higher in the central symbiotic region compared with the surrounding nodule cortex. Conversely, K, Cl and Ca are low in the central region of the symbiotic zone compared with the nodule cortex. Mg accumulates specifically in the inner and outer cortex inside sclerenchyma cells but not outside.

A sufficient supply of P is known to be important for symbiotic N_2 fixation in legume nodules. Concerning P application, Jabbar and Saud (2012) reported that the number and dry weight of soybean nodules are increased significantly by higher P application, up to 120 K gP/ha, under salt stress conditions. Tsvetkova and Georgiev (2003) reported that P deficiency treatment (0.1 mM phosphate) and an excess supply of phosphate (3 mM) in hydroponic soybean culture decreased the whole plant fresh and dry mass, and nodule weight, number and N_2 fixation activity compared with the optimum concentration of 1 mM.

1.3.4 Factors Affecting Soybean Yield

Soybean plants are very susceptible to environmental factors, such as climate and soil conditions. N_2 fixation by root nodules is very important for soybean growth and seed yield; however, it is difficult to obtain optimum conditions for nodulation and N_2 fixation, because nodulation and N_2 fixation are more susceptible to biotic and abiotic environmental factors than root growth and N absorption. Nodule formation and N_2 fixation are sensitive to external factors such as climate, soil properties, pests, etc., and internal factors such as competition among neighbor plants or competition among organs, pods, leaves, roots and nodules within a plant. Therefore, many stress conditions, such as drought stress, decrease in oxygen supply due to water logging, high or low pH, nutrient imbalance etc., may depress nodule formation and N_2 fixation activity. In addition, low population density of compatible bradyrhizobia, or the dominance of inefficient strains of indigenous bradyrhizobia in the field may decrease N_2 fixation activity. Inoculation of efficient strains of bradyrhizobia may promote soybean growth and seed yield.

Global warming may influence legume crop cultivation, including symbiosis with rhizobia (Sulieman and Tran 2015). From the Fifth Assessment Report of the United Nations Intergovernmental Panel on Climate Change (IPCC), warming of the atmosphere and ocean system is unequivocal, and there is a clear human influence on the climate (IPCC Fifth Assessment Report 2014). It was predicted that the global surface temperature increase by the end of twenty-first century is likely to exceed 2–4 °C relative to the late twentieth century. The key risks of global warming are identified with high confidence, span sectors and regions; such as (1) risk of death, injury, ill-health, or disrupted livelihoods in costal zones and small islands due to storm surges, coastal flooding, and sea level rise; (2) risk of severe ill-health for urban population due to inland flooding; (3) systemic risks due to extreme weather events leading to breakdown of infrastructure and critical services; (4) risk of mortality and morbidity during periods of extreme heat; (5) risk of food insecurity and the breakdown of food systems linked to warming, drought, flooding, and precipitation variability and extremes, particularly for poorer populations in urban and rural settings; (6) risk of loss of rural livelihoods and income due to insufficient access to drinking and irrigation water and reduced agricultural productivity, particularly for farmers and pastoralists with minimal capital in semi-rid regions; (7) risk of marine and coastal ecosystems and biodiversity; and (8) risk of loss of terrestrial and inland water ecosystems and biodiversity (IPCC Fifth Assessment Report 2014).

Concerning food security and food production systems, the IPCC report predicted that all aspects of food security are potentially affected by climate change, including food access, utilization, and price stability. For the major crops (wheat, rice and maize) in tropical and temperate regions, climate change without adaptation is projected to negatively impact production for local temperature increases of 2 °C or more above late twentieth century levels, although individual locations may benefit.

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