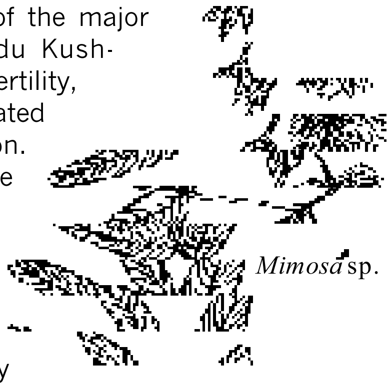
Chapter 1

Nitrogen-Fixing Plants

INTRODUCTION


Mimosa sp.

Environmental degradation is considered to be one of the major obstacles to sustainable development in the Hindu Kush-Himalayan (HKH) region. Soil erosion, declining soil fertility, declining land productivity, deforestation – and associated out migration – are problems seen across the region. Agriculture provides the major, and often only, source of livelihood of around 80% of the HKH population, but is becoming increasingly unsustainable as a result of increasing population pressure and degradation of the existing agricultural resources. Unsustainable agricultural development is one of the root causes of poverty in the region; and achieving the goal of poverty reduction will depend to a large extent on achieving sustainable improvement in agricultural practices. Tackling the problems of declining soil fertility, soil erosion, and lack of cash income is becoming ever more urgent.

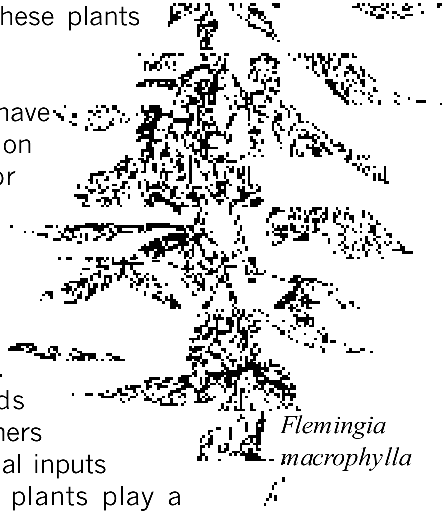
Nitrogen is a key component of protein and other important plant compounds, but it is often the most deficient nutrient in the soil, limiting plant growth and contributing to reduced agricultural yields throughout the world. The deficiency in soil nitrogen results from continual loss by such processes as microbial denitrification, soil erosion, leaching, chemical volatilisation, and – perhaps most important – through the removal of crops and residues from cropland. The nitrogen in agricultural soils needs to be replenished periodically to maintain an adequate level for crop production. Although the earth's atmosphere contains about 78% nitrogen, plants are usually unable to use this nitrogen directly. Nitrogen-fixing plants are the exception; these plants have an unusual advantage in being able to obtain gaseous nitrogen through the action of symbiotic nitrogen-fixing microorganisms that most commonly live within a specialised nodule on the roots of the plants. These microorganisms can take nitrogen in the form of N_2 from the air and convert it into the form that plants can use.

In former times, nitrogen levels were maintained in agricultural soils by addition of manure, use of careful crop rotation and co-cultivation systems including nitrogen-fixing plants, and ploughing in of crop residues, but in modern farming systems it is more commonly accomplished by adding chemically fixed nitrogen in the form of commercial inorganic fertilisers. Worldwide, the consumption of fertiliser nitrogen increased from 3.5 million metric tonnes in 1950 to 80 million metric tonnes in 1989 (TFI 1990), and 82 million metric tonnes in 2001/02 (TFI 2004). China and India, two of the ICIMOD member countries, rank first and third worldwide, respectively, in annual consumption of chemical fertiliser nitrogen (FADINAP 2004).

Although easy to apply, chemically fixed nitrogen fertiliser has many disadvantages compared to natural fertiliser. Chemical fertiliser nitrogen is used inefficiently by crops; in the HKH the efficiency of use varies from as low as around 30% in China (Peng 1999) to about 50% on average. In contrast to this, biologically fixed nitrogen can be used completely by crops. This means that about two metric tonnes of industrially fixed nitrogen at least are needed as fertiliser for crop production to equal the effects of one metric tonne of biologically fixed nitrogen. The vast amounts of chemically fixed nitrogen fertiliser used in the world are leading to an imbalance in the global nitrogen cycle, pollution of groundwater (sometimes to an alarming degree) and an increase in the cost of production inputs, among others.

The total annual terrestrial input of nitrogen from biological nitrogen fixation (BNF) is estimated to be somewhere between 139 and 170 million tonnes (Burns and Hardy 1975; Paul 1988 [cited in Peoples, Herridge, and Ladha 1995]; Pimentel et al. 1997; Ishizuka 1992). BNF is a significant factor in natural N cycles and is estimated to provide some 65% of the nitrogen currently used in agriculture. BNF nitrogen has considerable advantages in comparison with chemically fixed nitrogen, in particular that all of the biologically fixed nitrogen can be assimilated so that there is no groundwater pollution, and that it is cost free – and freely available as long as the appropriate plants can be grown. BNF nitrogen is expected to become increasing important in future crop productivity, particularly for sustainable systems (Thomas et al. 1997); the significance of BNF as a major mechanism for managing nitrogen recycling in agricultural and forest systems cannot be overemphasised.

In the HKH region, especially, where commercial chemical fertilisers are either unavailable or too expensive for local people to buy, and soil degradation is a major limiting factor in crop production, BNF can play a major role in agricultural development. However, in order to be able to exploit its potential to the full, it is important first to improve our understanding of BNF and its application to agricultural and forestry production. In this book we briefly describe the principles of biological nitrogen fixation, the different types and methods of action of nitrogen-fixing plants, and the major uses of these plants in mountain regions.



Flemingia macrophylla

Most of the technologies described in this book have been demonstrated at the Test and Demonstration Site maintained by the International Centre for Integrated Mountain Development (ICIMOD) at Godavari, near Kathmandu in the mid hills area of Nepal. This site is dedicated to developing, testing, and demonstrating appropriate technologies for sustainable agricultural development in the Hindu Kush-Himalayan region. No chemical fertilisers are used; the methods investigated are intended for use by the small farmers of the region who have little recourse to commercial inputs or capital intensive techniques. Nitrogen-fixing plants play a major role in ensuring the viability of such systems.

Biological Nitrogen Fixation

Biological nitrogen fixation (BNF) is a process in which atmospheric nitrogen is converted to ammonia by the enzyme nitrogenase. Essentially, there are two processes: symbiotic and non-symbiotic. In symbiotic processes, nitrogen-fixing microorganisms are associated with plants either within nodules attached to the plant roots, or in free association with the roots and/or leaves of the plants. In non-symbiotic processes the organisms involved are free-living. Some of the typical organisms involved are listed in Table 1. This publication is mainly concerned with nitrogen fixation by symbiotic processes, in particular by plants that have root nodules.

Table 1: Selected biological nitrogen-fixing organisms (modified from Ishizuka 1992 and Pokhriyal & Mathani 1989)

Symbiotic nitrogen-fixing systems
Legumes with *Rhizobium* and *Brachyzhirobium*
Non-legumes with *Frankia*
Lichens with *Nostoc*
Waterfern *Azolla* with *Anabaena*
Non-nodulated plants with root and leaf associated microorganisms like *Tripsacum* and *Azospirillum*
Non-symbiotic or free-living nitrogen fixers
Obligate aerobes, e.g., *Azotobacter vindelandii*
Facultative anaerobic bacteria, e.g., *Klebsiella pneumoniae*
Obligate anaerobes, e.g., *Clostridium pasteurianum*
Phototrophic bacteria, e.g., *Rhodospirillum rubrum*
Blue-green algae, e.g., *Nostoc muscorum* and *Anabaena cylindrical*

Nitrogen-Fixing Plants

Nitrogen-fixing plants are plants that support biological nitrogen fixation through a symbiotic association between the plants and nitrogen-fixing microorganisms. The great majority of these plants belong to the legume family, but there are also a number of non-leguminous species that can fix nitrogen from the atmosphere.

Legumes and Non-legumes

Legumes are the best known nitrogen-fixing plants, but a few non-leguminous species can also form a symbiotic relationship with microorganisms to fix atmospheric nitrogen. Legumes most commonly form nodules with *Rhizobium* bacteria, while non-leguminous plants form nodules with actinomycetes *Frankia*.

Leguminous species

Legumes are a large family of flowering plants – trees, shrubs, vines, and herbs – that produce podlike fruit (legumes) that split spontaneously along two seams to release the seeds they contain, which are attached along one of the seams. The legume family (or Fabaceae) contains about 650-750 genera with 13,000-18,000 species in three subfamilies: Caesalpinioideae, Mimosoideae, and Papilionoideae. The best known edible legumes are peas and beans (Papilionoideae), but legumes also include such plants as clover, lupins, wisteria, and some brooms (all Papilionoideae), acacia (Mimosoideae), and bauhinia and senna (Caesalpinioideae), among many others. There are both herbaceous and woody legumes; the Caesalpinioideae and Mimosoideae subfamilies are characterised by more woody plants and the Papilionoideae by more herbaceous plants.

Mutualistic symbiosis is a mutually beneficial relationship between two organisms. Symbiotic nitrogen fixation occurs in plants that harbour nitrogen-fixing bacteria within their tissues. Both the host plant and the bacteria can survive independently, but living together is beneficial to both. The host plant provides the bacteria with energy-rich carbohydrates and some other compounds, while the nitrogen-fixing bacteria supply the host plant with nitrogen in the form of ammonia. Nitrogen fixation can only take place when the bacteria are associated with the plant. The best-studied example is the association between legumes and bacteria of the genus Rhizobium.

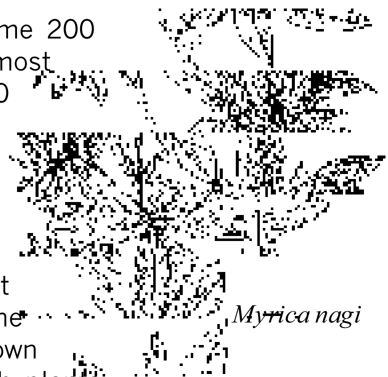
Most leguminous species can form a mutualistic symbiotic association with nitrogen-fixing bacteria, although the proportion in the subfamilies varies. In most cases the bacteria are contained in specialised root nodules, Of some 3,000 leguminous species examined, more than 90% were found to have root nodules – 98% of the Papilionoideae, 90% of the Mimosoideae, and about 30% of the Caesalpinioideae (Allen and Allen 1981). Of the 72 nitrogen-fixing species of Caesalpinioideae found in Allen and Allen's study, 44 were from the genus cassia (Cassia); it seems that most of the other members of the Caesalpinioideae are not nitrogen-fixing. Some of the more common leguminous plant genera in the HKH region are listed in Table 2.

Table 2: Some common leguminous plant genera in the HKH region

Sub-family	Genus	Common name
Caesalpinioideae	*Caesalpinia*	caesalpinia
	Delonix	flamboyant tree
	Gleditsia	honey locust
	Cassia	sena
	Tamarindus	tamarind
	Bauhinia	bauhinia
Mimosoideae	*Albizia*	albizia
	Acacia	acacia
	Leucaena	ipil-ipil
	Mimosa	sensitive plant/mimosa
	Prosopis	mesquite
Papalionoideae	*Sophora*	pogoda tree
	Meliotus	sweet clover
	Medicago	medic
	Trifolium	clover
	Glycine	soya bean
	Cajanus	pigeon pea
	Flemingia	flemingia
	Erythrina	coral bean
	Lespedeza	bush clover
	Campylotropis	clover shrub
	Desmodium	tick clover
	Indigofera	indigo
	Tephrosia	tephrosia
	Milletia	milletia
	Robinia	locust
	Sesbania	sesbania

Non-leguminous species

In addition to the leguminous species, there are some 200 species of non-leguminous nitrogen-fixing plants, almost all of them woody. Most are angiosperms – about 170 species of 17 genera in 8 families; typical species include alder, beefwood, and bayberry. There are also a few nitrogen-fixing gymnospermous species, especially members of the Cycadaceae, such as cycads (*Cycas, Encephalartos, Macrozamia*), and some other plants like podocarpus (*Podocarpus*). The most common non-leguminous nitrogen-fixing plants in the HKH region are listed in Table 3. Although less well-known than the legumes, they include some important cash plants like seabuckthorn (*Hippophae rhaminoides*) and elaeagnus (*Elaeagnus* spp.) which can play an important role in improving mountain livelihoods.



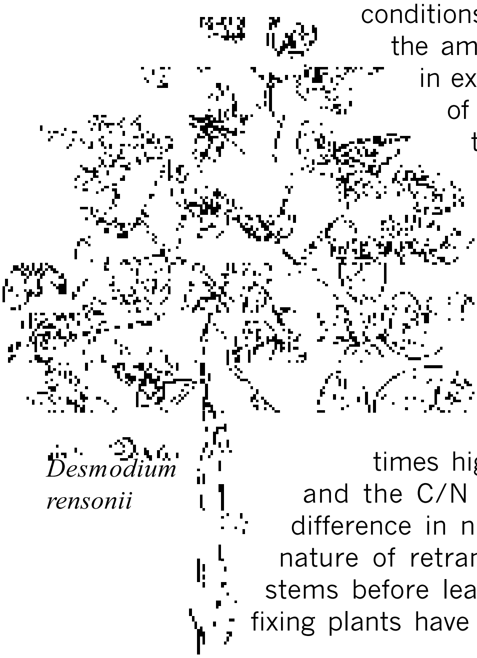
Myrica nagi

Table 3: Some common non-leguminous nitrogen-fixing plant genera in the HKH region		
Family	Genus	Common name
Betulaceae	*Alnus*	alder
Elaeagnaceae	*Elaeagnus*	wild olive
	Hippophae	seabuckthorn
Casuarinaceae	*Casuarina*	beefwood
Coriariaceae	*Coriaria*	coriaria
Rosaceae	*Dryas*	dryad
Myricaceae	*Myrica*	bayberry, sweet gale
Ulmaceae	*Trema*	trema

Methods of action

The nitrogen fixed by the nodule bacteria on the roots of nitrogen-fixing plants may enter the crops or soil in three ways. First, it may be used by the host plants themselves. Second, the nitrogen can pass directly into the soil, either by excretion or more often by the sloughing off of the plant roots and especially their nodules. Third, the nitrogen from legume residues enters the soil as the residues decay, and becomes available to the succeeding crops.

The actual amount of nitrogen fixed by nitrogen-fixing bacteria varies considerably and is determined by a number of factors. The conditions of the soil, especially aeration, drainage, moisture, pH, and the amount of active calcium, are of prime importance. The rates of fixation of nitrogen by nitrogen-fixing plants also vary widely, and the nitrogen fixed by a particular species will be different under different conditions. Addition of chemical fertilisers can decrease the amount of nitrogen fixed by organisms, as shown in experiments with clover (Walker et al. 1956). Use of large amounts of nitrogen fertiliser may reduce the efficiency of nitrogen-fixing plants. Field studies are needed to determine the amount of chemical fertiliser that can be used without impairing the nitrogen fixation capacity of nitrogen-fixing plants.



Desmodium rensonii

The fallen leaves of nitrogen-fixing plants have a higher nitrogen content than those of other plants: the nitrogen content of leaf litter from nitrogen-fixing plants was found to be 2-3 times higher than that of other plants (He et al. 1997) and the C/N ratio 2.5 times lower (He et al. 1999). The difference in nitrogen content is mainly due to the different nature of retranslocation of leaf nitrogen into branches and stems before leaf fall: nitrogen-fixing plants and non-nitrogen-fixing plants have a different strategy for conserving nitrogen. In the HKH region Alnus nepalensis and Albizia stipulata retranslocated 2-3 times less nitrogen than non-nitrogen fixing associate species thus retaining more in the leaves (Sharma et al. 1994, 1995). This enables more release of nitrogen to the soil from fallen leaves – nitrogen which is then available to non-nitrogen-fixing plants for uptake. Most leaf litter nitrogen enters the soil, and nitrogen from nitrogen-fixing plant litter enters the soil faster as the decomposition rate is faster than that of litter from other plants.

Both leguminous and non-leguminous nitrogen-fixing plants are important nitrogen-fixing sources and are the main contributors of nitrogen inputs in terrestrial ecosystems. They play a major role in global nitrogen cycling and balancing. Nitrogen fixed by leguminous plants is thought to account for about half the total biologically fixed nitrogen on earth (Zhou Xiangquan, 1983; Zhou Xiangquan and Han Shufeng, 1989). Until now, theoretical research on leguminous plants has mainly focused on herbaceous leguminous fodder species with little investigation of woody species, whereas applied research has focused on the woody species.

Nitrogen-fixing plants not only increase soil nitrogen content, they also improve the organic matter status of soil and thus improve such properties as water retention

Alnus nepalensis