

Review article.

The contributions of nitrogen-fixing crop legumes to the productivity of agricultural systems

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Abstract

Data collated from around the world indicate that, for every tonne of shoot dry matter produced by crop legumes, the symbiotic relationship with rhizobia is responsible for fixing, on average on a whole plant basis (shoots and nodulated roots), the equivalent of 30–40 kg of nitrogen (N). Consequently, factors that directly influence legume growth (e.g. water and nutrient availability, disease incidence and pests) tend to be the main determinants of the amounts of N₂ fixed. However, practices that either limit the presence of effective rhizobia in the soil (no inoculation, poor inoculant quality), increase soil concentrations of nitrate (excessive tillage, extended fallows, fertilizer N), or enhance competition for soil mineral N (intercropping legumes with cereals) can also be critical. Much of the N₂ fixed by the legume is usually removed at harvest in high-protein seed so that the net residual contributions of fixed N to agricultural soils after the harvest of legume grain may be relatively small. Nonetheless, the inclusion of legumes in a cropping sequence generally improves the productivity of following crops. While some of these rotational effects may be associated with improvements in availability of N in soils, factors unrelated to N also play an important role. Recent results suggest that one such non-N benefit may be due to the impact on soil biology of hydrogen emitted from nodules as a by-product of N₂ fixation.

Keywords: Biological nitrogen fixation, grain legumes, rhizobia, rotations, improved crop yields

1. Introduction

Although calculations of global contributions of biological nitrogen (N₂) fixation are subject to gross approximation, recent estimates indicate that the symbiotic

fixation of atmospheric N₂ by the symbiotic association between soil bacteria (rhizobia) and crop legumes is likely to be in the order of 20–22 million tonnes (20–22 Tg) of nitrogen (N) each year (Herridge et al., 2008). This fixed N directly influences agricultural productivity by providing N that can be accumulated in the fresh pods, seeds, and tubers

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Table 1. Estimates of the proportions (Ndfa) and amounts of shoot N fixed by widely-grown legume crops in different geographical regions of the world^a.

Legume species Region	Ndfa ^b (%)		Amount fixed (kg shoot N ha ⁻¹)	
	Range	Mean	Range	Mean
Soybean (<i>Glycine max</i>):				
Area grown = 93.4 Mha (yield = 215 Mt: USA 38%, Brazil 23%, Argentina 18%) ^c				
South Asia	44–88	74	21–197	88
South-East Asia	0–82	60	0–450	115
Africa	65–89	77	159–227	193
North America	13–80	50	14–311	144
South America	60–95	78	80–193	136
Overall mean		68		119
Common bean (<i>Phaseolus vulgaris</i>):				
Area grown = 25.1 Mha (yield = 18 Mt: Brazil 17%, India 16%) ^c				
Africa	10–51	30	8–58	30
North America	38–68	49	40–125	75
South America	0–73	37	0–125	40
Overall mean		39		48
Groundnut (<i>Arachis hypogea</i>):				
Area grown = 23.4 Mha (yield = 38 Mt: China 38%, India 15%, Nigeria 8%) ^c				
South Asia	42–92	66	99–152	116
South-East Asia	16–77	60	21–200	100
Africa	19–79	55	17–103	48
South America	54–78	67	68–116	75
Overall mean		62		98
Pea (<i>Pisum sativum</i>):				
Area grown = 10.4 Mha (yield = 11 Mt: Canada 29%, France 12%, China 12%) ^c				
West Asia	70–74	72	33–62	47
Europe	26–99	60	28–215	130
North America	33–75	59	11–196	83
Oceania	31–95	68	26–183	83
Overall mean		65		86
Cowpea (<i>Vigna unguiculata</i>):				
Area grown = 9.2 Mha (yield = 4.6 Mt: Nigeria 59%, Niger 14%, Myanmar 3%) ^c				
South Asia	33–77	58	57–125	84
Africa	15–89	52	3–201	63
South America	32–74	53	9–51	29
Overall mean		54		59
Chickpea (<i>Cicer arietinum</i>):				
Area grown = 6.6 Mha (yield = 8.4 Mt: India 76%, Pakistan 8%, Turkey 6%) ^c				
South Asia	25–97	60	18–80	36
West Asia	8–91	60	3–115	51
Europe	44–77	56	23–74	43
North America	47–60	54	24–84	54
Oceania	37–86	60	43–124	70
Overall mean		58		51
 Lentil (<i>Lens culinaris</i>):				
Area grown = 4.4 Mha (yield = 4.1 Mt: India 30%, Canada 24%, Turkey 13%) ^c				
South Asia	9–97	65	4–90	42
West Asia	58–68	64	110–152	122
Overall mean		65		82

Table 1. Continued.

Legume species Region	Ndfa ^b (%)		Amount fixed (kg shoot N ha ⁻¹)	
	Range	Mean	Range	Mean
Pigeonpea (<i>Cajanus cajan</i>):				
Area grown = 4.4 Mha (yield = 3.2 Mt: India 75%, Myanmar 15%, Kenya 3%) ^c				
South Asia	10–88	57	7–88	58
Faba bean (<i>Vicia faba</i>):				
Area grown = 2.7 Mha (yield = 4.3 Mt: China 42%, Ethiopia 10%, Egypt 9%) ^c				
West Asia	63–76	69	78–133	100
Europe	60–92	74	73–211	153
North America	60–92	74	13–252	118
Oceania	69–89	82	82–216	143
Overall mean		75		129

^aCollated from a range of published sources (Rennie and Kemp, 1983a,b; Peoples and Craswell, 1992; Herridge and Danso, 1995; Peoples et al., 1995b; Wani et al., 1995; Jensen, 1997; Schulz et al., 1999; Hafeez et al., 2000; Unkovich and Pate, 2000; Rochester et al., 2001; Turpin et al., 2002; Aslam et al., 2003; Hauggaard-Nielsen et al., 2003; Shah et al., 2003; Holdensen et al., 2007; Ncube et al., 2007; Ojiem et al., 2007; Adjei-Nsiah et al., 2008) and unpublished data of the authors. Data derived from N fertilized treatments have been excluded. ^bThe proportion of crop N derived from atmospheric N₂. ^cTotal global grain yield harvested, the major producing countries and their contribution to total production (FAO, 2008).

[in the case of species such as winged bean (*Psophocarpus tetragonolobus*), or Bambara groundnut (*Vigna subterranea*)], that are used as vegetables, or in the dried grain harvested for human or animal consumption. The N₂ fixed by legume crops can also represent an important renewable source of N for agricultural soils. However, the net inputs of fixed N into soils depend upon the amounts of N₂ fixed relative to the amounts of N removed in the protein-rich legume products which can represent between 45–75% of the N in the above-ground biomass of crop legumes (van Kessel and Hartley, 2000; Walley et al., 2007; Salvagiotti et al., 2008). With the N content of legume grain representing between 30–40 kg N in every tonne (t) for most legume pulses and 60–65 kg N per t for soybean (Salvagiotti et al., 2008), the 60 million t of pulses and 250 million t of legume oilseeds (soybean and groundnut) harvested globally each year (Table 1; Herridge et al., 2008), results in an annual removal of around 17 million t of N. One might expect some variation in the net returns of fixed N to soil as a result of local farming practices, the legume line used, or regional soil and climatic effects on the ability of different legume species to (i) grow and fix N₂ or (ii) partition N into grain (Evans et al., 2001; Maskey et al., 2001; Peoples et al., 2001; Ojiem et al., 2007).

However, the comparisons of global estimates of N₂ fixation (20–22 million t N) with the amounts of N

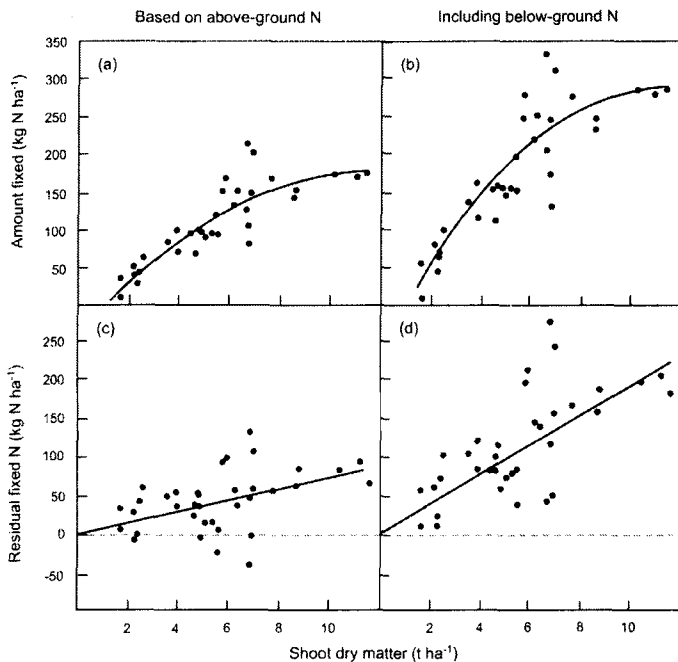


Figure 1. Data obtained from commercial faba bean crops illustrating the consequences of (i) ignoring the contribution of below-ground N (a,c), or (ii) including the contribution of below-ground N (b,d) on determinations of amounts of N_2 fixed (a,b) and residual fixed N remaining after grain harvest (i.e. fixed N - seed N removed). Calculations of positive measures of residual fixed N indicate a net input of fixed N, while negative values represent a net export of N from soil. The broken line represents the situation where the estimate of the amounts of N_2 fixed equals the seed N removed. Data modified from Rochester et al. (1998).

removed in grain each year (17 million t N) strongly suggest that the residual contribution of fixed N into agricultural systems following legume cropping might be relatively small. Despite this, the yields of crops are often greater when legumes are included in cropping sequences than when they are not (e.g. Peoples and Craswell, 1992; Chalk, 1998; Rochester et al., 2001; Kirkegaard et al., 2008). This review summarizes measurements of N_2 fixation obtained in different parts of the world, identifies the key variables that influence inputs of fixed N, and examines factors contributing to the observed benefits of legumes in crop rotations.

2. N_2 Fixation World-wide — Estimates and Quantification

The amount of N present in a legume crop is the product of its dry matter (DM) production and the N content of that DM:

$$\text{Legume N} = (\%N/100) \times (\text{legume DM})$$

A legume crop such as soybean yielding around 2.5 t grain ha^{-1} accumulates about 200 kg N ha^{-1} in its above-ground biomass over a growing season. That N must be provided from the soil, from symbiotic N_2 fixation, and/or from fertilizer N (Salvagiotti et al., 2008). The amount of N_2 fixed is determined by (i) the relative reliance of the crop upon N_2 fixation for growth [i.e. the proportion of the crop N derived from atmospheric N_2 , (%Ndfa)] and (ii) the amount of N accumulated in the legume:

$$\text{Amount fixed} = (\%Ndfa/100) \times (\text{legume N})$$

The formation of working symbioses between legumes and rhizobia is dependent upon many factors and does not occur as a matter of course. This is reflected in the range of experimental estimates of %Ndfa and amounts of N_2 fixed by the most widely-grown legume crops presented in Table 1. Yet, despite the measures of %Ndfa ranging from near zero to almost 100% for the various legume species growing in different parts of the world, there are marked similarities in the mean estimates of %Ndfa within a species across geographic regions. There also appear to be distinct groupings of legume species that exhibit similar capacities for N_2 fixation. For example, the overall average %Ndfa across regions for common bean (French bean) is <40%, compared with overall averages for most other legumes of between 54–65%, with soybean and faba bean at 68% and 75%, respectively (Table 1). Hardarson and Atkins (2003) came to a similar general conclusion about the relative rankings of the symbiotic performance of different crop legume species based on data collated from a series of FAO/IAEA co-ordinated research programmes undertaken in different countries, as did Walley et al. (2007) following a comprehensive review of legume research undertaken in the Northern Great Plains of Canada. However, the average estimate of %Ndfa calculated for soybean in Table 1 (68%) is higher than the value of 58% determined by Salvagiotti et al. (2008).

The experimental data indicate maximum daily rates of N_2 fixation of between 3 and 10 kg shoot N ha^{-1} (Jensen, 1997; Unkovich and Pate, 2000), delivering potential inputs >200 kg shoot N ha^{-1} (Table 1). Even though it is difficult to generalize about just how much N might be fixed by a specific legume species in a particular farming system or geographic region, the observed commonalities between legume species and mean %Ndfa discussed above seem also to be reflected in the measured inputs of fixed N. Therefore, on average, common bean might be expected to fix <50 kg shoot N ha^{-1} , the cool season pulses and cowpea between 50–80 kg shoot N ha^{-1} , while the legume oilseeds and faba bean are generally likely to fix >100 kg shoot N ha^{-1} (Table 1; see also Unkovich and Pate, 2000; Hardarson and Atkins, 2003).

The values presented in Table 1 have almost invariably been determined from measures of plant shoot biomass.

Below-ground contributions of fixed N have often been ignored. Research now suggests that N associated with nodules and roots may represent between 30% and 60% of the total N accumulated by legume crops (e.g. Rochester et al., 1998; Khan et al., 2003; Mahieu et al., 2007; McNeill and Fillery, 2008). Therefore, total inputs of fixed N are likely to be much greater than has been determined from shoot-based measurements such as those depicted in Table 1 (e.g. Fig. 1a,b; Khan et al., 2003). It follows that, when below-ground contributions of fixed N are included in N budgets compared to when they are not, different conclusions would be drawn about the potential for legume crops to return fixed N to soil following grain harvest (e.g. Fig. 1c,d; Peoples et al., 2001).

Some of the data presented in Table 1 were also derived from research trials in which specific treatments had been imposed to generate differences in (i) legume growth, and/or (ii) %Ndfa values and as an experimental means of studying factors that regulate N₂ fixation. Therefore, these values may not necessarily be a useful guide to what is actually occurring in farmers' fields. Fortunately, procedures such as the ureide method and the ¹⁵N natural abundance technique are now available and can be utilized to provide on-farm measures of N₂ fixation (Peoples and Herridge, 2000; Unkovich et al., 2008). However, it should be recognized that there are many inherent assumptions behind the application of these methodologies to commercial legume crops, particularly with those methodologies based on the analysis of the natural abundance of ¹⁵N (Unkovich and Pate, 2000; Peoples et al., 2002a; Unkovich et al., 2008). For example, potential sources of error include (i) the use of weeds and the choice of 'reference species' to measure the ¹⁵N composition of soil mineral N (Nyemba and Dakora, 2005; Ojiem et al., 2007), (ii) rhizobial strain-induced differences in isotopic fractionation during N₂ fixation (particularly when farmers' crops are nodulated by multiple, unknown strains of rhizobia; Okito et al., 2004), and (iii) excessive site variability (Holdensen et al., 2007). Nonetheless, such approaches can be expected to provide sensible, semi-quantitative estimates of N₂ fixation under most situations (Peoples and Herridge, 2000; Unkovich et al., 2008).

Examples of estimates of N₂ fixation by legumes in farmers' fields are summarized in Table 2. More farmers' crops have been examined in some regions than in others. Although these don't always match the main production areas identified for specific crops in Table 1, such on-farm data can be used to develop a picture of the relative importance of N₂ fixation to the N nutrition of crop legumes. The values in Table 2 indicate different inputs of fixed N between legumes and geographic regions; however, mean estimates of %Ndfa for commercial crops were often comparable to the mean estimates for experimental crops for the same species given in Table 1. The on-farm data suggest that short-duration warm season crops such as

black gram and green gram tended to have a lower reliance on N₂ fixation than many other species (Table 2). A smaller N demand by these crops, and possibly greater N mineralization of the soil organic N pool, as a result of elevated soil temperatures and higher moisture levels during crop growth, may be factors that contribute to that lower %Ndfa (Maskey et al., 2001).

3. Variables Influencing Inputs of Fixed N – Soil N, Soil Rhizobia and Rhizobial Inoculation

Soil mineral N

The %Ndfa of a legume is not a characteristic solely determined by the association between legume genotype and its rhizobia. It also reflects the interaction between plant-available soil N and legume growth (Unkovich and Pate, 2000). Soil nitrate and N₂ fixation are complementary in meeting the N requirements for growth by a legume crop, and the inhibitory effect of nitrate on nodulation and N₂ fixation processes is well documented (e.g. Herridge et al., 1984; Streeter, 1988; Peoples et al., 1995b). High concentrations of soil nitrate, induced by such factors as excessive tillage, and applications of fertilizer N have been shown experimentally to delay the formation of nodules and the onset of N₂ fixation and to reduce both %Ndfa and the amount of N₂ fixed (Jensen, 1997; van Kessel and Hartley, 2000; Salvagiotti et al., 2008). The same outcomes apply to farmers' crops. For example, elevated concentrations of soil mineral N generated through consecutive years of legume cropping or extended periods of fallow have been shown to suppress N₂ fixation in commercial soybean, chickpea or faba bean crops in Australia (Peoples et al., 1995a; Schwenke et al., 1998; Peoples et al., 2001). Reduced reliance on fixed N reflected applications of fertilizer N by farmers to groundnut crops in Nepal and Vietnam (Maskey et al., 2001; Hiep et al., 2002; Hoa et al., 2002). Conversely, strategies that (i) reduce soil nitrate concentrations such as sowing legumes immediately after N-hungry crops (Doughton et al., 1993; Peoples et al., 1995a; Peoples et al., 2001), or that (ii) increase competition for soil mineral N by means such as intercropping legumes and cereals (e.g. Rerkasem et al., 1988; Jensen 1996; Hauggaard-Nielsen et al., 2003) will generally increase %Ndfa.

Data collated for soybean indicate that very little N would be fixed by soybean receiving fertilizer N at rates of 200–300 kg N ha⁻¹ (Salvagiotti et al., 2008). Experimentation with pea in France indicated that nitrate inhibition of N₂ fixation was absolute (i.e. seasonal %Ndfa = 0) when soil mineral N at sowing exceeded 380 kg nitrate-N ha⁻¹; symbiotic N₂ fixation was not initiated until soil mineral N concentrations dropped below 56 kg N ha⁻¹ (Voisin et al., 2002). Similarly, research trials undertaken

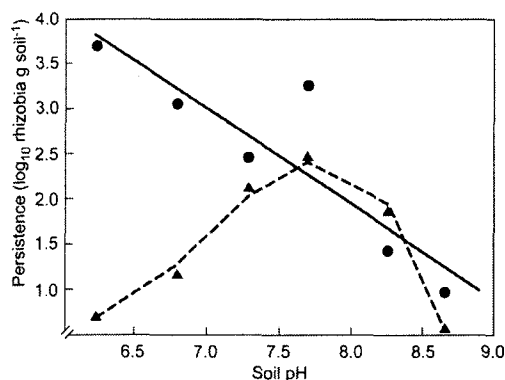


Figure 2. Contrasting profiles of persistence in vertosols for two strains of *Bradyrhizobium japonicum* used as soybean inocula. Persistence of strain CB1809 (●—●) is good at low soil pH, but declines as pH increases, while the persistence of CC709 (▲- -▲) is poor at low and high soil pH, but relatively good when soil pH is neutral (after Brockwell and Evans, 2005).

Table 2. Examples of the proportion of plant N derived from N₂ fixation (Ndfa) and estimates of the amounts of N₂ fixed by crop legumes in farmers' fields.

Legume and region ^a	Number of fields	Mean Ndfa (%)	Shoot N fixed (kg N ha ⁻¹)
Soybean			
South Asia	22	62	39
South-East Asia	43	71	148
Africa	14	58	na ^b
South America	42	64	182
Oceania	33	53	179
Overall mean		62	137
Common bean			
Africa	10	36	na ^b
South America	1	25	15
Overall mean		31	15
Groundnut			
South Asia	18	58	123
South-East Asia	60	47	83
Africa	61	50	na ^b
Overall mean		52	103
Pea			
Europe	9	65	57
Oceania	8	75	160
Overall mean		70	108
Cowpea			
Africa	89	73	20
Chickpea			
South Asia	102	75	59
West Asia	39	58	na ^b
Oceania	15	28 ^c	20 ^c
Overall mean		54	40
Lentil			
South Asia	57	71	53
West Asia	37	71	na ^b
Oceania	4	79	90
Overall mean		74	71

Table 2. Continued.

Legume and region ^a	Number of fields	Mean Ndfa (%)	Shoot N fixed (kg N ha ⁻¹)
Pigeonpea			
South Asia	5	65	na ^b
Africa	16	92	59
Overall mean		78	59
Faba bean			
South Asia	2	85	na ^b
Oceania	56	68	95
Overall mean		77	95
Black gram (<i>Vigna mungo</i>)			
South Asia	83	47	26
Green gram (<i>V. radiata</i>)			
Oceania	65	28	na ^b
Bambara groundnut (<i>V. subterranea</i>)			
Africa	16	54	na ^b

^aValues derived from information presented in Aslam et al. (1997); Shah et al. (1997); Rochester et al. (1998); Schwenke et al. (1998); Maskey et al. (2001); Peoples et al. (2001); Hiep et al. (2002); Hoa et al. (2002); Herridge et al. (2005); Adu-Gyamfi et al. (2007); Naab et al. (2009); Pule-Meulenberg and Dakora (2009), and includes unpublished data by the authors. ^bna = data not available. ^cData confounded by drought and high concentrations of soil nitrate following long periods of fallow (Schwenke et al., 1998).

with productive chickpea in Australia showed that little or no N₂ was fixed once the soil contained >350 kg nitrate-N ha⁻¹ (Doughton et al., 1993), although data collected from commercial chickpea crops with lower biomass suggested the critical value may be closer to 200 kg nitrate-N ha⁻¹ (Schwenke et al., 1998). While the on-farm results for chickpea may have been complicated by low rainfall, it was noteworthy that neighboring faba bean crops, sampled as part of the same survey, maintained much higher levels of %Ndfa than chickpea at equivalent concentrations of soil nitrate (Schwenke et al., 1998). Experimental data also led Turpin et al. (2002) to conclude that faba bean maintains a higher dependence on N₂ fixation for growth and fixes more N than does chickpea under the same concentrations of soil mineral N.

In addition to evidence for differences between legume species in their nodulation and N₂ fixation responses to soil mineral N, data from irrigated soybean indicate that symbiotic performance following the presence of high concentrations of soil nitrate at sowing (e.g. 260 kg nitrate-N ha⁻¹) may be modified by choice of soybean cultivar and rate of rhizobial inoculation (Peoples et al., 1995b).

Rhizobia

In its simplest form, the symbiosis is established when indigenous rhizobia infect the roots of legumes to produce

nodules. For instance, farmers in South Asia have grown pulses for centuries. As a legacy of this, rhizobia have become established in many soils, mostly by natural means, but sometimes by seed inoculation with commercial inoculant cultures. Nonetheless, there are also soils where rhizobia are absent, or where the strains that are present are unsuitable for the legume that the farmer intends to grow. Moreover, even in soils with effective resident rhizobia, the populations may be too small to guarantee prompt formation of root nodules and, as a consequence, optimal N_2 fixation (e.g. Herridge et al., 2005).

Cropping history and persistence of rhizobial strains

Generally, the particular legume species grown most recently is a major determinant of the type and size of rhizobial populations in the soil (Thies et al., 1995). For instance, populations of *Bradyrhizobium* spp. in alfisols at six sites in Karnataka State, India, were invariably high when a host pulse was part of recent cropping systems (Table 3; Hegde, 1994). On the other hand, where a particular host legume has not been recently cropped, the appropriate strains of rhizobia are more likely to be absent from the soil and inoculation will be needed to ensure satisfactory and effective nodulation.

There are exceptions to this generalization. For example, CB1809, a *B. japonicum* strain for soybean, survived in large numbers in mildly acidic vertosols for up to 5 years during several cropping sequences that included irrigated rice (*Oryza sativa*), but excluded the host plant, soybean (Roughley et al., 1995). But CB1809 survived progressively less well as soil alkalinity increased (Fig. 2; Brockwell and Evans, 2005), and data collected from commercial soybean fields located on alkaline clay soils indicate that CB1809 may not persist from one cropping season to the next (Peoples et al., 1995a). There is evidence that the ability to persist in soil may be a strain characteristic (Fig. 2).

Although regarded as facultative organisms, root-nodule bacteria are much obligated to a host plant for their existence and persistence. There is considerable proliferation on the root surfaces of host legumes and, within the nodules themselves, the bacteria enjoy an environment akin to pure culture and attain very large populations. In sharp contrast, rhizobia released into the soil, as the nodules of annual legumes decay and disintegrate, encounter largely stressful conditions and, usually, populations diminish rapidly.

Amongst stressful edaphic factors that bear on rhizobial survival are soil pH, temperature, aridity, excess of heavy metals, low clay content, soil salinity and fallowing (Giller, 2001; Slattery et al., 2001; Howieson and Ballard, 2004). Nearly all farmers' fields will at some stage endure one or more of the stresses listed above. Despite the principle of rhizobial ecology that useful populations of rhizobia remain in the soil when the same or a symbiotically related legume

has been a component of the immediate past history of the land (e.g. Table 3), rhizobial numbers may decline rapidly in soil under unfavorable conditions. For example, Marshall et al. (1993) noted that, in acidic soils, populations of *Rhizobium leguminosarum* bv. *viciae* were frequently $<100 \text{ g soil}^{-1}$ (0–10 cm) within 1 year of the growth of a well nodulated crop of field peas.

The proposition that numbers of appropriate rhizobia in many soils may be small at the time of sowing food legumes is supported by the results of four investigations (Table 4). Examinations were made of the rhizobial composition of 429 soil samples collected in 20 countries. Overall, approximately 54% of all soils had <100 rhizobia g soil^{-1} , and another 13% had between 100 and 1000 g soil^{-1} . Further support for this proposition is forthcoming from the results of a number of on-farm inoculation trials undertaken in different countries around the world that indicate that even the so-called promiscuous *Vigna* species often benefit from inoculation (Table 5).

Numerical aspects of strain competition

Resident rhizobia represent a competitive barrier to inoculant rhizobia. The size of the pre-existing population of rhizobia in the soil is a prime determinant of the feasibility of inoculation – with very small populations, inoculation is mandatory whereas, with large populations, it is often futile (e.g. Thies et al., 1991). Opinions vary, though not greatly, about the threshold size of populations of resident rhizobia below which legume inoculation is likely to contribute to nodulation, N_2 fixation and plant productivity. Following a comprehensive survey of the literature, Howieson and Ballard (2004) concluded that legumes will very often respond to inoculation where the rhizobial community is less than 100 cells g soil^{-1} . Herridge (2008) proposed a slightly higher threshold value (300 rhizobia g soil^{-1} , 0–10 cm) below which inoculation is likely to be beneficial. However, there is evidence from Brazil even in the presence of apparently adequate numbers of effective rhizobia in soil (>1000 rhizobia g^{-1}), that soybean's symbiotic performance (and grain yield) can be enhanced by re-inoculation each year (Hungria et al., 2006).

Numerical aspects of competition between resident and inoculant rhizobia are simply illustrated. A resident population of 300 rhizobia g soil^{-1} is equivalent to approximately 5×10^{11} rhizobia ha^{-1} (0–10 cm). To achieve a similar population through seed inoculation, it would be necessary to sow 500,000 seeds ha^{-1} , with an inoculation rate of one million rhizobia seed $^{-1}$. The inoculant (peat) culture would need to contain 5×10^9 rhizobia g peat^{-1} . Were the inoculant culture to contain only 1×10^8 rhizobia g peat^{-1} , the same rate of peat application and sowing would be equivalent to merely 6 rhizobia g soil^{-1} . It is evident that inoculant quality, i.e. numbers of rhizobia in the culture, is a critical determinant of inoculation success. The best inoculants (i.e. those with the highest rhizobial

Table 3. Populations of *Bradyrhizobium* spp. in alfisols at six sites in Karnataka State, India, as influenced by cropping system.

Cropping system	Rhizobia g soil ⁻¹ ^a
Cereal-legume rotation	34,000
Cereal-legume intercrop	7,000
Sole legume	63,000
Sole non-legume	87

^aRhizobia counted with a serial-dilution, nodulation-frequency, plant-infection test (Brockwell, 1963) using *Macroptilium atropurpureum* as the test plant. Data derived from Hegde (1994).

content) tend to be produced in countries that have quality control authorities (Brockwell and Bottomley, 1995; Herridge, 2008).

On-farm experience with rhizobial inoculation

Despite the results of numerous trials that indicate enhanced growth and grain yield in response to inoculation of many legume crops (Table 5), and reports from regions such as South Asia of poor nodulation in >40% of farmers' crops of chickpea (e.g. Namdeo and Gupta, 1994; Wani et al., 1995; Bhattarai et al., 1997), lentil (Bhattarai et al., 1997), groundnut (Joshi, 1994), and pigeon pea (Khurana and Dudeja, 1994), adoption by farmers of inoculation technology varies widely around the world.

The global use of legume inoculants can be divided between the relatively sophisticated markets of North and South America, Europe and Australia which are dominated by a small number of manufacturers producing consistently high quality inoculants, and the less sophisticated and more fragmented production of inoculants in Asia and Africa of variable quality (Singleton et al., 1997; Herridge, 2008).

Table 5. Summary of inoculation responses for a range of legume species^a.

Species	Number of trials	Significant yield response to inoculation compared to local farmer practice (% of trials)
Soybean	42	64
Common bean	10	10
Groundnut	31	45
Cowpea	11	64
Chickpea	87	49
Lentil	50	86
Pigeon pea	35	63
Green gram	78	67
Black gram	33	64

^aData from more than 20 countries collected by Singleton et al., (1992) combined with additional results obtained from on-farm trials undertaken in Bangladesh (Sattar et al., 1997), India (Namedo and Gupta, 1994; Khurana et al., 1997), and Nepal (Bhattarai et al., 1997).

In the Americas, Europe and Australia inoculant manufacturing has frequently developed as a result of the large-scale introduction of new and exotic legumes with rhizobial specificity. Examples include soybean in Brazil, pea and lentil in Canada, and lupin (*Lupinus* spp.) in Australia. Dramatic responses to legume inoculation in these systems helped facilitate the development of a legume inoculant market (Herridge, 2008). Using inoculant when it is unnecessary is probably more common in these countries than not using inoculants when they are needed. Inoculation is a form of insurance where a small premium (cost of inoculation) protects a farmer against the possibility of a N-deficient crop that can result in reduced grain yield and lower income (Herridge, 2008).

Table 4. Frequency of low populations of naturalized rhizobia capable of nodulating crop legumes.

Location	Rhizobia	Proportion of soils with		Reference
		<1000 rhizobia g ⁻¹	<100 rhizobia g ⁻¹	
17 countries 305 soil samples	<i>Bradyrhizobium</i>	60%	47%	Singleton et al., 1992
	For soybean	80%	75%	
	For common bean	80%	66%	
Bangladesh 16 locations	For cowpea	44%	25%	Khanam et al., 1994
	For chickpea	81%	62%	
	For lentil	62%	44%	
	For pigeonpea	56%	19%	
Nepal 6 locations	For chickpea	33%	17%	Bhattarai, 1994
Thailand 11 provinces, 102 soil samples	For soybean	64%	36%	Toomsan et al., 1994
	For groundnut	36%	18%	

Table 6. Comparisons of shoot dry matter (DM) accumulation, and the proportion (Ndfa) and amount of shoot N fixed by various food legume crops growing in Thailand, Nepal, and Australia.

Location and legume	Season or days to maturity	Shoot DM (t ha ⁻¹)	N ₂ fixation	
			Ndfa (%)	Amount (kg N ha ⁻¹)
Chiang Mai, Thailand				
Soybean ^a	Late rainy	2.02	82	41
Green gram ^a	Late rainy	3.66	89	65
Black gram ^a	Late rainy	5.40	80	103
Soybean ^b	Late rainy	2.81	72	70
Soybean ^b	Dry	5.47	73	126
Soybean ^b	Early rainy	11.93	72	215
Nepalgunj, Nepal				
Chickpea ^c	Winter	2.12	67	52
Lentil ^c	Winter	3.34	86	90
Breeza, Australia				
Cowpea ^d	77	3.56	38	24
Green gram ^d	84	3.72	15	9
Black gram ^d	100	3.29	37	21
Soybean ^d	147	9.64	90	254
Pigeon pea ^d	147	2.84	44	16

^aMean of 2 cultivars; unpublished data of Sampet, Herridge and Peoples. ^bMean of 5 cultivars; unpublished data of Rerkasem and Peoples. ^cUnpublished data for rainfed crops of Maskey, Bhattarai, Peoples, and Herridge. ^dUnpublished data for irrigated crops of Herridge and Peoples.

In Asia and Africa, limited local production and distribution of inoculants (Sattar et al., 1997) and poor inoculant quality (Singleton et al., 1997) are the major constraints. There may also be a lack of knowledge about inoculants by farmers and extension staff (Khurana et al., 1997; Hoa et al., 2002), and an inability to convince farmers of the merits of inoculation because of inconsistencies in crop response or an inability to demonstrate visible differences between inoculated and uninoculated treatments (Joshi, 1994; Khurana and Dudeja, 1994). Some of these limitations could be addressed with increased effort in training and education and especially with improved inoculant quality, emphasis in R&D on rhizobial strain selection and methods of inoculant production and application, private-sector investment and stricter quality control (Herridge, 2008). However, economic restrictions that bear on resource-poor farmers may remain an insurmountable problem. The use of promiscuously-nodulating soybean lines that nodulate and fix N₂ with naturalized rhizobia and, therefore, require no inoculation, is an approach being evaluated in Africa; it represents a potential solution to the poor adoption of inoculant technology (Mpeperekki et al., 2000; Sanginga, 2003). There seems no good reason why similar strategies should not be applicable to other legume crops.

4. Variables Influencing Inputs of Fixed N – Factors Mediated by Crop Biomass

Although the levels of %Ndfa are important, provided there are adequate numbers of effective rhizobia in the soil and concentrations of soil mineral N are not too high, N₂ fixation will be overwhelmingly regulated by legume growth rather than by %Ndfa (Peoples et al., 2002b). Most legume crops generally fix between 15–25 kg shoot N for every tonne of shoot DM accumulated (Fig. 3), with an average of around 20 kg shoot N t shoot DM⁻¹. Similar relationships appear to hold for both experimental crops (e.g. Pilbeam et al., 1997; Evans et al., 2001) and for farmers' crops (Rochester et al., 1998; Maskey et al., 2001; Peoples et al., 2001). Since fixed N will also be contained in roots and nodules, the average value for total N fixed may be the equivalent of 30 kg N fixed t shoot DM⁻¹. Common bean is likely to be an exception to this generalization, fixing only about 10 kg shoot N t DM⁻¹ (15 kg total N fixed t shoot DM⁻¹ when contributions of below-ground N are included). On the other hand, chickpea is reported to accumulate a higher proportion of total plant N in nodules and roots than many other legumes (Unkovich and Pate, 2000; Khan et al., 2003) so that total inputs of fixed N from chickpea might approach 40 kg N t shoot DM⁻¹.

Crop agronomy

Little can be done about the suppression of N₂ fixation by temperature extremes (Hardarson and Atkins, 2003), or drought (Schwenke et al., 1998; Thomas et al., 2004). However, almost every other factor that has been identified as influencing inputs of fixed N by farmers' crops through a direct impact on crop growth potential (e.g. crop nutrition, weed control, disease, pests, or cropping sequence and intensity; Peoples and Herridge, 2000) can be addressed or manipulated by a farmer through management. Therefore, basic improvements in crop agronomy probably hold the greatest promise as a means of enhancing N₂ fixation through increasing legume biomass (Peoples et al., 2002b). A significant strategy involves the use of legume genotypes adapted to the prevailing soil and environmental conditions. For example, species and cultivar comparisons of upland crops during the mid-late rainy season at Chiang Mai in north-west Thailand demonstrated that black gram (mashbean) was better adapted to the environment, grew better and consequently fixed more N than either green gram (mungbean) or soybean (Table 6). Similar studies comparing growth and symbiotic performance of different legume crops have also been undertaken for rainfed crops following rice at Nepalgunj in the western Terai of Nepal, and with irrigated summer crops growing in Australia. Data from the Nepalese study indicated that lentil consistently accumulated more shoot biomass and fixed more N than

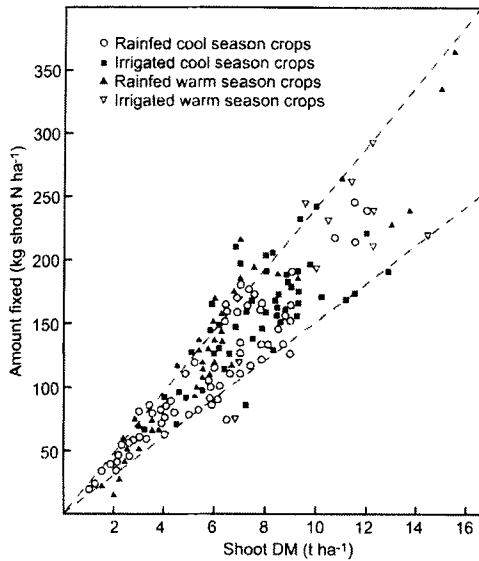


Figure 3. Examples of the relationship between amounts of shoot N fixed (kg N ha^{-1}) and shoot dry matter production (DM, t ha^{-1}) for legume crops (modified from Herridge et al., 2008). The upper broken line indicates the $25 \text{ kg N fixed t DM}^{-1}$ relationship, while the lower broken line shows $15 \text{ kg N fixed t DM}^{-1}$ relationship. The figure uses data from rainfed and irrigated warm season crops (monocropped and intercropped soybean, cowpea, green gram, and black gram), and cool season crops (pea, chickpea, lentil, faba bean, and lupin) growing in South Asia (Schulz et al., 1999; Maskey et al., 2001; Aslam et al., 2003; Shah et al., 2003; Maskey and Bhattarai, unpublished), West Asia (Beck et al., 1991; Kurdali et al., 1997), South-East Asia (Rerkasem and Sampet, unpublished), Europe (Zapata et al., 1987; Beck et al., 1991; Jensen, 1997), North America (Rennie and Dubetz, 1986; Smith et al., 1987), South America (Boddey et al., 1990; Alves and Boddey, unpublished), and Australia (Rochester et al., 1998; Peoples et al., 2001; Rochester et al., 2001; Peoples and Herridge, unpublished).

chickpea over a 3-year period which implied that lentil was better suited to the local soil and environmental conditions, and/or was more tolerant of the prevailing pests and diseases (Table 6). In the case of the Australian investigation, soybean (long-duration crop, 140 d to physiological maturity) had a much greater opportunity to accumulate DM and fix N_2 than shorter-season crops such as cowpea, green gram or black gram (Table 6). By contrast, another long-duration crop, pigeonpea, grew poorly and fixed relatively little N (Table 6). This reflected the erratic nodulation and yield observed in the alkaline soils of the region, which has been attributed to iron deficiency (Brockwell et al., 1991; Herridge and Holland, 1993).

Planting time is another factor that can influence legume growth. At Chiang Mai, for instance, it is possible to grow soybean during the early wet, the late wet, and the dry seasons. Although soybean's reliance upon N_2 fixation may not alter greatly, soybean's capacity for growth and consequently the amounts of N_2 fixed differ considerably

between these 3 growing seasons (Table 6). Similar large effects due to variation in sowing time have also been observed for chickpea in Syria and France (Beck et al., 1991).

Other management related approaches designated to reduce the impact of pests and diseases, or to enhance crop growth are discussed elsewhere (Beck et al., 1991; Peoples et al., 1995b; van Kessel and Hartley, 2000). However, the prospects of actually realizing such increases in N_2 fixation in farmers' fields through improvements in agronomy must be considered within the context of the present constraints to the adoption of existing knowledge. For example, research has demonstrated that nutritional deficiencies induced by poor supply of available phosphorus commonly restrict legume growth and N_2 fixation (Giller and Cadisch, 1995; Hardarson and Atkins, 2003). Yet the implementation of the simple fertilizer strategies needed to ameliorate such soil constraints may not necessarily be straightforward. Farmers in developing countries are currently struggling with problems of supply and the rapidly escalating costs of phosphatic fertilizers. Even if transport or knowledge barriers can be overcome in developing countries the economic challenge of addressing nutrient deficiencies is likely to remain overwhelming difficult. On-farm studies have indicated relationships between crop biomass production, N_2 fixation and legume population (Hoa et al., 2002; Naab et al., 2009). Sub-optimal density, poor vigor and disease have been implicated as important factors contributing to low inputs of fixed N in farmers' fields in South Asia (Aslam et al., 1997; Shah et al., 1997; Peoples and Herridge, 2000; Maskey et al., 2001). Yet access to (i) better quality seed to ensure reliable germination, (ii) improved varieties with greater tolerance of pests and diseases, or (iii) the latest seeding technologies is likely to be very limited for resource-poor farmers. Other common farming practices may also restrict legume growth potential. For example, N_2 fixation and contributions of residual fixed N to soil in northern Thailand would be maximized for soybean if it was grown during the early wet season when water supply and temperatures are both optimal (Table 6). But, as in many countries throughout Asia, the most favorable growing season is reserved by farmers for rice.

Few countries have cropping ratios of cereal:legume less than 10:1 (Peoples et al., 2002b; Crews and Peoples, 2004). Therefore, even in the absence of access to fertilizers or quality seed, there still appears to be considerable potential for enhancing inputs of fixed N through the inclusion of more legumes in farming systems. Increased areas of legumes might be achieved by including more leguminous crops in rotations, or by the introduction of short-duration legume green manures or 'catch crops' such as green gram or Chinese milk vetch (*Astragalus sinicus*) in the fallow period between crops, or by the use of legumes to rehabilitate areas of degraded land. Intercropping legumes and non-legumes is common in parts of Asia,

Africa and South America. It is an approach that could be applied more widely. While intercropping generally results in a reduction in the amount of N₂ fixed relative to a legume monocrop, it represents an agronomically important input of N compared to a sole cereal crop (Rerkasem et al., 1988; Jensen, 1996; Hauggaard-Nielsen et al., 2003). It also enhances the efficiency of utilization of available nutrients and water (van Kessel and Hartley, 2000; Jensen and Hauggaard-Nielsen, 2003). However, such strategies have limitations that restrict their widespread use. Changes to Asian and African farming practices so that legume residues are retained rather than removed after grain harvest for animal feed or fuelwood (in the case of pigeonpea) would also have a large impact on the subsequent benefits of fixed N to following crops and the overall N balance of cropping systems (e.g. Adu-Gyamfi et al., 2007). Data collected by the authors from South and South-East Asia suggest that the potential return of 50–90 kg fixed N ha⁻¹ can be lost when above-ground vegetative residues are not conserved (Rerkasem, Maskey and Bhattaria; unpublished data).

5. Benefits of Legumes in Crop Rotations

It is well documented that yields of non-legume crops can often be improved by including a legume in the cropping sequence (e.g. Peoples and Craswell, 1992; Stevenson and van Kessel, 1996; Chalk, 1998; Aslam et al., 2003; Shah et al., 2003; Jensen et al., 2004; Kirkegaard et al., 2008). However, despite (i) net returns of fixed N in excess of 100 kg fixed N ha⁻¹ in some farming systems (Evans et al., 2001), (ii) evidence of legume residues making major contributions to the soil organic matter pool (Schwenke et al., 2002), thereby providing enhanced potential for N mineralization (Wani et al., 1995), and (iii) being responsible for increased concentrations of soil mineral N (Herridge et al., 1995; Chalk, 1998; Rochester and Peoples, 2005), cereal yield responses are not always wholly attributable to improvements in soil N supply (e.g. Stevenson and van Kessel, 1996; Sanginga, 2003). Both N and non-N factors contribute to the observed improvements in crop productivity after legumes. For example, when cotton (*Gossypium hirsutum*) was grown in a rotation that included vetch (*Vicia sativa*) green manure crops, the yield of lint, in the absence of fertilizer N, was 20% greater than that achieved by continuous cotton or a wheat-cotton sequence (Fig. 4).

However, cotton yields in all 3 systems converged with increasing rates of fertilizer N. These data suggest that improved N availability was primarily responsible for the rotational effect of the vetch. The pattern of crop response was different when cotton followed faba bean grown for grain. Lint yield was 45% higher than either of the non-legume treatments in the absence of fertilizer N (Fig. 4).

This was despite the net inputs of fixed N by faba bean being smaller than those achieved by the vetch green manure crops (Rochester and Peoples, 2005). In this case, the levels of lint yield in the faba bean-cotton system did not change greatly with increasing rates of fertilizer N (Fig. 4). Such responses usually indicate that factors other than N are contributing to crop performance. Chalk (1998) compared yield responses to increasing rates of applied fertilizer N similar to those depicted in Fig. 4 for 26 wheat-wheat and lupin-wheat rotations undertaken in Australia. He concluded that, in over 60% of the experiments (16 of the 26 comparisons), non-N-effects derived from the lupin either dominated the rotational effect, or were important contributing factors in the subsequent yield improvement by wheat. The possible sources of these rotational benefits will be examined in further detail below.

Uptake of legume N by following crops

The fate of N in legume residues is often measured using ¹⁵N-labeled leguminous materials (discussed by Crews and Peoples, 2005). Such studies indicate generally that <30% of the legume N is taken up by a subsequent crop (Table 7; see also Giller and Cadisch, 1995; Fillery, 2001; Crews and Peoples, 2005). A notable feature of the decomposition and release of N from legume organic material is that, after a period of time, the subsequent rate of mineralization can be quite slow, regardless of the initial quality of the residues, so that the legume N not mineralized during the first season becomes available only very slowly thereafter (usually <5–10% per year) for successive crops (Fillery, 2001). The relatively slow, low recovery of legume N by following crops, particularly in temperate regions, has led to suggestions that legumes are an inefficient short-term source of N (Hesterman et al., 1987; Harris et al., 1994). Certainly there are many examples in Table 7 where <15% of the N in a following crop appear to be derived from the prior legume. However, there are also situations where legume sources have provided a significant proportion (20–33%) of the next crop's N requirements (Table 7).

Typically the ¹⁵N-labeled legume inputs utilized in experiments such as those presented in Table 7 represent only shoot material. This ignores the potentially large amounts of below-ground legume N associated with, or derived from, roots and nodules (Rochester et al., 1998; Khan et al., 2002). Under Australian field conditions wheat (*Triticum aestivum*) has been reported to utilize between 3–10% of the residual below-ground N from a previous lupin crop (McNeill and Fillery, 2008), or 8% and 15% of the below-ground N of prior faba bean and chickpea crops, respectively (Khan, 2000). In the case of faba bean and chickpea, this uptake of below-ground N contrasted with an uptake by wheat of just 3% of the residual shoot N (Khan, 2000).

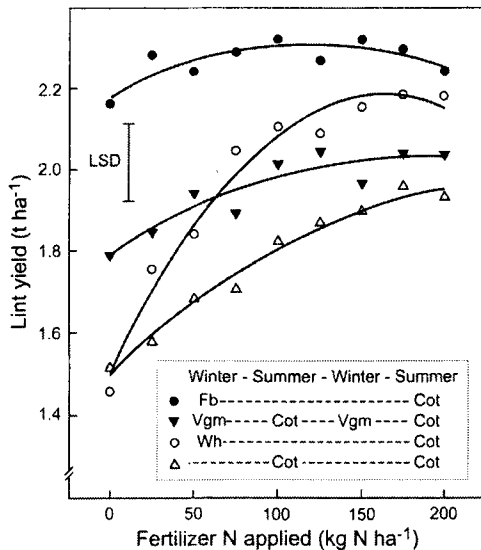


Figure 4. Australian field data comparing the yield response of irrigated cotton to increasing rates of fertilizer N in cropping sequences that either omit legumes (open symbols) or include legumes (closed symbols). The open triangle symbols (Δ) indicate continuous cotton (Cot), the open circles (\circ) growing a wheat crop (Wh) in winter-spring each second year, the closed inverted triangles (\blacktriangledown) indicate a short duration vetch green manure (Vgm) each winter, and the closed circles (\bullet) indicate growing a faba bean crop (Fb) in winter-spring every second year [the growing season of the wheat and faba bean crops (April–October, November harvest) is too long to accommodate sowing cotton (September) in the same calendar year]. Derived from unpublished data of Rochester.

Other studies suggest that below-ground legume N plays a key role in contributing to the soil pool of particulate organic matter (Schwenke et al., 2002) and may be the source of between 30–75% of the total mineral N accumulating after legumes (Evans et al., 2003). Thus the below-ground pool of legume N appears to be an important source of N for following crops. Paradoxically, it has often not even been considered.

Studies that estimate uptake efficiencies of labeled N from recently applied legume residues also have a tendency to underestimate the overall N-supplying capacity of a legume-based system. This is likely to be the result of N ‘pool substitution’ whereby the newly applied ^{15}N -labeled legume N is immobilized in the microbial biomass and unlabeled N is mineralized. The importance of pool substitution was illustrated by Murphy et al. (1998) in a ^{15}N experiment in Western Australia. In the second year of a lupin-wheat rotation, they found gross N mineralization in the top 10 cm of soil to be 120 kg N ha^{-1} , and net N mineralization (i.e. gross mineralization-immobilization) to be only 59 kg N ha^{-1} , 69% of which (41 N kg) originated from the soil microbial pool. These data suggest that most of the N initially released from lupin residues was immobilized and thus inaccessible to the wheat crop in the

short term. This was compensated for by mineralization of older (unlabeled) microbial-N that subsequently became available for crop uptake. Microbial utilization of labile legume N was also a feature of a field study in Canada where 65–75% of the ^{15}N -labelled legume N was detected in the microbial biomass 7 months after incorporation of the legume material (Bremer and van Kessel, 1992a).

The net result of such processes is that calculations based on crop recovery of ^{15}N -labeled leguminous material are often lower than determinations of ‘agronomic’ N benefits derived from including a legume in a rotation. This is demonstrated in the data from a study of faba bean-wheat and barley (*Hordeum vulgare*)-wheat cropping sequences presented in Table 8 where the measured N benefit to wheat from the prior faba bean crop was 4-fold higher than predicted from the wheat’s uptake of faba bean ^{15}N . The interpretation of such data may be further confounded by a carryover of soil nitrate unutilized by the legume crop (Herridge et al., 1995), and possibly a greater degree of immobilization of soil mineral N by the high C:N ratio of the barley stubble with the barley-wheat treatment than with legume residues (e.g. Green and Blackmer, 1995). Lower disease incidence in wheat after faba bean than in wheat after barley, and thus resultant healthier root systems better able to utilize existing soil N or applied N, may also be implicated (Stevenson and van Kessel, 1996).

Rotational benefits not related to N

Impacts on soil structure or nutrient and water availability

Examples of the effect of including legumes in a cropping sequence on the availability of nutrients other than N is relatively limited, although there is evidence that legume species such as chickpea, pigeon pea and white lupin (*Lupinus albus*) can mobilize fixed forms of soil phosphorus by the secretion of organic acids such as citrate and malate (and other compounds) from their roots (Hocking, 2001) and influence supply of plant-available phosphorus for subsequent crops (Nuruzzaman et al., 2005). Tap-rooted legume species can also assist the roots of following crops to explore a larger soil volume through improvements in soil aggregate structure and organic carbon (Rochester et al., 2001; Aslam et al., 2003; Shah et al., 2003), the penetration of soil hardpans, and by providing a continuous network of residual root channels and macropores in the subsoil (e.g. Lesturgez et al., 2004; McCallum et al., 2004). Species such as pea use less water than other crops (Merrill et al., 2007). Such carryover of available soil water after legumes has been identified as an important factor contributing to higher yields by following wheat crops (Miller et al., 2002).

Impacts on soil biology

In addition to reducing the inoculum potential of some cereal soil-borne pathogens as a result of growing legumes

can also influence the populations of specific rhizosphere organisms which may compete, antagonize or suppress pathogens (Stevenson and van Kessel, 1996; Kirkegaard et al., 2008). Some legumes appear to reduce the survival of certain species of nematodes, encourage mycorrhizal associations that assist nutrient uptake, and stimulate the activity of a plethora of soil organisms such as earthworms (Jensen and Hauggaard-Nielsen, 2003; Lupwayi and Kennedy, 2007). Legumes in rotations also result in greater microbial activity and diversity in soils (Lupwayi and Kennedy, 2007). Some symbioses also influence the composition of the microbial population in the legume's rhizosphere *via* the release of molecular hydrogen (H_2) as a by-product of symbiotic N_2 fixation in legume nodules.

About 35% of the energy consumed in the overall nitrogenase activity goes towards H_2 production (Hunt and Layzell, 1993). In some legume systems, the rhizobial bacteria possess a hydrogenase uptake system (uptake hydrogenase, designated Hup^+) that is able to recycle almost all of the H_2 evolved and recover most of the energy that might otherwise be lost (Evans et al., 1988). The Hup^+ trait appears to be common in nodules formed by *Bradyrhizobium* spp. representing 64–77% of host-strain combinations tested (Evans et al., 1988; Arp, 1992). However, less than one-quarter of *B. japonicum* strains effective on soybean appear to be Hup^+ (Uratsu et al., 1982). The characteristic also seems to be rare in legumes nodulated by strains of *Rhizobium*, *Sinorhizobium*, or *Mesorhizobium* spp. (Evans et al., 1988; Arp, 1992). In those symbiotic associations that either lack the hydrogenase enzyme (Hup^-), or have low Hup activity, the H_2 produced diffuses out of the nodules into the soil. Measurements of H_2 evolution from Hup^- nodules in the field show that >200,000 litres of H_2 gas per ha may be released into the soil over a growing season by soybean fixing around 200 kg N ha^{-1} (Peoples et al., 2008). Whether a particular rhizobial strain-legume host combination is Hup^+ or Hup^- seems to be controlled by the host. For example, when either of the rhizobial strains CB756 or 32H1 were applied to several *Vigna* species, the nodules were found to be Hup^- on green gram, but were Hup^+ on cowpea and black gram (Gibson et al., 1981). There may also be differences between legume species in the extent to which H_2 is evolved from Hup^- nodules during the N_2 -fixing process. Gibson et al. (1981) reported that the ratio of H_2 evolved: N_2 fixed varied from 4–5:1 for lupin nodules, 2:1 for pea and cowpea nodules, and 1.5:1 for soybean nodules.

In the case of Hup^- symbioses the exposure of soil to the H_2 released from the nodules results in the rapid buildup of populations of micro-organisms in the immediate vicinity of the legume roots that are able to oxidize the H_2 so that, in a short space of time, all the H_2 emitted is consumed within a few centimeters of the nodules and none escapes from the soil surface (Dong et al., 2003). There appear to be

substantial consequences of the H_2 emissions, and its uptake by the soil microflora, on soil characteristics. Experiments undertaken in controlled conditions and in the field have pointed to improvements in plant growth in soils previously exposed to H_2 (see Fyson and Oaks, 1990; Dong et al., 2003; Kirkegaard et al., 2008). Whatever the factors involved in this stimulation in plant growth, field studies that have compared crop performance following soybean symbioses that either recycled (Hup^+) or emitted H_2 from nodules (Hup^-) have indicated that the Hup^- trait significantly ($P < 0.05$) increased grain yield of a succeeding barley crop by 48% (2.9 and 4.3 t ha^{-1} for Hup^+ and Hup^- ; Dean et al., 2006), or a succeeding maize (*Zea mays*) crop by 32% (1.43 and 1.89 t ha^{-1} for Hup^+ and Hup^- ; Peoples et al., 2008). If these findings can be confirmed for other crops, and in other environments, there would be strong reason to recommend that future decisions on the choice of rhizobial strains for inoculants should consider ensuring that the resultant symbioses are Hup^- to facilitate these additional yield advantages in following food crops.

6. Conclusions and Future Prospects

While the calculated inputs of fixed N by food legumes and the carryover of fixed N for the benefit of following crops may seem relatively small when compared to the 85 million t N applied as fertilizer each year, there are a number of environmental and rotational advantages in relying upon N_2 fixation rather than fertilizer N to produce high-quality protein (Jensen and Hauggaard-Nielsen, 2003; Crews and Peoples, 2004; Kirkegaard et al., 2008). Strategies are available to improve N_2 fixation beyond what is currently being achieved. Provided that a legume crop is abundantly nodulated and effectively fixing N_2 , enormous benefits in terms of crop production and N_2 fixed can be derived from the application of good agronomic principles. However, in many parts of the world, the relevant technologies are either not in the hands of the farmers, or they cannot be adopted because of lack of knowledge or infrastructure, economic constraints, or operational imperatives.

These issues have been raised before (Giller and Cadisch, 1995; Peoples et al., 2002b; Crews and Peoples, 2004), but the ability to overcome constraints at the farm level, or to undertake applied N_2 fixation research that will be of direct benefit to farmers, continues to deteriorate rather than improve. The poor countries become poorer in this regard and the training and support programs like NifTAL that were instrumental in advancing N_2 fixation in the past (particularly in the developing world) have been wound up or redirected. Unhappily, N_2 fixation research and development currently receives little support from the influential CGIAR institutes. There is a need for some strong policy intervention to redress this trend.

Table 7. Examples of field experiments undertaken to determine the extent of utilization of ¹⁵N-labelled legume N by a following crop^a.

Legume residue Species	Amount applied (kg N ha ⁻¹)	Following crop		Proportion of crop N derived from legume (%)	Reference
		Species	Legume N taken up (% applied)		
Soybean	180	Rice	11	19	Norman et al., 1990
	21–27	Rice	15–23	4–7	Toomsan et al., 1995
	150	Oats	8	33	Bergersen et al., 1992
	132	Cotton	16	10	Rochester and Peoples, unpubl.
Groundnut	74–166	Rice	8–22	10–30	Toomsan et al., 1995
Pea	45	Oats ^b	31	8	Hauggaard-Nielsen and Peoples, unpubl.
	130	Barley ^c	6–15	12–13	Jensen, 1994
Lentil	49	Wheat	12	6	Stevenson and van Kessel, 1997
	45	Wheat	6	1	Bremer and van Kessel, 1992b
Faba bean	73	Barley	17	19	Muller and Sundman, 1988
	43	Cotton	11	2	Rochester and Peoples, unpubl.
Lupin (<i>Lupinus angustifolius</i>)	36–86	Wheat	21–27	6–18	Russell and Fillery, 1999

^aData obtained from experiments undertaken in different locations in South-East Asia, North America, Europe, and Australia. ^bTotal uptake of pea N by oats (*Avena sativa*) was determined from analyses of 2 harvests of above-ground material. The first was taken prior to grazing by livestock, and the second represented the oat re-growth. ^cWinter and spring barley comparison. Spring barley recovered a smaller % of pea residue N than did winter barley.

Table 8. Comparison of observed agronomic N benefits for wheat following a faba bean crop using ¹⁵N-based estimates of direct wheat uptake of legume-N^a.

Parameter	Cropping sequence	
	Faba bean-wheat	Barley-wheat
Residue N remaining from faba bean or barley (kg N ha ⁻¹)	96 ^b	73 ^b
Wheat N at maturity (kg N ha ⁻¹)	97	59
Wheat N benefit from legume (kg N ha ⁻¹)	38 ^c	
Non-isotopic estimate of recovery of legume N (%)	40 ^d	
¹⁵ N-based estimate of recovery of legume N (%)	11 ^e	

^aAdapted from data of Khan (2000). ^bIncludes shoot vegetative N remaining after grain harvest and an estimate of below-ground N. ^cCalculated as: (wheat N after faba bean) – (wheat N after barley) = 97–59 = 38 kg N ha⁻¹. ^dCalculated as: 100 × (wheat N benefit) / (faba bean residue N) = 100 × (38)/(96) = 40%. ^eCalculated from the measured recovery of the legume residue ¹⁵N present in the wheat crop.

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