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## Review article

# Models of biological nitrogen fixation of legumes. A review

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**Abstract** – Leguminous crops have the ability to fix nitrogen (N) biologically from the atmosphere. This can benefit not only the legumes themselves but also any intercropped or subsequent crops, thus reducing or removing the need to apply N fertilizers. Improved quantification of legume biological nitrogen fixation (BNF) will provide better guidance for farmers on managing N to optimise productivity and reduce harmful losses to the environment. There are many techniques available for the direct quantitative measurement of legume BNF in the field and in controlled environments. However, these are time-consuming and therefore expensive, and generate data relevant only to the time and place of measurement. Alternatively, legume BNF can be estimated by either empirical models or dynamic mechanistic simulation models. Comparatively, simulation by a dynamic model is preferable for quantifying legume BNF, because of its capability to simulate the response of N fixation to a wide range of environmental variables and legume growth status. Currently there is no published review of the approaches used to simulate, rather than measure, legume BNF. This review of peer-reviewed literature shows that most simulation models estimate the N fixation rate from a pre-defined potential N fixation rate, adjusted by the response functions of soil temperature, soil/plant water status, soil/plant N concentration, plant carbon (C) supply and crop growth stage. Here, we highlight and compare the methods used to estimate the potential N fixation rate, and the response functions to simulate legume BNF, in nine widely-cited models over the last 30 years. We then assess their relative strengths in simulating legume BNF with varying biotic and abiotic factors, and identify the discrepancies between experimental findings and simulations. After this comparison, we identify the areas where there is the potential to improve legume BNF simulation in the future. These include; (1) consideration of photosynthetic C supply, (2) refining the various effects of soil mineral N concentration, (3) characterization and incorporation of excess soil water stress and other factors into models, and (4) incorporation of the effects of grazing, coexistence and competition with intercrops and weeds into models to improve their practical relevance to sustainable agricultural systems. This review clarifies, for the first time, the current progress in legume BNF quantification in simulation models, and provides guidance for their further development, combining fundamental experimental and modelling work.

**nitrogen fixation / soil mineral nitrogen / legume / simulation / review**

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## 1. INTRODUCTION

Increasing demand for agricultural production typically results in greater fertilizer application (Whitehead, 1995; Wood, 1996). Nitrogen (N) applied in fertilizers or manures which is not taken up by crops can be released into the atmosphere as nitrogenous greenhouse gases (Flechard et al., 2007) or leached into ground water (Stout et al., 2000; Trindade et al., 2001), with resulting environmental implications. Rather than relying purely on applications of N fertilizer, alternative N sources are needed to help develop more sustainable farming systems. Legumes have the potential to fulfil this requirement due to their unique ability to fix N biologically from the atmosphere, benefiting not only the legumes themselves but also the intercropped or subsequent crops.

The ability to fix atmospheric N comes from the symbiotic relationship between legumes and rhizobia, bacteria in soil, through which the legume supplies energy and carbon (C) to rhizobia through the products of photosynthesis, and rhizobia provides the legume with N, mainly in the form of ammonium (Howard and Rees, 1996). The symbiosis initiates from the infection of legume roots by *Rhizobium*, forming root nodules where N fixation occurs. The N fixation process needs the nitrogenase enzyme, as a catalyst for the reaction to split the nitrogen molecule and change it into ammonia with energy and electron supply (Postgate, 1982). Garg and Geetanjali (2007) reviewed the processes and signalling involved in symbiotic N fixation in legume nodules at a micro scale.

This kind of symbiosis exists in many types of legumes, including grain legumes, forage legumes, and some leguminous trees. Grain legumes and forage legumes are used to build up soil N fertility in sustainable farming systems (Hossain et al., 1996; Jensen, 1997; McCallum et al., 1999), especially in temperate zones. The benefit to soil N fertility from grain/forage legumes is positively correlated with N fixation (Evans et al., 1989, 2001) assuming that the plant residues are incorporated into soil after crop harvest. Therefore, quantifying legume biological N fixation (BNF) is very important for a better understanding of how to design sustainable farming systems, where subsequent crops benefit from legume N fixation, and where N losses and thus environmental pollution may be reduced.

There are many techniques available for measuring legume BNF in the field and in controlled environments (Goh et al., 1978; Sheehy et al., 1991; Herridge et al., 2008; Carlsson and Huss-Danell, 2008). Most of these methods involve destructive sampling of plants or disturb the original soil structure, except for the acetylene ( $C_2H_2$ ) reduction assay (Carlsson and Huss-Danell, 2008). However, the  $C_2H_2$  reduction assay can only be used over a short time period (minutes or hours), and legume N fixation rate varies seasonally with growth stage of

the plant, so the time of measurement affects precision and reliability (Carlsson and Huss-Danell, 2008). These methods are able to reveal the response of N fixation to varying factors in real situations, but they are limited to the conditions prevailing at the time of measurement and cannot be used to predict N fixation.

Thus, there is a need for methods to predict legume BNF quantitatively to help make decisions about developing and managing sustainable farming schemes. Estimating N fixation from crop yield or empirical models based on historical crop data can be used, but these are restricted to a particular site for the same crop. Modelling is potentially a better tool to understand and quantify legume BNF as it is based on knowledge of the mechanisms involved, climate and management, and can accurately represent variations in legume BNF under different situations. However, models require very detailed information on the environment and plant genetic performance, in addition to historical crop datasets for calibration and validation. This means that models can not be applied to a given site unless this data is available.

A further complication is that the mathematical functions used to simulate legume BNF vary between models. For example, the rate of potential N fixation is estimated either by plant N demand and uptake (Bouniols et al., 1991; Cabelguenne et al., 1999), nodule biomass (Boote et al., 2002; Wu and McGechan, 1999), root biomass (Thornley et al., 1995; Soussana et al., 2002) or aboveground biomass (Sinclair, 1986; Robertson et al., 2002). Also the response of N fixation to soil mineral N concentration in the root zone, which is closely correlated to N fixation and thus to the benefit to soil N fertility from legumes (Evans et al., 1987, 1989), differs between models. N fixation is either very sensitive (Wu and McGechan, 1999; Schwinning and Parsons, 1996) or tolerant (Sharpley and Williams, 1990; Bouniols et al., 1991) to high soil N concentration in different models.

In this paper we explore and compare the modelling methods published for quantifying legume BNF by grain and forage legumes at the plot/field scale. More specifically, we contrast the different schemes and functions used to simulate the response of N fixation to biotic and abiotic factors and analyse their relative strengths and weaknesses. We then identify gaps in the current models and make suggestions to improve the simulation of legume BNF in future models.

## 2. FACTORS INFLUENCING LEGUME BIOLOGICAL NITROGEN FIXATION

The complex process of legume BNF is affected by environmental conditions such as temperature, water content, N

concentration, root zone pH, plant nutrient status including C and N substrates in roots, and genetic variation in potential N fixation capacity. It is also affected by plant nutritional status such as phosphorus (P) and potassium (K) levels that control nodule growth and nitrogenase activity directly or indirectly (Havelka et al., 1982). In this section we focus only on how legume BNF responds to environmental conditions and plant C and N substrates with a view to improving models.

### 2.1. Temperature

Generally, soil temperature inhibits legume BNF through its control on nodulation, nodule establishment, and nitrogenase activity when it is either too high or too low (Roughley and Dart, 1970; Roughley, 1970; Whitehead, 1995). Therefore, minimum and maximum soil temperatures, and the range of temperature between these which are favourable for N fixation, could be used to define the response of N fixation to soil temperature.

Soil temperature in the root zone is one of controlling factors for nodulation and nodule establishment. For example, the nodulation of arrowleaf clover (*Trifolium vesiculosum* Savi.) is accelerated at a root temperature of 25 °C compared with that growing at both 18 °C and 32 °C (Schomberg and Weaver, 1992). However, the response of nodule establishment to soil temperature differs between species and varieties. In soybean (*Glycine max* (L.) Merr.), more nodules are produced in the early growth stage at 25 °C, while 20 °C is optimal for nodule size after nodule generation is completed compared to 15 °C and 30 °C (Lindemann and Ham, 1979). In contrast, nodule establishment is enhanced with increasing temperature in the range of 10–35 °C for white clover (*Trifolium repens* L.) regardless of the varieties and the rhizobia strains (Richardson and Syers, 1985; Whitehead, 1995).

Nitrogenase activity responds slightly differently to soil temperature between species. There are a large number of studies on the response of N fixation to temperature in legume crops, some of which are summarized in Table I. Minimum temperatures for N fixation differ among species from 2 °C to 10 °C, and normally tropical and subtropical legumes have higher minimum temperatures than temperate species. Nitrogenase activity is high around 12–35 °C and reaches maximum at 20–25 °C in most legumes. N fixation in subterranean clover (*Trifolium subterraneum* L.) is very active at a wide range of temperatures, from 5 °C to 30 °C, but declines dramatically with low temperature and almost stops at 2 °C. Generally, 35 °C or 40 °C is the upper limit of temperature for legume BNF.

### 2.2. Soil water status

In a similar manner to soil temperature, soil water content in the root zone controls N fixation through nodule establishment and nodule activity, plus gas permeability (Sprent, 1976; Weisz et al., 1985; Weisz and Sinclair, 1987; Sinclair et al., 1987). Soil water deficit inhibits N fixation (Whitehead, 1995; Goh and Bruce, 2005), and the inhibition is reinforced

as drought stress becomes more intense (Albrecht et al., 1984). N fixation by peanut (*Arachis hypogaea* L.) grown in soil at two-thirds of field water capacity is reduced by 18–40% compared to that in well-watered soil, and by 44–69% when the soil water is one-third of field capacity (Pimratch et al., 2008). In addition, water-logging can seriously reduce N fixation through depression of the establishment and activity of nodules (Havelka et al., 1982).

It is not easy to quantify the relationship between N fixation and soil water status precisely, due to the limitations of experimental controls, stress periods and plant recovery (Engin and Sprent, 1973; Ledgard and Steele, 1992). Pimratch et al. (2008) tried to determine N fixation under drought stress with multiple-linear regression but the correlation coefficients between the reduction of N fixation under drought compared to under field capacity and the soil water deficit was not statistically significant. A sigmoid function has been used to describe the response of N fixation by common bean (*Phaseolus vulgaris* L.), black gram (*Vigna mungo* (L.) Hepper) and cowpea (*Vigna unguiculata* (L.) Walp) to soil water stress, showing a sharper decline in N fixation as soil water stress becomes more severe (Serraj and Sinclair, 1998; Sinclair et al., 1987).

### 2.3. N concentration in the root zone

It has been widely reported that soil mineral N in the root zone inhibits legume nodulation (Abdel Wahab et al., 1996; Herridge et al., 1984), nodule establishment (Imsande, 1986) and nitrogenase activity (Purcell and Sinclair, 1990; Eaglesham, 1989) as it costs less energy for legumes to take up N from soil than fix N biologically from the atmosphere (Cannell and Thornley, 2000; Phillips, 1980; Ryle et al., 1979; Wood, 1996).

Normally, the severity of inhibition of N fixation by soil mineral N increases with soil mineral N content (Macduff et al., 1996; Lamb et al., 1995; Waterer and Vessey, 1993a; Chalifour and Nelson, 1987). It has been reported that a certain concentration of mineral N in the root zone, defined as “starter N”, stimulates nodule establishment and N fixation compared to non-mineral N in some circumstances. And the concentrations of “starter N” that stimulate legume BNF vary widely with cultivar and growth conditions but are normally less than 4mM for ammonium ( $\text{NH}_4^+$ ) and less than 2 mM for nitrate ( $\text{NO}_3^-$ ) (Bethlenfalvay et al., 1978; Schomberg and Weaver, 1992; Gulden and Vessey, 1997; Gan et al., 2004). However, the time of external N application in relation to legume growth stage affects nodule growth and N fixation, and the later N is applied, the less nodule growth and N fixation is inhibited in field pea (*Pisum sativum* L.) (Jensen, 1986; Waterer and Vessey, 1993b). This is probably due to nodules being well-established in the early stages before N is applied. Moreover, the inhibition of N fixation by  $\text{NO}_3^-$  was more severe than that of  $\text{NH}_4^+$  in white clover, field pea and soybean (Svenning et al., 1996; Bollman and Vessey, 2006; Gan et al., 2004) even though high amounts of either  $\text{NH}_4^+$  or  $\text{NO}_3^-$  retarded N fixation.

**Table I.** Response of N fixation to soil temperature (°C) for legume species.

Species	Minimum	Optimum range	Maximum	Reference
Alfalfa ( <i>Medicago sativa</i> L.)	2	20–25		Waughman (1977)
	2	35	40	Dart and Day (1971)
Arrowleaf clover ( <i>Trifolium vesiculosum</i> )		25		Schomberg and Weaver (1992)
Barrel medic ( <i>Medicago truncatula</i> Gaertn.)	2	20	35	Dart and Day (1971)
Big-leaved lupin ( <i>Lupinus polyphyllus</i> Lindl.)	1.5	25		Waughman (1977)
Birdsfoot trefoil ( <i>Lotus corniculatus</i> L.)	10	25–27	35	Rao (1977)
			35	Piha and Munns (1987)
Common bean ( <i>Phaseolus vulgaris</i> L.)			40	Michiels et al. (1994)
Common vetch ( <i>Vicia sativa</i> L.)	2	20	40	Dart and Day (1971)
Cowpea ( <i>Vigna unguiculata</i> (L.) Walp)	5	40		Dart and Day (1971)
	2	20–30	40	Dart and Day (1971)
Faba bean ( <i>Vicia faba</i> L.)	5	20		Waughman (1977)
	5	15–25		Halliday (1975)
Field/garden pea ( <i>Pisum sativum</i> L.)	0.5	25		Waughman (1977)
		20–26		Lie (1971)
Guar ( <i>Cyamopsis tetragonoloba</i> Guar)			37–40	Pate (1961)
Narrowleaf lupin ( <i>Lupinus angustifolius</i> L.)	10	20–30		Halliday (1975)
Peanut ( <i>Arachis hypogaea</i> L.)			40	Kishinevsky et al. (1992)
Purple vetch ( <i>Vicia atropurpurea</i> Desf.)		24		Pate (1961)
	2	25	35	Dart and Day (1971)
Red clover ( <i>Trifolium pratense</i> L.)		12–26		Small and Joffe (1968)
		27		Kuo and Boersma (1971)
Soybean ( <i>Glycine max</i> L.)		20–25		Lindemann and Ham (1979)
	5	20–35	40	Dart and Day (1971)
	2	5–30		Dart and Day (1971)
Subterranean clover ( <i>Trifolium subterraneum</i> L.)	5	12–32		Gibson (1971)
		15		Roughley and Dart (1969)
	5	13–26		Halliday and Pate (1976)
		26		Small and Joffe (1968)
White clover ( <i>Trifolium repens</i> L.)		21		Masterson and Murphy (1976)
	9			Frame and Newbould (1986)

#### 2.4. Carbon demand for fixation

Photosynthate partitioned to roots supports nodule growth, provides energy for N fixation, maintains a functional population of rhizobia, and allows the synthesis of amino compounds produced from N fixation (Minchin and Pate, 1973; Layzell et al., 1979; King et al., 1986). Even though it is difficult to distinguish the proportion of CO<sub>2</sub> generated by N fixation from that generated by respiration for nodule growth and maintenance (Warembourg and Roumet, 1989), the correlation between the rate of CO<sub>2</sub> produced from either nodulated roots or nodules and N fixation rate may be used to evaluate C consumption by N fixation (Lawrie and Wheeler, 1973; Mahon, 1977a, b; Warembourg and Roumet, 1989).

The C cost per unit fixed N (gC per g N fixed) varies widely with species, growth stage and environmental conditions, and ranges from 1.4 to 8.5 (Minchin and Pate, 1973; Phillips, 1980; Minchin et al., 1981; Sheehy, 1987; Schulze et al., 1999). For example, it is reported that the C cost is 1.54 gC g N fixed<sup>-1</sup> in cowpea and 3.64 gC g N fixed<sup>-1</sup> in white lupin (*Lupinus albus* L.) from nodules during early vegetative stage (Layzell et al., 1979; Layzell et al., 1981) while it is 6.3–6.8 gC g N fixed<sup>-1</sup> for soybean, cowpea and white clover from nodulated roots at periods of intense N fixation (Ryle et al., 1979). The C

cost determined by CO<sub>2</sub> released from nodulated roots is generally higher than that from nodules as the former includes root respiration. The C cost of N fixation also varies with growth stages (Ryle et al., 1979; Twary and Heichel, 1991), but it is a matter of debate that the C cost increases (Warembourg and Roumet, 1989; Voisin et al., 2003) or decreases (Adgo and Schulze, 2002) with the course of legume life cycle. In addition, the strain of *Rhizobium* may affect the C cost significantly. For example, the C cost in alfalfa (*Medicago sativa* L.) nodules formed by strain P207 is an average 9.4 molC molN<sup>-1</sup> which is 59% higher than that of nodules formed by strain I02F51, 5.9 molC molN<sup>-1</sup> (Twary and Heichel, 1991).

#### 2.5. Seasonal regulation of legume biological nitrogen fixation

The rate of legume BNF changes with physiological growth stages. It is low in the early growth stages while nodules are establishing (Lawrie and Wheeler, 1973) and reaches a maximum value between early flowering and early seed-filling, depending on the species and growing conditions (Lawn and Brun, 1974; Klucas, 1974; Nelson et al., 1984; Jensen, 1987). After the peak, N fixation decreases dramatically or even

**Table II.** Parameter values used to estimate N fixation in equation (1).

Species	Condition	$N_{con}$ (%)	$\%Ndfa$ (%)	$R_{root}$	Reference
White clover	Stock camp	4.9	61	–	
White clover	Gentle slope	4.9	82	–	
Subterranean clover	Gentle slope	4.9	82	–	Ledgard et al. (1987)
Subterranean clover	Steep slope	4.9	82	–	
Slender birds-foot trefoil	Steep slope	4.9	82	–	
Lucerne	Cut 1–2 years	2.7	74	0.25	
Red clover	Cut 1–2 years	3.0	74	0.25	
White clover	Intercropping with grass, cut 1–2 years	4.3	95	0.25	
White clover	Intercropping with grass, grazed 1–2 years	4.3	80	0.25	Høgh-Jensen et al. (2004)
Red clover	Intercropping with grass, cut 1–2 years	3.0	95	0.25	
Red clover	Intercropping with grass, grazed 1–2 years	3.0	80	0.25	
White clover	Intercropping with grass, cut >2 years	4.3	95	0.25	
White clover	Intercropping with grass, grazed >2 years	4.3	75	0.25	
White clover	1st cut	3.94	92.3	For all cuts, 0.27 (year 1 and 2), 0 (year 3)	Korsaeth and Eltun (2000)
	2nd cut	3.49	92.3		
Red clover	1st cut	3.14	92.3		
	2nd cut	2.91	92.3		
Grey peas		2.80	65.4	0.045	
Common vetch		3.00	65.4	0.27	

ceases during seed-filling (Herridge and Pate, 1977; Beverly and Jarrell, 1984; Sinclair et al., 1987), due to nodule senescence (Lawrie and Wheeler, 1973) and poor C supply as a result of the strong demand for seed dry matter accumulation (Herridge and Pate, 1977; Voisin et al., 2003, 2007).

### 3. QUANTIFICATION OF LEGUME BIOLOGICAL NITROGEN FIXATION

Legume BNF may be quantified by direct measurement, estimation based on yield or with empirical models, or simulation by crop models. The methods used to measure N fixation directly so far, such as the acetylene reduction/hydrogen increment assay, N difference,  $^{15}\text{N}$ -labelling and ureide, have been thoroughly reviewed (Herridge et al., 2008; Carlsson and Huss-Danell, 2008). The review of these methods is beyond our focus, and in this paper we describe and compare only the methods to estimate and simulate N fixation.

#### 3.1. Estimation with empirical models

A static estimation of N fixation during the whole growing season may use either economic yield or above-ground dry matter. The equation is:

$$N_{fix} = \alpha \cdot DM \cdot f_{leg} \cdot N_{con} \cdot \%Ndfa \cdot (1 + R_{root}) \quad (1)$$

where  $DM$  is the yield or aboveground dry matter,  $f_{leg}$  is the proportion of legume if it is intercropped,  $N_{con}$  is the N concentration in the legume,  $\%Ndfa$  is the proportion of total plant

N derived from  $\text{N}_2$  fixation, and  $R_{root}$  is the ratio of the fixed N belowground to the fixed N aboveground. Values for the parameters  $f_{leg}$ ,  $N_{con}$  and  $R_{root}$  for different species at a range of sites are summarized in Table II.  $\alpha$  is a parameter that has different definitions according to author. For example,  $\alpha$  may represent the decline in  $\%Ndfa$  under high soil mineral N conditions, in terms of net mineral N input ( $\text{g N m}^{-2}$ ), and is calculated as:

$$\alpha = 1 - \beta \cdot N_{net.inorg} \quad (2)$$

where  $\beta$  evaluates the sensitivity of legume BNF to soil mineral N (Korsaeth and Eltun, 2000). This is set to 0.028 for white clover (*Trifolium repens* L.) and red clover (*Trifolium pratense* L.), 0.043 for grey peas (*Pisum arvense* L.) and common vetch (*Vicia sativa* L.). Alternatively,  $\alpha$  is used as a modifier to compensate for the proportion of fixed N transferred to a companion crop, consumed by grazing animals, or lost by immobilization (Høgh-Jensen et al., 2004).

The second method to estimate N fixation is by empirical models based on the correlation of fixed N in the final yield against variables, such as harvested dry matter or the proportion of legume in mixed leys. A linear equation has been fitted to the measured data of mixed white clover and grass swards at different sites from four countries. This showed a significant correlation between fixed N and the extra dry matter of mixed leys, compared with corresponding pure grass either for cut swards or grazed swards (Watson and Goss, 1997). Kristensen et al. (1995) found fixed N at harvest increased linearly with clover dry matter content in mixed swards through statistically analyzing the experimental data from different sites with

**Table III.** Values of parameters,  $c$  and  $d$ , in linear empirical models expressed in equation (3).

Condition	$c$	$d$	Model reference
Cut swards	6.8	0.067	Watson and Goss (1997)
Grazed swards	-168.1	0.067	
1–2 cropping years	18	4.47	Kristensen et al. (1995)
3–5 cropping years	19	2.77	

distinct soil types and irrigation schemes. The equation for the estimation of N fixation ( $\text{kg N ha}^{-1}$ ) is summarized as,

$$N_{fix} = c + d \cdot Leg \quad (3)$$

where  $Leg$  is the extra dry matter increment ( $\text{kg ha}^{-1}$ ) in white clover mixed leys compared with a pure grass ley in Watson's estimation, and clover dry matter content (%clover) in clover mixed leys for Kristensen's study (1995), respectively; and  $c$  and  $d$  are parameters the values of which are summarized in Table III.

The first method described above is apparently a direct estimation of N fixation, and the parameter values can be easily measured on site or estimated from the literature. It does not strictly require an adequate dataset for multiple years to determine the parameters, so it is easy to use. However, when determining the parameter values, data from years of abnormal weather should be avoided, and the properties of the soil should be relatively stable year-on-year. As these equations are independent from environmental factors such as soil properties and weather conditions, they are only applicable and accurate for similar sites and average weather conditions. In addition, the parameter values need to be adjusted if the equations are used for different sites or legumes.

In contrast, the second method is based on statistical correlation and assumes that N fixation has a strong linear relationship to the variables. It is more flexible to use and can be applied to one specific site or multiple sites with different soil types, depending on how the empirical relationship is developed and which sites the data were obtained from. This approach has a higher data requirement compared to the first method, and the data should be representative and adequate to guarantee the correlation and determine the parameter values. However, as with the first method, these approaches are restricted to specific sites because the equation is not able to represent the interaction between plant and environment mechanistically.

### 3.2. Mechanistic simulation of legume biological nitrogen fixation as a subroutine in crop models

The simulation of legume BNF in soybean developed by Duffy et al. (1975), probing into the biophysiochemical transformations of N in tile-drained soil, might be the earliest mechanistic model involving leguminous N fixation. The rate of N fixation by soybean in the model depends on the root growth rate, which is derived from the sigmoid relationship with the number of days after planting.

In more recent simulation models of BNF in legumes, the most popular method to estimate the rate of legume BNF is a potential or maximum fixation rate modified by the influence of environmental factors. The potential fixation rate is estimated based on either a demand-uptake mechanism or on the dry matter of plant tissues, and is varied with plant growth stages. The environmental factors normally include soil temperature, soil or plant water content, soil mineral N or substrate N concentration in plant tissues and substrate C concentration in the plant. Other factors, such as soil pH, salinity and the supply of other nutrients, have not been included in models to date.

In this paper we review the most-used recent simulation models in which a legume N fixation function has been implemented (Tab. IV). As crop models may be used under different circumstances, the estimation of N fixation may have various versions within the same model. The general expression of the calculation in the majority of the reviewed models can be written as:

$$N_{fix} = N_{fixpot} f_T f_W f_N f_C f_{gro} \quad (4)$$

while in the EPIC and STICS models it is:

$$N_{fix} = N_{fixpot} f_T \min(f_W, f_N) f_{gro} \quad (5)$$

where,  $N_{fixpot}$  is the potential N fixation rate ( $\text{g N fixed day}^{-1}$ ),  $f_T$  is the influence function of soil temperature,  $f_W$  is a soil water deficit or flooding function,  $f_N$  is the function of soil mineral N or root substrate N concentration,  $f_C$  is the function of substrate C concentration in plant or root,  $f_{gro}$  is the influence factor of growth stage and  $\min$  is the mathematical function to take a minimum value between  $f_W$  and  $f_N$ . There is an extra function,  $f_a$ , representing the limitation by anoxia in the STICS model.

#### 3.2.1. Potential N fixation rate

There are two definitions on a potential legume BNF rate used in the models based either on the difference between N demand and uptake by a legume, or on the N fixation capacity of legume nodules.

The EPIC and APSIM models use variations of the first definition to estimate potential N fixation rate. The EPIC model assumes that the total plant N demand is equal to the potential N fixation (Bouniols et al., 1991; Cabelguenne et al., 1999). APSIM defines critical N concentrations for plant tissues and uses these to estimate N demand by maintaining non-stressed N levels in plant tissues and supporting the N demand of new tissues. This N demand is met by either N uptake from soil and/or N fixation. The former has a higher priority because the process is less energy consuming than N fixation (Macduff et al., 1996); N fixation is only calculated if N uptake can not meet the plant N demand. Thus the potential N fixation is assumed to be the difference between plant N demand and N uptake (Herridge et al., 2001; Robertson et al., 2002).

The second definition is based on the strong relationship between N fixation and either nodule size/biomass (Weisz et al., 1985; Voisin et al., 2003) or root biomass (Voisin et al.,

**Table IV.** Simulation models that include legume BNF, and the factors considered in each model.\* indicates model named here after the first author's name.  $f_T$ ,  $f_W$ ,  $f_N$ ,  $f_C$  and  $f_{gro}$  are the factor of soil temperature, soil/plant water, soil/plant nitrogen, plant carbon and plant growth stage, respectively, used in equations (4, 5).

Model	Factors					Simulated legume specie	Reference
	$f_T$	$f_W$	$f_N$	$f_C$	$f_{gro}$		
Sinclair Model*	✓					soybean	Sinclair (1986)
EPIC	✓			✓		soybean, cowpea, black gram	Sinclair et al. (1987)
Hurley Pasture Model	✓	✓	✓	✓		soybean	Sharpley and Williams (1990); Bouniols et al. (1991); Cabelguenne et al. (1999)
Schwinning Model*			✓	✓		white clover	Thornley (1998); Thornley and Cannell (2000); Thornley (2001)
CROPGRO	✓	✓		✓	✓	white clover	Eckersten et al. (2006)
SOILN	✓	✓	✓			white clover	Schwinning and Parsons (1996); Schmid et al. (2001)
APSIM	✓	✓		✓		soybean, peanut, drybean, velvet bean, faba bean, cowpea	Boote et al. (1998); Sau et al. (1999); Hartkamp et al. (2002); Boote et al. (2002, 2008)
Soussana Model*			✓			white clover	Wu and McGechan (1999)
STICS	✓	✓	✓	✓		white clover	Herridge et al. (2001); Robertson et al. (2002)
						field pea and other legumes	Soussana et al. (2002)
							Brisson et al. (2009); Corre-Hellou et al. (2007, 2009)

2007). As the biomass of both nodules and roots are difficult to measure in the field, some studies have used above-ground biomass to replace nodule/root biomass, based on the relationship between these two variables (Denison et al., 1985; Bell et al., 1994; Yu et al., 2002). The potential fixation rates used in the models are shown in Table V.

### 3.2.2. Impact of soil temperature

The impact of soil temperature on N fixation rate is assumed to follow a four-threshold-temperature function in most of the reviewed models:

$$f_T = \begin{cases} 0 & (T < T_{min} \text{ or } T > T_{max}) \\ \frac{T-T_{min}}{T_{optL}-T_{min}} & (T_{min} \leq T \leq T_{optL}) \\ 1 & (T_{optL} \leq T \leq T_{optH}) \\ \frac{T_{max}-T}{T_{max}-T_{optH}} & (T_{optH} < T < T_{max}) \end{cases} \quad (6)$$

where  $T$  is soil temperature ( $^{\circ}\text{C}$ ),  $T_{min}$  is the minimum temperature below which N fixation ceases,  $T_{optL}$  and  $T_{optH}$  define the optimal temperature range within which the response function to soil temperature is the unit, and  $T_{max}$  is the maximum temperature above which fixation stops. The values of these four temperatures vary among models and are shown in Table VI.

A slightly different function is used in the Hurley Pasture model to simulate the influence of temperature on N fixation and most plant rate responses of white clover (Thornley, 1998):

$$f_T = T^2(45 - T) \times 10^{-4}. \quad (7)$$

There are variations in the response functions adopted in the reviewed models. For example, the functions for white clover

in Hurley Pasture and SOILN differ in curve shapes and threshold temperatures (Fig. 1). There is a much wider range of temperature for N fixation in Hurley Pasture than SOILN, perhaps because the function in the Hurley Pasture model is not specially assigned to the N fixation module, but is used to simulate most plant processes. The function reaches unit at  $20^{\circ}\text{C}$  for both models and the maximum value of the function occurs at  $30^{\circ}\text{C}$  in Hurley Pasture, while the function is zero in SOILN at the same temperature. The value of the function in Hurley Pasture is higher than SOILN when the soil temperature is below  $10^{\circ}\text{C}$ , but this reverses when soil temperature is in the range  $10$  to  $20^{\circ}\text{C}$ . Over  $20^{\circ}\text{C}$  there is a large difference between Hurley Pasture and SOILN. This may be because Hurley Pasture was developed in southern Britain, where the temperature is often between  $4$  and  $16^{\circ}\text{C}$  (Thornley and Cannell, 2000) and thus it might not perform well at much higher temperatures.

### 3.2.3. Impact of soil water status

Soil water stress in the form of either a deficit or excess in the root zone could inhibit nodule nitrogenase activity. The effect of water deficit is considered in some models, but only few models take account for the influence of excess water. An exponential or linear equation derived from experimental data to represent the response of legume BNF rate to soil water deficit is incorporated into most of the reviewed models.

In STICS, the soil is divided into depth layers, and the proportion of these soil layers where water contents are above permanent wilting point is used as the water deficit factor.

In Sinclair's model, an empirical sigmoid relationship between relative nodule nitrogenase activity, expressed as  $\text{C}_2\text{H}_2$ , and transpirable soil water was developed based on

**Table V.** Potential fixed N rate used in the models. Value in APSIM varies with growth stage.

Model	Species	Maximum specific N fixation rate	Unit	Based on	Reference
Sinclair Model	soybean	$0.55-0.7 \times 10^{-3}$	g N gshoot $\text{DM}^{-1} \text{d}^{-1}$	shoot biomass	Sinclair (1986); Sinclair et al. (1987)
	cowpea	$0.7-0.8 \times 10^{-3}$	g N gshoot $\text{DM}^{-1} \text{d}^{-1}$	shoot biomass	
	black gram	$0.7-0.75 \times 10^{-3}$	g N gshoot $\text{DM}^{-1} \text{d}^{-1}$	shoot biomass	
	legumes	$1.0-6.0 \times 10^{-3}$	g N gshoot $\text{DM}^{-1} \text{d}^{-1}$	shoot biomass	
APSIM	soybean, chick pea, mungbean, peanut, lucerne	N demand-uptake	g N $\text{d}^{-1}$	N demand after N uptake	(APSIM source code: <a href="http://apsrunet.apsim.info/svn/development/trunk/apsim/">http://apsrunet.apsim.info/svn/development/trunk/apsim/</a> ) Herridge et al. (2001); Robertson et al. (2002)
EPIC	soybean	total N demand	g N $\text{d}^{-1}$	total N demand	Bouniols et al. (1991); Cabelguenne et al. (1999)
Hurley Pasture Model	clover	0.175	g N gnodule $\text{DM}^{-1} \text{d}^{-1}$	nodule biomass	Brugge and Thornley (1984)
	whiteclover	$0.05-1.0 \times 10^{-3}$	g N groot $\text{DM}^{-1} \text{d}^{-1}$	root biomass	Thornley, 1998; Thornley and Cannell (2000); Thornley (2001)
	pea	0.012–0.027	g N groot $\text{DM}^{-1} \text{d}^{-1}$	root biomass	Eckersten et al. (2006)
CROPGRO	faba bean	0.05	g N gnodule $\text{DM}^{-1} \text{d}^{-1}$	nodule biomass	Boote et al. (2002)
	soybean	0.045	g N gnodule $\text{DM}^{-1} \text{d}^{-1}$	nodule biomass	
SOILN	white clover	0.1106	g N gnodule $\text{DM}^{-1} \text{d}^{-1}$	nodule biomass	Wu and McGechan (1999)
Soussana Model	white clover	0.04	g N groot $\text{DM}^{-1} \text{d}^{-1}$	root biomass	Soussana et al. (2002)
STICS	legumes	–	g N gshoot $\text{DM}^{-1} \text{d}^{-1}$	aboveground growth rate	Brisson et al. (2009)
	field pea	0.028	g N gshoot $\text{DM}^{-1} \text{d}^{-1}$	aboveground growth rate	Corre-Hellou et al. (2007); Corre-Hellou et al. (2009)

**Table VI.** Values of threshold temperatures ( $^{\circ}\text{C}$ ) used in three models that use equation (6) to simulate the effect of temperature on legume BNF.

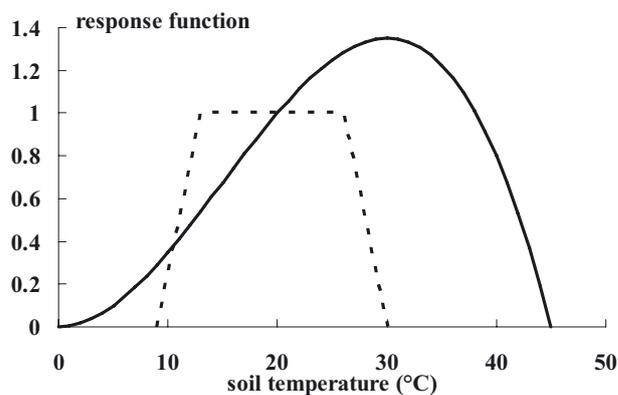
Model	Species	$T_{min}$	$T_{optL}$	$T_{optH}$	$T_{max}$	Reference
CROPGRO	soybean	5	20	35	44	Boote et al. (2008)
	cowpea	5	20	35	44	
	velvet bean	5	23	35	44	
	dry bean	4	19	35	44	
	peanut	7	23	34	44	
	faba bean	1	16	25	40	
SOILN	white clover	9	13	26	30	Wu and McGechan (1999)
STICS	legumes	0	15	25	35	Brisson et al. (2009)

experimental data (Sinclair, 1986; Sinclair et al., 1987):

$$f_w = -1 + \frac{2}{1 + e^{(-m \cdot f_{TSW} + n)}} \quad (8)$$

where  $f_{TSW}$  is the fraction of transpirable soil water, and the total transpirable soil water content is defined as the difference between field capacity and the soil water content when the transpiration rate from drought-stressed plants decreases to less than 10% of that from well-watered plants (Sinclair, 1986);  $m$  and  $n$  are constants which defines the sensitivity of legume BNF to low soil water content. Values of  $m$  and  $n$  are 6 and 0 for soybean, 9 and 0.03 for both cowpea and black gram, respectively.

A linear function is incorporated into APSIM, EPIC (Sharpley and Williams, 1990; Bouniols et al., 1991;

**Figure 1.** The response of N fixation rate in white clover to temperature in the Hurley Pasture Model (solid line) and SOILN (dotted line).

Cabelguenne et al., 1999) and SOILN (Wu and McGechan, 1999):

$$f_w = \begin{cases} 0 & (W_f \leq W_a) \\ \phi_1 + \phi_2 \cdot W_f & (W_a < W_f < W_b) \\ 1 & (W_f \geq W_b) \end{cases} \quad (9)$$

where  $W_f$  is the relative available soil water, expressed as the ratio of available soil water content to that at field capacity,  $\phi_1$  and  $\phi_2$  are coefficients, and  $W_b$  is a critical value of  $W_f$  above

**Table VII.** Parameter values in the response function to soil water deficit.

Model	APSIM											EPIC	SOILN
	soybean	peanut	navy bean	mung bean	lupin	lucerne	faba bean	cow pea	chick pea	butterfly pea	field pea	soybean	White clover
$\phi_1$	-0.33	0	0	0	0	0	0	0	0	0	0	-0.82	0.216
$\phi_2$	1.67	2.5	2	2	2	1.25	2	2	2	2	2	1.82	0.789
Wb	0.8	0.4	0.5	0.5	0.5	0.8	0.5	0.5	0.5	0.5	0.5	1	1
Wa	0.2	0	0	0	0	0	0	0	0	0	0	0.45	-

which N fixation is not inhibited by soil water content and  $W_a$  is the threshold below which N fixation is totally restricted by soil water deficit. The values for the parameters are shown in Table VII.

Although a linear expression similar to equation (9) is used in CROPGRO to simulate the impact of water stress on N fixation, the variable  $W_f$  is defined as the ratio of root water uptake to transpiration demand (Boote et al., 2008). Furthermore, the value of  $W_f$  on the current day is kept to compare with the average of  $W_f$  for the last eight days, to account for the prolonged impact of any previous severe drought on N fixation. The final value of  $W_f$  is equal to the lesser of these two values.

The upper soil layer above 30 cm depth is prone to be dry or wet when drought or irrigation occurs, and this causes a lag between water potential in the root nodules and that in the soil (Sprent, 1972; Albrecht et al., 1984). Therefore, water potential within roots could be a more stable indicator to quantify the relationship between water stress and N fixation. In the Hurley Pasture model, it is assumed that N fixation is controlled through chemical activity in roots, which is further influenced by root water potential and soil temperature. Thus the effect of water on N fixation is combined together with temperature (Thornley, 1998):

$$f_W = e^{20 \cdot \left[ \frac{18 \cdot \phi_{rt}}{8314 \cdot (T_s + 273.15)} \right]} \quad (10)$$

where  $\phi_{rt}$  is root water potential ( $\text{J kg}^{-1}$ ) and  $T_s$  is soil water temperature ( $^{\circ}\text{C}$ ).

Compared with water deficit, the influence of excessive water on the simulation of N fixation has received very little attention in published studies. In Sinclair's model, a simple assumption is adopted that N fixation is set to zero once flooding happens (Sinclair, 1986; Sinclair et al., 1987). In STICS, the restraint of legume BNF by excessive water is considered as a stress from anoxia, which is calculated as the proportion of soil layers that are in anaerobic conditions in the nodulation zone (Brisson et al., 2009). The evaluation of N fixation inhibition by water excess is incorporated into CROPGRO (Boote et al., 2008) by computing the fraction of pore space filled with water. When pore space is fully filled with water, N fixation is inhibited, but the validation of this rule to date is insufficient.

### 3.2.4. Effect of soil mineral N or internal substrate N

A natural logarithmic function to represent the effect of soil mineral N content on N fixation by white clover is incorpo-

rated in SOILN model:

$$f_N = \begin{cases} 1 - 0.0784 \ln N_s & (N_s \geq 1) \\ 1 & (N_s < 1) \end{cases} \quad (11)$$

where  $N_s$  is soil mineral N concentration ( $\text{mg N m}^{-3}$ ).

As N uptake and N fixation are the main N source for legumes, the influence of soil mineral N on legume BNF rate could be presented indirectly by its influence on N uptake from soil. In Schwinning's model, such a scheme is used:

$$f_N = \varepsilon \times (1.0 - f_{Nup}) = \varepsilon \times \left( 1.0 - f_{max} \frac{1}{1 + K_N/N_s} \right) \quad (12)$$

where  $\varepsilon$  is the efficiency of legume BNF and a value of 0.6 was used for white clover,  $f_{max}$  is a maximal fraction of N derived from soil mineral N uptake, which is assumed to be 0.85 for white clover to make sure that N fixation still retains even in high soil mineral N concentrations in the root zone;  $K_N$  is the nitrate content ( $\text{g N m}^{-2}$ ) at which an N uptake rate reaches half its maximal rate and  $N_s$  is the actual soil nitrate content ( $\text{g N m}^{-2}$ ).

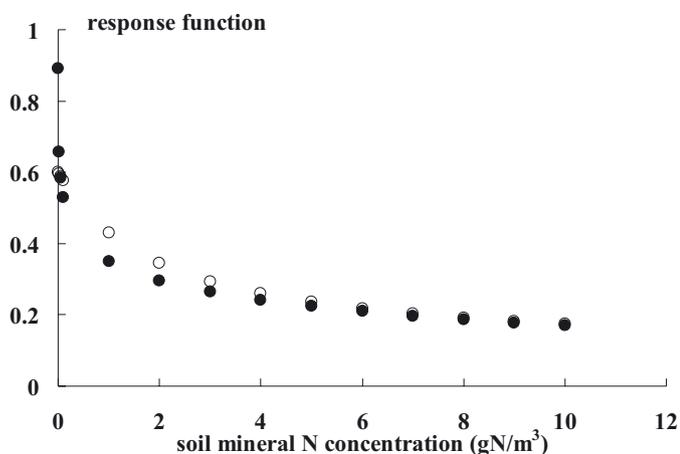
Even though the schemes in SOILN and Schwinning's model are different and  $f_N$  in Schwinning's model is never greater than 0.6 due to the limitation by legume BNF efficiency, they describe a similar trend of N fixation response to soil mineral N by white clover. The values of  $f_N$  from these two functions are very close while soil mineral N is higher than  $0.05 \text{ g N m}^{-2}$  (Fig. 2).

The impact of soil mineral N is assessed as a linear function when soil nitrate concentration ( $N_{sNitra}$ ) is between 10 and  $30 \text{ g N m}^{-3}$  within 0.3 m top soil in EPIC (Sharpley and Williams, 1990; Cabelguenne et al., 1999):

$$f_N = \begin{cases} 1 & (N_{sNitra} \leq 10) \\ 1.5 - 0.05 N_{sNitra} & (10 < N_{sNitra} < 30) \end{cases} \quad (13)$$

A similar function is incorporated in STICS as in EPIC, with a different value of  $N_{sNitra}$ . Moreover, in STICS, nodulation progress is also inhibited by high soil nitrate concentration, which is represented by a reduction of potential N fixation rate. Once the soil nitrate concentration in the nodulation zone is greater than a critical value,  $N_{fixpot}$  equals a baseline value; otherwise,  $N_{fixpot}$  is kept at the normal value (Brisson et al., 2009).

Both the Hurley Pasture Model and Soussana's model use plant substrate N concentration to simulate the influence of N



**Figure 2.** N fixation response to soil mineral N concentration by white clover in the Schwinning Model (closed circle) and SOILN (open circle), assuming the nodulation zone is 40 cm deep.

nutritional status on N fixation rate:

$$f_N = \frac{1}{1 + N_{inter}/K_r} \quad (14)$$

where  $N_{inter}$  ( $\text{g N g}^{-1} \text{r.wt}$ ) is root substrate N concentration in the Hurley Pasture Model or plant substrate N concentration in Soussana's model, respectively; and  $K_r$  is a coefficient inhibiting N fixation at high internal N concentration and it is set to 0.02 for white clover and 0.01 for field pea in the Hurley Pasture Model (Thornley, 1998, 2001; Eckersten et al., 2006), 0.004 for both a normal cultivar and a low-soil-N-uptake breeding cultivar, and 0.0004 for a low-N-fixation breeding cultivar in Soussana's model for white clover (Soussana et al., 2002).

### 3.2.5. Influence of plant substrate C or C supply

Biological N fixation has a high energy demand and the rate of legume BNF depends on the C supply, which is the energy source for N fixation. Experimental observations demonstrate that the enhancement of photosynthetic C allocated to roots which is available for N fixation and nodule establishment increases nodule numbers and N fixation rate (Voisin et al., 2003; Haase et al., 2007; Kirizii et al., 2007). However, among the reviewed models only the Hurley Pasture and CROPGRO models implemented this mechanism.

In the Hurley Pasture Model, a Michaelis-Menten function is used to demonstrate the effect of root substrate C on the N fixation of white clover and field pea:

$$f_c = \frac{1}{1 + K_c/C_r} \quad (15)$$

where  $C_r$  is legume root C substrate concentration ( $\text{gC g}^{-1} \text{r.wt}$ ), and  $K_c$  is a Michaelis-Menten constant, set to be 0.01 for white clover (Thornley, 2001) and 0.035 for field peas (Eckersten et al., 2006).

In CROPGRO, photosynthetic carbohydrate supports not only the energy consumption of N fixation but is also the substrate supply for nodule biomass accumulation. Carbohydrate allocated to nodulated roots is divided into three parts with priorities: requirement for minimum nodule growth, the cost for N fixation and the requirement for nodule normal growth (Fig. 3). First, it needs to guarantee the minimum daily nodule growth (step 1). Then the carbohydrate left over after the reduction for the minimum nodule growth is assumed to be potentially available for N fixation to determine potential N fixation rate (step 2), and in this step carbohydrate is not truly used to fix N until the actual N fixation rate is determined (step 3). If there is any carbohydrate left after the third step, the remainder will be used to produce more nodules (step 4) (Boote et al., 2008).

### 3.2.6. Changes in N fixation with plant growth stage

The quantitative effect of plant growth stage on legume BNF rate is considered in very few models and in general the process is stopped forcibly after the legume attains a certain growth stage. For example, N fixation ceases at the beginning of seed growth for cowpea and black gram, but continues until the end of seed-filling for soybean in Sinclair's model (Sinclair et al., 1987), whereas it stops at physiological maturity in CROPGRO (Boote et al., 2008).

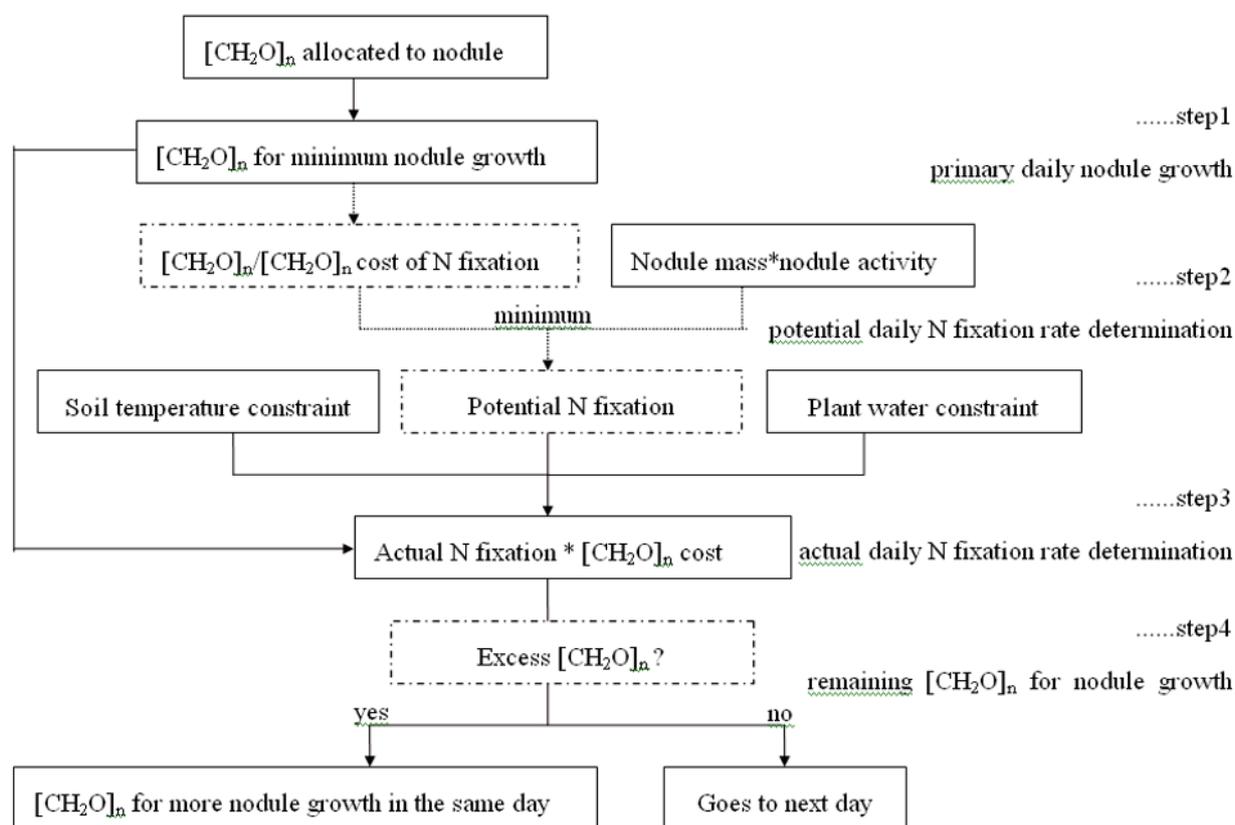
A more specific function, similar to the temperature response function, is incorporated into EPIC and STICS to simulate the seasonal change of N fixation (Sharpley and Williams, 1990; Bouniols et al., 1991; Cabelguenne et al., 1999):

$$f_{gro} = \begin{cases} 0 & (g < g_{min} \text{ or } g > g_{max}) \\ \frac{g - g_{min}}{g_{optL} - g_{min}} & (g_{min} \leq g \leq g_{optL}) \\ 1 & (g_{optL} \leq g \leq g_{optH}) \\ \frac{g_{max} - g}{g_{max} - g_{optH}} & (g_{optH} < g < g_{max}) \end{cases} \quad (16)$$

where  $g_{min}$  is a period before which no fixation happens because of inadequate nodulation, expressed as the percentage of total growing period required;  $g_{optL}$  and  $g_{optH}$  are the beginning and end time within which legume BNF rate is not limited by growth stage; and  $g_{max}$  is the time after which N fixation ceases because of nodule senescence. The values of the parameters for soybean are set to 10%, 30%, 60%, and 80% of the life cycle by Bouniols et al. (1991), and 15%, 30%, 55% and 75% by Cabelguenne et al. (1999). In the STICS model,  $g_{min}$  and  $g_{max}$  are the times of nodule initiation and senescence respectively, and  $g_{optL}$  and  $g_{optH}$  correspond to 27% and 80% of nodule life (Brisson et al., 2009).

### 3.3. Summary of approaches

In addition to direct field measurements, estimates of legume BNF can be based on harvested yield or be derived



**Figure 3.** The representation of daily carbohydrate routes in nodules used in CROPGRO (Boote et al., 2008).  $[\text{CH}_2\text{O}]_n$  in dotted line means that the amount of  $[\text{CH}_2\text{O}]_n$  here is only used for calculation, neither for fixation nor nodule growth.

from simple empirical models. However, these approaches generate data that is strictly valid only for the prevailing conditions at the time and place of measurement, with limited potential for use in wider-scale predictions. Simulation of legume BNF by models that incorporate the kinetics of N fixation may be the best approach as they may help us to understand the nature of the detailed relationships between N fixation and environmental and plant factors. Thus they permit more accurate simulation of legume BNF under a much broader range of farming systems and environmental conditions than other approaches.

Our review has found that the method used to simulate legume BNF most frequently in recent publications is to modify potential N fixation rate by factors such as soil temperature, soil/plant water, soil/plant N, plant C and plant growth stage. However, which of these factors are included and the functions used to relate them to the potential N fixation rate differ between models. Despite this range of approaches, the simulation of legume BNF in recent models may be conveniently summarized as:

(i) Potential N fixation rate may be estimated based on plant N demand and N uptake from the soil, in which case a critical plant N concentration needs to be defined appropriately for each species. Alternatively, if the potential N fixation rate is estimated from nodule/root/aboveground dry matter,

then the maximum specific N fixation rate has to be estimated experimentally, as this value varies widely between species and sites.

- (ii) The influence of soil temperature on legume BNF is commonly represented by a four-threshold-temperature function, or a sigmoidal cubic function in the Hurley Pasture Model (Thornley, 1998). The values of these four threshold-temperatures vary with species and cultivar.
- (iii) The relationship between legume BNF rate and soil water deficit, alternatively represented by low plant water status in some models, is described by either exponential or linear equations. The choice of equations has only a small effect on the output, unless some species are more sensitive than others to water deficit and this is likely as species will have been bred for different environment. However, the effect of excessive water on N fixation is over-simplified or absent in all models and this factor needs more attention.
- (iv) The sensitivity of legume BNF to a factor representing general N availability, e.g. soil mineral N or plant N content, is the function that differs most between different models. In the EPIC model (Sharpley and Williams, 1990; Cabelguenne et al., 1999), legume BNF is unaffected until soil mineral N in the root zone is higher than  $10 \text{ g N m}^{-3}$  whereas in SOILN (Wu and McGechan, 1999) and Schwinning's model (1996) legume BNF is highly sensitive to even small amounts of soil mineral N. This latter

pattern is also seen in the Hurley Pasture Model (Thornley, 2001) and Soussana's model (2002), which use root and plant N concentrations respectively instead of soil mineral N concentration.

- (v) Factors that increase plant C status have a positive effect on legume BNF. The relationships are sometimes represented by Michaelis-Menten functions, or are included in more complex simulations together with nodule growth and the synthesis of the products of legume BNF.
- (vi) The change in legume BNF with legume growth stage follows a function that contains four threshold values in most of the models. However, in APSIM, the same effect is achieved by using different values of potential specific N fixation rate for each growth stage.

#### 4. DISCUSSION

There has been much progress in simulating N fixation by forage and grain legumes since the first simple model by Duffy et al. (1975). However, the analysis presented above has revealed areas of the simulation schemes that are in need of refinement, and identified some potentially important factors omitted from all current models. These areas, and suggestions for their improvement, are discussed below.

It is preferable to define the key parameter of potential N fixation rate based on nodule mass rather than on the plant N deficit after soil N uptake. This is because normally legume BNF is not completely inhibited by soil mineral N and some species and cultivars continue to fix N even when soil mineral N is relatively high (Lamb et al., 1995; Blumenthal and Russelle, 1996). However, not all the models have the potential to estimate nodule biomass accurately. In these cases root or shoot biomass is used instead of nodule biomass to estimate potential N fixation rate, and this is based on the strong relationships between nodule size and plant size (Denison et al., 1985; Sinclair, 1986), and between nodule biomass and root biomass (Macduff et al., 1996). On this basis a constant ratio of nodule biomass to root or shoot biomass is assumed, and linear functions are used to calculate the potential N fixation rates in these models. In practice, however, this ratio varies with legume growth stage (Schiltz et al., 2005; Bollman and Vessey, 2006) and is affected by other environmental factors, such as soil mineral N (Voisin et al., 2002; Bollman and Vessey, 2006). Thus the constant ratio of nodule biomass to root or shoot biomass assumed in some models does not represent the real situation and would be better defined as a temporal factor that changes with environmental conditions. The importance of this is shown by the fact that even when the nodule biomass is available and is used to estimate potential legume BNF, the relationship between legume BNF rate and nodule mass varies with growth stage (Voisin et al., 2003).

The above section has shown how measurements of plant or nodule biomass may be used to modify the maximum potential legume BNF to provide an estimate of legume BNF in the field. However, a potentially more accurate approach is to use the amount and allocation pattern of photosynthetically-fixed C in the plant. Carbon is required for nodule growth, as an en-

ergy source for legume BNF and for the synthesis of N compounds produced from legume BNF (Minchin and Pate, 1973; Voisin et al., 2003), and many studies have shown a strong correlation between legume BNF and the C respired by nodules, nodulated roots (Mahon, 1977a, b; Ryle et al., 1979), or photosynthetic allocation (Lawrie and Wheeler, 1973; Voisin et al., 2003, 2007). Of all the models considered here, C sink is closely related to nodule growth and legume BNF only in CROPGRO (Boote et al., 2008). Thus improved experimental evidence of C allocation and usage by nodules and incorporation of these processes more widely into models would do much to improve estimates of potential legume BNF.

As mentioned above, soil mineral N in the rhizosphere inhibits legume root nodulation and legume BNF, and this inhibition is more severe under higher N concentrations, although moderate levels of soil mineral N stimulate nodulation and N fixation in some circumstances. It is difficult to give the critical value of soil N concentration below which nodulation and N fixation are stimulated (Munns, 1970; Streeter, 1988; Waterer et al., 1992; Waterer and Vessey, 1993b), and the issue about "starter N" is still controversial (Bethlenfalvay and Phillips, 1978; Bethlenfalvay et al., 1978; Waterer and Vessey, 1993b; Gulden and Vessey, 1997). Taken together, these factors explain why this stimulating effect of soil mineral N is not incorporated into any of the current models of legume BNF, and suggest that much further experimental work may be needed before it is possible to do so. The lack of inclusion of this process is a probable cause of the large discrepancy between modelled and actual legume BNF in some circumstances.

A further process that is absent from the current models of legume BNF is that they cannot distinguish the different inhibitory effects of soil nitrate and ammonium in the rhizosphere. Experimentally it has been shown that legume BNF is less sensitive to ammonium than nitrate (Svenning et al., 1996; Bollman and Vessey, 2006; Gan et al., 2004), but all models use soil nitrate or total mineral N concentration.

Our analysis of the currently-used models of legume BNF has revealed that the inhibition of legume BNF by soil mineral N concentration might be overestimated in the model that uses nodule biomass to determine the potential N fixation rate,  $N_{fixpot}$ . Considering equation (4),  $N_{fixpot}$  is the potential N fixation rate, calculated as the amount of fixed N per unit of nodule biomass multiplied by nodule biomass. Thus if nodule biomass is reduced by high soil mineral N concentration,  $N_{fixpot}$  will also be reduced. However, this model also uses  $f_N$ , the factor that relates legume BNF to soil mineral N concentration. This factor is derived experimentally from measurements of legume BNF under different levels of soil mineral N concentration, and by default this includes any inhibitory effect of soil mineral N concentration on legume BNF. Thus in STICS (Brisson et al., 2009), the effect of soil mineral N concentration on nodule biomass is effectively incorporated twice. We suggest that to avoid this problem, future models could remove the effect of nodule biomass on  $f_N$  by basing estimates of the latter on measurements taken from plants with well developed nodules. The effect of high soil mineral N concentration on nodule biomass should be included as it is currently, by its effect on  $N_{fixpot}$ .

In both experimental work and models of legume BNF, the effect of the water status of either soil or plant on legume BNF has concentrated on cases of water deficit rather than flooding. Despite many studies on the effect of soil water deficit on legume BNF, the nature of the relationship between N fixation and water soil status is poorly characterised. A linear or exponential function has been widely used in modelling studies to date except in the Hurley Pasture Model. CROPGRO includes an eight-day moving average of a water factor, which is used to simulate the prolonged effect of drought on N fixation. This is consistent with the conclusion that the inhibition of N fixation by drought in peanut and cowpea is not alleviated immediately once the drought releases (Venkateswarlu et al., 1990). Some studies have found that drought stress on N fixation is caused more by the inhibition of photosynthesis than by drought directly (Huang et al., 1975), which highlights the importance of the link between N fixation and photosynthesis. However, some studies have shown that N fixation is more sensitive than photosynthesis to drought (Sprent, 1976) and N fixation may decline before transpiration or photosynthesis decreases (Sinclair et al., 1987; Sall and Sinclair, 1991; Purcell et al., 1997).

Stress from excessive water is omitted or oversimplified in all models, even though the proportion of water in pore spaces has been suggested as an approach to evaluate the restriction on nodule nitrogenase activity by excessive water in CROPGRO. More studies are needed on the effect of excess water on legume BNF in the future, both experimentally and in simulations.

In summary, the difficulty of simulating legume BNF is because of the large variance in N fixation between sites and species, and over time. Biological N fixation is a highly complex process which integrates plant and soil processes in the macro-environment with the micro-environmental processes of rhizobial bacteria in nodules (Nelson and Child, 1981; Bolaños et al., 1994; Rice et al., 2000). It is difficult to represent these two completely different but closely related processes in one model. Also, there is currently inadequate information to quantify reliably the relationship of legume BNF with those factors such as soil water and plant C. This is because legume BNF is not only sensitive to temperature, water, N and C cycling, which have been incorporated into models, but also to soil pH (Rehcgigl et al., 1987; Peoples et al., 1995; Ibekwe et al., 1997; Rice et al., 2000; Le Roux et al., 2008), O<sub>2</sub> permeability (Cowan, 1978; Weisz and Sinclair, 1987; Faurie and Soussana, 1993; Serraj et al., 2001), salinity (Serraj and Drevon, 1998) and other nutrition regimes (Lynd et al., 1984; Collins et al., 1986; Bolaños et al., 1994; Chaudhary et al., 2008), which are currently absent from models despite frequently being cited as important factors.

Furthermore, in terms of the practical uses and benefits of N fixed by legumes, realistic field scenarios should be considered. In temperate agricultural systems, mixed pastures of legumes and grasses are common; intercrops of forage and grain legumes with cereal crops for animal feed are increasing in importance, especially in low-input and organic farming systems (Hauggaard-Nielsen et al., 2001, 2003; Andersen et al., 2004; Corre-Hellou et al., 2006). In these systems the in-

teractions, both competitive and facilitative, between the components alter legume BNF in mixtures compared to monocultures (Malézieux et al., 2009). Intercropping enhances the proportion of legume N derived from fixation due to the higher competition for soil N. However, the total legume BNF is often reduced as a consequence of its dependence on not only soil N but also legume growth. In addition to deliberate intercropping, weeds have a similar influence on legume BNF. This may have a big influence on yield and N accumulation of the main crops in pure legume stands if the weeds are relatively competitive (Hauggaard-Nielsen et al., 2001, 2006). Schwinning and Parsons (1996) and Thornley (2001) incorporate the interaction between legumes and grasses in their models, which have been reviewed by Malézieux's (2009). Therefore, these simulation schemes of interspecies competition are not reviewed here as we have focused on legumes only.

Apart from intercropping, other field managements such as grazing by livestock, especially in forage legume grasslands, affects N fixation through its effects on both legume growth and soil mineral N cycling due to urine and dung inputs. Thornley and Cannell (2000) simulated mineral N availability in grazed grassland with legume BNF through the effect on leaf area by grazing in the Hurley Pasture Model. Schwinning and Parson (1996) implement not only the effect of grazing on leaf cover but also the urine deposits from animals into their model. However, their simulation scheme of N fixation is simpler than in other models as it considers only the direct influence of soil mineral N on BNF. Many of these dynamic and competitive aspects with grazing and other crops should be better incorporated into future models that aim to have real agricultural relevance.

## 5. CONCLUSION

This review critically interprets the methods used to quantify legume BNF by the most commonly used experimental and modelling approaches. In particular, it highlights and compares the functions used to simulate legume BNF by different models and assesses their relative strengths in predicting N fixation with varying biotic and abiotic factors. We found that:

- (i) As a base to estimate legume BNF, all models use the potential N fixation rate although they differ in how they estimate this. Different functions are incorporated into each of the models to estimate how the maximum potential N fixation rate is restrained by soil temperature, soil water, soil/plant N concentration, plant C status and legume growth stages.
- (ii) Estimations of potential N fixation rate based on above ground biomass or root biomass are not as reliable as those based on nodule biomass (although see (iii) below). C supply from photosynthesis is recommended as the factor best able to explain the potential N fixation rate.
- (iii) The stimulating effect on legume BNF at relatively low levels of soil mineral N should be distinguished from the inhibition of legume BNF by soil mineral N. The simulated inhibition of legume BNF by soil mineral N might be

exaggerated if potential N fixation is modified by functions that include nodule biomass and the effect of soil mineral N on potential N fixation ( $f_N$ ).

- (iv) More experimental work is needed to characterise the effect of both soil water deficit and excess soil water on legume BNF. The responses of legume BNF to other factors currently absent from all models, such as soil pH and O<sub>2</sub> permeability, need to be included and reinforced with adequate experimental work.
- (v) Intercropping of legumes, either with grain crops or in grasslands, as well as the presence of grazing livestock, affect legume BNF in the fields. Models of legume BNF should take better account of these important practical uses of legumes.

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