



HAL
open science

Models of biological nitrogen fixation of legumes. A review

Liu, Wu, John Baddeley, Christine Watson

► **To cite this version:**

Liu, Wu, John Baddeley, Christine Watson. Models of biological nitrogen fixation of legumes. A review. *Agronomy for Sustainable Development*, 2011, 31 (1), pp.155-172. 10.1051/agro/2010008 . hal-00930470

HAL Id: hal-00930470

<https://hal.science/hal-00930470>

Submitted on 11 May 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Review article

Models of biological nitrogen fixation of legumes. A review

Yanyan LIU^{1,2*}, Lianhai WU^{2**}, John A. BADDELEY², Christine A. WATSON²

¹ College of Resources and Environmental Sciences, China Agricultural University, Beijing 100193, China

² Crop and Soil Systems Research Group, Scottish Agricultural College, Craibstone Estate, Aberdeen, AB21 9YA, UK

(Accepted 9 February 2010)

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

Contents

1	Introduction	2
2	Factors influencing legume biological nitrogen fixation	2
	2.1 Temperature	3
	2.2 Soil water status	3
	2.3 N concentration in the root zone	3
	2.4 Carbon demand for fixation	4
	2.5 Seasonal regulation of legume biological nitrogen fixation	4
3	Quantification of legume biological nitrogen fixation	5
	3.1 Estimation with empirical models	5
	3.2 Mechanistic simulation of legume biological nitrogen fixation as a subroutine in crop models	6
	3.2.1 Potential N fixation rate	6
	3.2.2 Impact of soil temperature	7

* Corresponding author: yanyan.liu@cau.edu.cn

** Current address: North Wyke Research, Okehampton, Devon EX20 2SB, UK.

3.2.3	Impact of soil water status	7
3.2.4	Effect of soil mineral N or internal substrate N	9
3.2.5	Influence of plant substrate C or C supply	10
3.2.6	Changes in N fixation with plant growth stage	10
3.3	Summary of approaches	11
4	Discussion	12
5	Conclusion	13

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 (NH_4^+) and less than 2 mM for nitrate (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 NO_3^- was more severe than that of 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 NH_4^+ or 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 CO₂ generated by N fixation from that generated by respiration for nodule growth and maintenance (Warembourg and Roumet, 1989), the correlation between the rate of CO₂ 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⁻¹ in cowpea and 3.64 gC g N fixed⁻¹ 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⁻¹ for soybean, cowpea and white clover from nodulated roots at periods of intense N fixation (Ryle et al., 1979). The C

cost determined by CO₂ 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⁻¹ which is 59% higher than that of nodules formed by strain I02F51, 5.9 molC molN⁻¹ (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}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 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. α is a parameter that has different definitions according to author. For example, α may represent the decline in $\%Ndfa$ under high soil mineral N conditions, in terms of net mineral N input (g N m^{-2}), and is calculated as:

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

where β 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, α 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 (kg N ha^{-1}) is summarized as,

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

where Leg is the extra dry matter increment (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	✓	✓	✓	✓		soybean, chickpea, peanut, mungbean, lucerne	Herridge et al. (2001); Robertson et al. (2002)
						white clover	Soussana et al. (2002)
						field pea and other legumes	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°C for both models and the maximum value of the function occurs at 30°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°C , but this reverses when soil temperature is in the range 10 to 20°C . Over 20°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°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 C_2H_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 d^{-1}	N demand after N uptake	(APSIM source code: http://apsrunet.apsim.info/svn/development/trunk/apsim/) Herridge et al. (2001); Robertson et al. (2002)
EPIC	soybean	total N demand	g N 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.

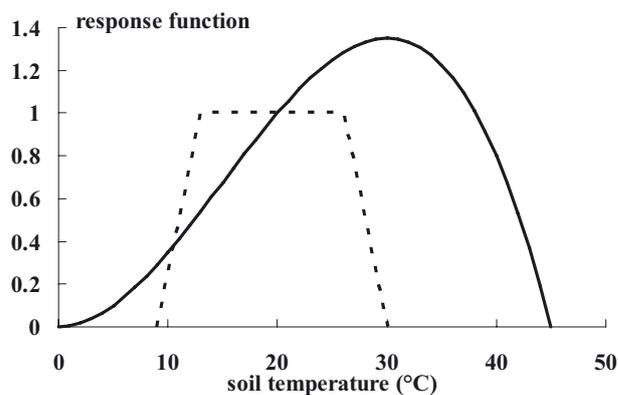
Model	Species	T_{min}	T_{optL}	T_{optH}	T_{max}	Reference
CROPGRO	soybean	5	20	35	44	Boote et al. (2008)
	cowpea	5	20	35	44	
	velvet bean	5	23	35	44	
	dry bean	4	19	35	44	
	peanut	7	23	34	44	
	faba bean	1	16	25	40	
SOILN	white clover	9	13	26	30	Wu and McGechan (1999)
STICS	legumes	0	15	25	35	Brisson 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, ϕ_1 and ϕ_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
ϕ_1	-0.33	0	0	0	0	0	0	0	0	0	0	-0.82	0.216
ϕ_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 ϕ_{rt} is root water potential (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 (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 ε 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 (g N m^{-2}) at which an N uptake rate reaches half its maximal rate and N_s is the actual soil nitrate content (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 g N m^{-2} (Fig. 2).

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

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

A similar function is incorporated in STICS as in EPIC, with a different value of $N_{sNitrate}$. 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

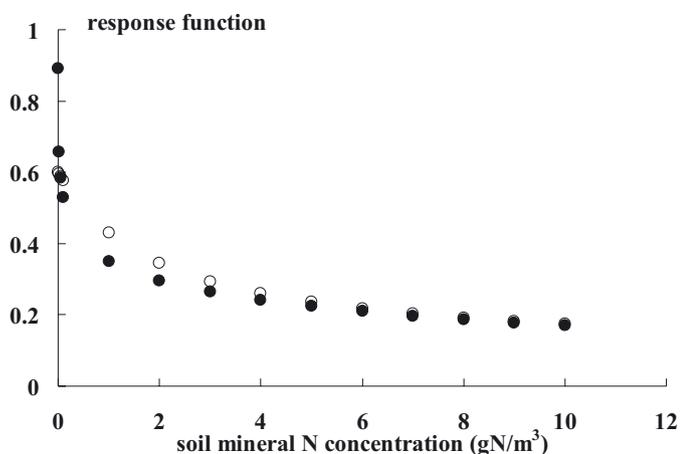


Figure 2. N fixation response to soil mineral N concentration by white clover in the Schwinning Model (closed circle) and SOILN (open circle), assuming the nodulation zone is 40 cm deep.

nutritional status on N fixation rate:

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

where N_{inter} (g N g^{-1} 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 (gC g^{-1} 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

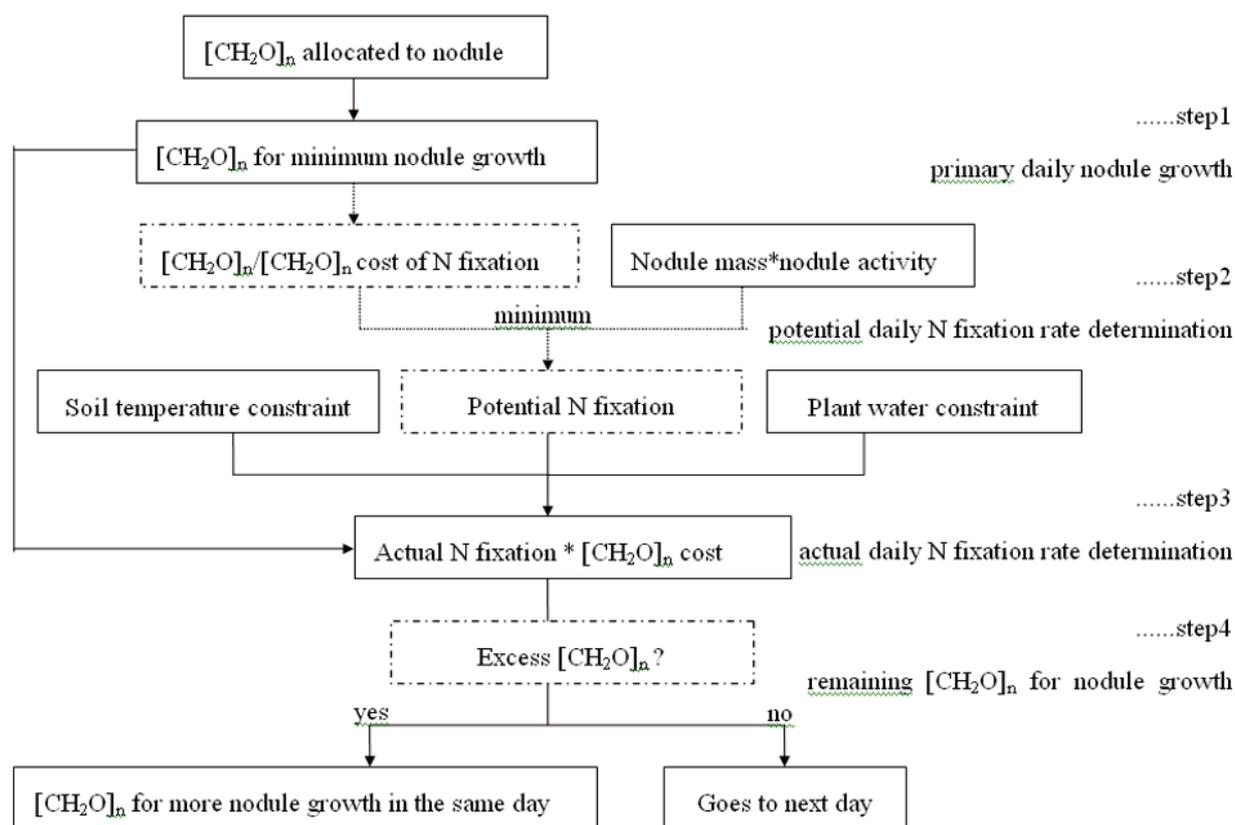


Figure 3. The representation of daily carbohydrate routes in nodules used in CROPGRO (Boote et al., 2008). $[\text{CH}_2\text{O}]_n$ in dotted line means that the amount of $[\text{CH}_2\text{O}]_n$ here is only used for calculation, neither for fixation nor nodule growth.

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 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 (Rechcigl et al., 1987; Peoples et al., 1995; Ibekwe et al., 1997; Rice et al., 2000; Le Roux et al., 2008), O₂ 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 O₂ 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.

Acknowledgements: Y. Liu thanks the China Scholarship Council for financial support. SAC receives funding from the Rural and Environment Research and Analysis Directorate (RERAD) of the Scottish Government. The authors thank Drs M. Robertson and P. Devoil for the source code of the AP-SIM model, and N. Brisson and M. Launay for details of the STICS model.

REFERENCES

- Abdel Wahab A.M., Zahran H.H., Abd-Alla M.H. (1996) Root-hair infection and nodulation of four grain legumes as affected by the form and the application time of nitrogen fertilizer, *Folia Microbiol.* 41, 303–308.
- Adgo E., Schulze J. (2002) Nitrogen fixation and assimilation efficiency in Ethiopian and German pea varieties, *Plant Soil* 239, 291–299.
- Albrecht S.L., Bennett J.M., Boote K.J. (1984) Relationship of nitrogenase activity to plant water stress in field-grown soybeans, *Field Crops Res.* 8, 61–71.
- Andersen M.K., Hauggaard-Nielsen H., Ambus P., Jensen E.S. (2004) Biomass production, symbiotic nitrogen fixation and inorganic N use in dual and tri-component annual intercrops, *Plant Soil* 266, 273–287.
- Bell M.J., Wright G.C., Suryantini, Peoples M.B. (1994) The N₂-fixing capacity of peanut cultivars with differing assimilate partitioning characteristics, *Aust. J. Agric. Res.* 45, 1455–1468.
- Bethlenfalvay G.J., Phillips D.A. (1978) Interactions between symbiotic nitrogen fixation, combined-N application, and photosynthesis in *Pisum sativum*, *Physiol. Plant.* 42, 119–123.
- Bethlenfalvay G.J., Abu-Shakra S.S., Phillips D.A. (1978) Interdependence of nitrogen nutrition and photosynthesis in *Pisum sativum* L. I. effect of combined nitrogen on symbiotic nitrogen fixation and photosynthesis, *Plant Physiol.* 62, 127–130.
- Beverly R.B., Jarrell W.M. (1984) Cowpea response to N form, rate, and timing of application, *Agron. J.* 76, 663–668.
- Blumenthal J.M., Russelle M.P. (1996) Subsoil nitrate uptake and symbiotic dinitrogen fixation by alfalfa, *Agron. J.* 88, 909–915.
- Bolaños L., Esteban E., de Lorenzo C., Fernández-Pascual M., de Felipe M.R., Gárate A., Bonilla I. (1994) Essentiality of boron for symbiotic dinitrogen fixation in pea (*Pisum-sativum*) rhizobium nodules, *Plant Physiol.* 104, 85–90.
- Bollman M.I., Vessey J.K. (2006) Differential effects of nitrate and ammonium supply on nodule initiation, development, and distribution on roots of pea (*Pisum sativum*), *Can. J. Bot.* 84, 893–903.
- Boote K.J., Hoogenboom G., Jones J.W., Ingram K.T. (2008) Modeling nitrogen fixation and its relationship to nitrogen uptake in the CROPGRO model, in: Ma L., Ahuja L.R., Bruulsema T.W. (Eds.), *Quantifying and Understanding Plant Nitrogen Uptake for Systems Modeling*. CRC Press, Florence, USA, pp. 13–46.
- Boote K.J., Jones J.W., Hoogenboom G., Pickering N.B. (1998) The CROPGRO model for grain legumes, in: Tsuji G.Y., Hoogenboom G., Thornton P.K. (Eds.), *Understanding Options for Agricultural Production*, pp. 99–128.
- Boote K.J., Mínguez M.I., Sau F. (2002) Adapting the CROPGRO legume model to simulate growth of faba bean, *Agron. J.* 94, 743–756.
- Bouniols A., Cabelguenne M., Jones C.A., Chalamet A., Charpentreau J.L., Marty J.R. (1991) Simulation of soybean nitrogen nutrition for a silty clay soil in southern France, *Field Crops Res.* 26, 19–34.
- Brisson N., Launay M., Mary B., Beaudoin N. (2009) Nitrogen transformations, in: Brisson N., Launay M., Mary B., Beaudoin N. (Eds.), *Conceptual basis, formalisations and parameterization of the STICS crop model*, Quæ, Versailles Cedex, France, pp. 141–165.
- Brugge R., Thornley J.H.M. (1984) Shoot root nodule partitioning in a vegetative legume – A model, *Ann. Bot.* 54, 653–671.
- Cabelguenne M., Debaeke P., Bouniols A. (1999) EPICphase, a version of the EPIC model simulating the effects of water and nitrogen stress on biomass and yield, taking account of developmental stages: validation on maize, sunflower, sorghum, soybean and winter wheat, *Agric. Syst.* 60, 175–196.
- Cannell M.G.R., Thornley J.H.M. (2000) Modelling the components of plant respiration: some guiding principles, *Ann. Bot.* 85, 45–54.
- Carlsson G., Huss-Danell K. (2008) How to quantify biological nitrogen fixation in forage legumes in the field, in: Dakora F.D., Chimphango S.B.M., Valentine A.J., Elmerich C., Newton W.E. (Eds.), *Biological Nitrogen Fixation: Towards Poverty Alleviation through Sustainable Agriculture*, Springer Netherlands, Dordrecht, pp. 47–48.
- Chalifour F.P., Nelson L.M. (1987) Effects of continuous combined nitrogen supply on symbiotic dinitrogen fixation of faba bean and pea inoculated with different rhizobial isolates, *Can. J. Bot.* 65, 2542–2548.
- Chaudhary M., Adu-Gyamfi J., Saneoka H., Nguyen N., Suwa R., Kanai S., El Shemy H., Lightfoot D., Fujita K. (2008) The effect of phosphorus deficiency on nutrient uptake, nitrogen fixation and photosynthetic rate in mashbean, mungbean and soybean, *Acta Physiol. Plant.* 30, 537–544.
- Collins M., Lang D.J., Kelling K.A. (1986) Effects of phosphorus, potassium, and sulfur on alfalfa nitrogen-fixation under field conditions, *Agron. J.* 78, 959–963.
- Corre-Hellou G., Brisson N., Launay M., Fustec J., Crozat Y. (2007) Effect of root depth penetration on soil nitrogen competitive interactions and dry matter production in pea–barley intercrops given different soil nitrogen supplies, *Field Crops Res.* 103, 76–85.
- Corre-Hellou G., Faure M., Launay M., Brisson N., Crozat Y. (2009) Adaptation of the STICS intercrop model to simulate crop growth and N accumulation in pea–barley intercrops, *Field Crops Res.* 113, 72–81.
- Corre-Hellou G., Fustec J., Crozat Y. (2006) Interspecific competition for soil N and its interaction with N₂ fixation, leaf expansion and crop growth in pea–barley intercrops, *Plant Soil* 282, 195–208.
- Cowan M. (1978) The influence of nitrate, water potential and oxygen tension on nitrogen fixation in detached pea roots, *Plant Foods Hum. Nutr.* 28, 65–69.
- Dart P., Day J. (1971) Effects of incubation temperature and oxygen tension on nitrogenase activity of legume root nodules, *Plant Soil* 35, 167–184.
- Denison R.F., Weisz P.R., Sinclair T.R. (1985) Variability among plants in dinitrogen fixation (acetylene reduction) rates by field-grown soybeans, *Agron. J.* 77, 947–950.

- Duffy J., Chung C., Boast C., Franklin M. (1975) A simulation model of biophysicochemical transformations of nitrogen in tile-drained corn belt soil, *J. Environ. Qual.* 4, 477–486.
- Eaglesham A.R.J. (1989) Nitrate inhibition of root-nodule symbiosis in doubly rooted soybean plants, *Crop Sci.* 29, 115–119.
- Eckersten H., Af Geijersstam L., Torssell B. (2006) Modelling nitrogen fixation of pea (*Pisum sativum* L.), *Acta Agric. Scand. B* 56, 129–137.
- Engin M., Sprent J.I. (1973) Effects of water stress on growth and nitrogen-fixing activity of *Trifolium repens*, *New Phytol.* 72, 117–126.
- Evans J., McNeill A.M., Unkovich M.J., Fettel N.A., Heenan D.P. (2001) Net nitrogen balances for cool-season grain legume crops and contributions to wheat nitrogen uptake: a review, *Aust. J. Exp. Agric.* 41, 347–359.
- Evans J., O'Connor G.E., Turner G.L., Coventry D.R., Fettel N., Mahoney J., Armstrong E.L., Walsgott D.N. (1989) N₂ fixation and its value to soil N increase in lupin, field pea and other legumes in south-eastern Australia, *Aust. J. Agric. Res.* 40, 791–805.
- Evans J., Turner G.L., O'Connor G.E., Bergersen F.J. (1987) Nitrogen fixation and accretion of soil nitrogen by field-grown lupins (*Lupinus angustifolius*), *Field Crops Res.* 16, 309–322.
- Faurie O., Soussana J.F. (1993) Oxygen-induced recovery from short-term nitrate inhibition of N₂ fixation in white clover plants from spaced and dense stands, *Physiol. Plant.* 89, 467–475.
- Flechar C.R., Ambus P., Skiba U., Rees R.M., Hensen A., van Amstel A., van den Pol-van Dasselaar A., Soussana J.-F., Jones M., Clifton-Brown J., Raschi A., Horvath L., Neftel A., Jocher M., Ammann C., Leifeld J., Fuhrer J., Calanca P., Thalman E., Pilegaard K., Di Marco C., Campbell C., Nemitz E., Hargreaves K.J., Levy P.E., Ball B.C., Jones S.K., van de Bulk W.C.M., Groot T., Blom M., Domingues R., Kasper G., Allard V., Ceschia E., Cellier P., Laville P., Henault C., Bizouard F., Abdalla M., Williams M., Baronti S., Berretti F., Grosz B. (2007) Effects of climate and management intensity on nitrous oxide emissions in grassland systems across Europe, *Agric. Ecosyst. Environ.* 121, 135–152.
- Frame J., Newbould P. (1986) Agronomy of white clover, *Adv. Agron.* 40, 1–88.
- Gan Y., Stulen I., van Keulen H., Kuiper P.J.C. (2004) Low concentrations of nitrate and ammonium stimulate nodulation and N₂ fixation while inhibiting specific nodulation (nodule DW g⁻¹ root dry weight) and specific N₂ fixation (N₂ fixed g⁻¹ root dry weight) in soybean, *Plant Soil* 258, 281–292.
- Garg N., Geetanjali (2007) Symbiotic nitrogen fixation in legume nodules: process and signaling. A review, *Agron. Sustain. Dev.* 27, 59–68.
- Gibson A. (1971) Factors in the physical and biological environment affecting nodulation and nitrogen fixation by legumes, *Plant Soil* 35, 139–152.
- Goh K.M., Bruce G.E. (2005) Comparison of biomass production and biological nitrogen fixation of multi-species pastures (mixed herb leys) with perennial ryegrass-white clover pasture with and without irrigation in Canterbury, New Zealand, *Agric. Ecosyst. Environ.* 110, 230–240.
- Goh K.M., Edmeades D.C., Robinson B.W. (1978) Field measurements of symbiotic nitrogen fixation in an established pasture using acetylene reduction and a ¹⁵N method, *Soil Biol. Biochem.* 10, 13–20.
- Gulden R., Vessey J.K. (1997) The stimulating effect of ammonium on nodulation in *Pisum sativum* L. is not long lived once ammonium supply is discontinued, *Plant Soil* 195, 195–205.
- Haase S., Neumann G., Kania A., Kuzyakov Y., Römheld V., Kandeler E. (2007) Elevation of atmospheric CO₂ and N-nutritional status modify nodulation, nodule-carbon supply, and root exudation of *Phaseolus vulgaris* L., *Soil Biol. Biochem.* 39, 2208–2221.
- Halliday J. (1975) An interpretation of seasonal and short term fluctuations in nitrogen fixation, PhD thesis, University of Western Australia, Perth.
- Halliday J., Pate J.S. (1976) The acetylene reduction assay as a means of studying nitrogen fixation in white clover under sward and laboratory conditions, *Grass Forage Sci.* 31, 29–35.
- Hartkamp A.D., Hoogenboom G., White J.W. (2002) Adaptation of the CROPGRO growth model to velvet bean (*Mucuna pruriens*): I. Model development, *Field Crops Res.* 78, 9–25.
- Hauggaard-Nielsen H., Ambus P., Jensen E.S. (2001) Interspecific competition, N use and interference with weeds in pea-barley intercropping, *Field Crops Res.* 70, 101–109.
- Hauggaard-Nielsen H., Ambus P., Jensen E.S. (2003) The comparison of nitrogen use and leaching in sole cropped versus intercropped pea and barley, *Nutr. Cycl. Agroecosyst.* 65, 289–300.
- Hauggaard-Nielsen H., Andersen M.K., Jørnsgaard B., Jensen E.S. (2006) Density and relative frequency effects on competitive interactions and resource use in pea-barley intercrops, *Field Crops Res.* 95, 256–267.
- Havelka U.D., Boyle M.G., Hardy R.W.F. (1982) Biological nitrogen fixation, in: Stevenson F.J. (Ed.), *Nitrogen in Agricultural Soils*, ASA, Madison, Wisconsin, USA, pp. 365–422.
- Herridge D.F., Pate J.S. (1977) Utilization of net photosynthate for nitrogen fixation and protein production in an annual legume, *Plant Physiol.* 60, 759–764.
- Herridge D.F., Roughley R.J., Brockwell J. (1984) Effect of rhizobia and soil nitrate on the establishment and functioning of the soybean symbiosis in the field, *Aust. J. Agric. Res.* 35, 149–161.
- Herridge D.F., Turpin J.E., Robertson M.J. (2001) Improving nitrogen fixation of crop legumes through breeding and agronomic management: analysis with simulation modelling, *Aust. J. Exp. Agric.* 41, 391–401.
- Herridge D.F., Peoples M.B., Boddey R.M. (2008) Global inputs of biological nitrogen fixation in agricultural systems, *Plant Soil* 311, 1–18.
- Høgh-Jensen H., Loges R., Jørgensen F.V., Vinther F.P., Jensen E.S. (2004) An empirical model for quantification of symbiotic nitrogen fixation in grass-clover mixtures, *Agric. Syst.* 82, 181–194.
- Hossain S.A., Strong W.M., Waring S.A., Dalal R.C., Weston E.J. (1996) Comparison of legume-based cropping systems at Warra, Queensland. II. Mineral nitrogen accumulation and availability to the subsequent wheat crop, *Aust. J. Soil Res.* 34, 289–297.
- Howard J.B., Rees D.C. (1996) Structural basis of biological nitrogen fixation, *Chem. Rev.* 96, 2965–2982.
- Huang C.Y., Boyer J.S., Vanderhoef L.N. (1975) Limitation of acetylene reduction (nitrogen fixation) by photosynthesis in soybean having low water potentials, *Plant Physiol.* 56, 228–232.
- Ibekwe A.M., Angle J.S., Chaney R.L., van Berkum P. (1997) Enumeration and N₂ fixation potential of *Rhizobium leguminosarum* biovar *trifolii* grown in soil with varying pH values and heavy metal concentrations, *Agric. Ecosyst. Environ.* 61, 103–111.
- Imsande J. (1986) Inhibition of nodule development in soybean by nitrate or reduced nitrogen, *J. Exp. Bot.* 37, 348–355.
- Jensen E.S. (1986) The influence of rate and time of nitrate supply on nitrogen fixation and yield in pea (*Pisum sativum* L.), *Fert. Res.* 10, 193–202.
- Jensen E.S. (1987) Seasonal patterns of growth and nitrogen fixation in field-grown pea, *Plant Soil* 101, 29–37.

- Jensen E.S. (1997) The role of grain legume N₂ fixation in the nitrogen cycling of temperate cropping systems, DSc Thesis, Riso-R-885, Riso National Laboratory, Denmark.
- King B.J., Layzell D.B., Canvin D.T. (1986) The role of dark carbon dioxide fixation in root nodules of soybean, *Plant Physiol.* 81, 200–205.
- Kirizii D.A., Vorobei N.A., Kots' S.Ya. (2007) Relationships between nitrogen fixation and photosynthesis as the main components of the productivity in alfalfa, *Russ. J. Plant. Physiol.* 54, 589–594.
- Kishinevsky B.D., Sen D., Weaver R.W. (1992) Effect of high root temperature on *Bradyrhizobium*-peanut symbiosis, *Plant Soil* 143, 275–282.
- Klucas R.V. (1974) Studies on soybean nodule senescence, *Plant Physiol.* 54, 612–616.
- Korsaeth A., Eltun R. (2000) Nitrogen mass balances in conventional, integrated and ecological cropping systems and the relationship between balance calculations and nitrogen runoff in an 8-year field experiment in Norway, *Agric. Ecosyst. Environ.* 79, 199–214.
- Kristensen E.S., Høgh-Jensen H., Kristensen I.S. (1995) A simple model for estimation of atmospherically-derived nitrogen in grass-clover systems, *Biol. Agric. Hortic.* 12, 263–276.
- Kuo T., Boersma L. (1971) Soil water suction and root temperature effects on nitrogen fixation in soybeans, *Agron. J.* 63, 901–904.
- Lamb J.F.S., Barnes D.K., Russelle M.P., Vance C.P., Heichel G.H., Henjum K.I. (1995) Ineffectively and effectively nodulated alfalfas demonstrate biology nitrogen fixation continues with high nitrogen fertilization, *Crop Sci.* 35, 153–157.
- Lawn R.J., Brun W.A. (1974) Symbiotic nitrogen fixation in soybeans. I. effect of photosynthetic source-sink manipulations, *Crop Sci.* 14, 11–16.
- Lawrie A.C., Wheeler C.T. (1973) The supply of photosynthetic assimilates to nodules of *Pisum Sativum* L. in relation to the fixation of nitrogen, *New Phytol.* 72, 1341–1348.
- Layzell D.B., Pate J.S., Atkins C.A., Canvin D.T. (1981) Partitioning of carbon and nitrogen and the nutrition of root and shoot apex in a nodulated legume, *Plant Physiol.* 67, 30–36.
- Layzell D.B., Rainbird R.M., Atkins C.A., Pate J.S. (1979) Economy of photosynthate use in nitrogen-fixing legume nodules: observations on two contrasting symbioses, *Plant Physiol.* 64, 888–891.
- Le Roux M.R., Khan S., Valentine A.J. (2008) Organic acid accumulation may inhibit N₂ fixation in phosphorus-stressed lupin nodules, *New Phytol.* 177, 956–964.
- Ledgard S.F., Steele K.W. (1992) Biological nitrogen fixation in mixed legume/grass pastures, *Plant Soil* 141, 137–153.
- Ledgard S.F., Brier G.J., Littler R.A. (1987) Legume production and nitrogen fixation in hill pasture communities, *New Zeal. J. Agric. Res.* 30, 413–421.
- Lie T.A. (1971) Symbiotic nitrogen fixation under stress conditions, *Plant Soil* 35, 117–127.
- Lindemann W.C., Ham G.E. (1979) Soybean plant growth, nodulation, and nitrogen fixation as affected by root temperature, *Soil Sci. Soc. Am. J.* 43, 1134–1137.
- Lynd J.Q., Hanlon E.A., Jr., Odell G.V., Jr. (1984) Nodulation and nitrogen fixation by arrowleaf clover: effects of phosphorus and potassium, *Soil Biol. Biochem.* 16, 589–594.
- Macduff J.H., Jarvis S.C., Davidson I.A. (1996) Inhibition of N₂ fixation by white clover (*Trifolium repens* L.) at low concentrations of NO₃⁻ in flowing solution culture, *Plant Soil* 180, 287–295.
- Mahon J.D. (1977a) Respiration and the energy requirement for nitrogen fixation in nodulated pea roots, *Plant Physiol.* 60, 817–821.
- Mahon J.D. (1977b) Root and nodule respiration in relation to acetylene reduction in intact nodulated peas, *Plant Physiol.* 60, 812–816.
- Malézieux E., Crozat Y., Dupraz C., Laurans M., Makowski D., Ozier-Lafontaine H., Rapidel B., de Tourdonnet S., Valantin-Morison M. (2009) Mixing plant species in cropping systems: concepts, tools and models. A review, *Agron. Sustain. Dev.* 29, 43–62.
- Masterson C.L., Murphy P.M. (1976) Application of the acetylene reduction technique to the study of nitrogen fixation by white clover in the field, in: Nutman P.S. (Eds.), *Symbiotic Nitrogen Fixation in Plants*, Cambridge University Press, London, UK, pp. 299–316.
- McCallum M.H., Peoples M.B., Connor D.J. (1999) Contributions of nitrogen by field pea (*Pisum sativum* L.) in a continuous cropping sequence compared with a lucerne (*Medicago sativa* L.)-based pasture ley in the Victorian Wimmera, *Aust. J. Agric. Res.* 51, 13–22.
- Michiels J., Verreth C., Vanderleyden J. (1994) Effects of temperature stress on bean-nodulating *Rhizobium* strains, *Appl. Environ. Microbiol.* 60, 1206–1212.
- Minchin F.R., Pate J.S. (1973) The carbon balance of a legume and the functional economy of its root nodules, *J. Exp. Bot.* 24, 259–271.
- Minchin F.R., Summerfield R.J., Hadley P., Roberts E.H., Rawsthorne S. (1981) Carbon and nitrogen nutrition of nodulated roots of grain legumes, *Plant Cell Environ.* 4, 5–26.
- Munns D. (1970) Nodulation of *Medicago sativa* in solution culture V. calcium and pH requirements during infection, *Plant Soil* 32, 90–102.
- Nelson L.M., Child J.J. (1981) Nitrogen-fixation and hydrogen metabolism by *Rhizobium-leguminosarum* isolates in pea root nodules, *Can. J. Microbiol.* 27, 1028–1034.
- Nelson D.R., Bellville R.J., Porter C.A. (1984) Role of nitrogen assimilation in seed development of soybean, *Plant Physiol.* 74, 128–133.
- Pate J.S. (1961) Temperature characteristics of bacterial variation in legume symbiosis, *Nature* 192, 637–639.
- Peoples M.B., Lilley D.M., Burnett V.F., Ridley A.M., Garden D.L. (1995) Effects of surface application of lime and superphosphate to acid soils on growth and N₂ fixation by subterranean clover in mixed pasture swards, *Soil Biol. Biochem.* 27, 663–671.
- Phillips D.A. (1980) Efficiency of symbiotic nitrogen fixation in legumes, *Annu. Rev. Plant Physiol.* 31, 29–49.
- Piha M.I., Munns D.N. (1987) Sensitivity of the common bean (*Phaseolus vulgaris* L.) symbiosis to high soil temperature, *Plant Soil* 98, 183–194.
- Pimratch S., Jogloy S., Vorasoot N., Toomsan B., Patanothai A., Holbrook C.C. (2008) Relationship between biomass production and nitrogen fixation under drought-stress conditions in peanut genotypes with different levels of drought resistance, *J. Agron. Crop Sci.* 194, 15–25.
- Postgate J.R. (1982) Biological nitrogen fixation: fundamentals, *Philos. Trans. R. Soc. B* 296, 387–375.
- Purcell L.C., Sinclair T.R. (1990) Nitrogenase activity and nodule gas permeability response to rhizospheric NH₃ in soybean, *Plant Physiol.* 92, 268–272.
- Purcell L.C., de Silva M., King C.A., Kim W.H. (1997) Biomass accumulation and allocation in soybean associated with genotypic differences in tolerance of nitrogen fixation to water deficits, *Plant Soil* 196, 101–113.
- Rao V.R. (1977) Effect of root temperature on the infection processes and nodulation in *Lotus* and *Stylosanthes*, *J. Exp. Bot.* 28, 241–259.
- Rechcigl J.E., Edmisten K.L., Wolf D.D., Reneau R.B. Jr. (1987) Influence of calcium, nitrogen, and pH on alfalfa root growth and nitrogen fixation using the implanted soil mass technique, *Agron. J.* 79, 926–928.

- Rice W.A., Clayton G.W., Olsen P.E., Lupwayi N.Z. (2000) Rhizobial inoculant formulations and soil pH influence field pea nodulation and nitrogen fixation, *Can. J. Soil Sci.* 80, 395–400.
- Richardson A.C., Syers J.K. (1985) Edaphic limitations and soil nutrient requirements of legume-based forage systems in temperate regions of New Zealand, in: Barnes R.F., Ball P.R., Broughman R.W., Marten G.C., Minson D.J. (Eds.), *Forage Legumes for Energy-efficient Animal Production*, USDA, Washington, pp. 89–94.
- Robertson M.J., Carberry P.S., Huth N.I., Turpin J.E., Probert M.E., Poulton P.L., Bell M., Wright G.C., Yeates S.J., Brinsmead R.B. (2002) Simulation of growth and development of diverse legume species in APSIM, *Aust. J. Agric. Res.* 53, 429–446.
- Roughley R.J. (1970) The influence of root temperature, *Rhizobium* strain and host selection on the structure and nitrogen-fixing efficiency of the root nodules of *Trifolium subterraneum*, *Ann. Bot.* 34, 631–646.
- Roughley R.J., Dart P.J. (1969) Reduction of acetylene by nodules of *Trifolium subterraneum* as affected by root temperature, *Rhizobium* strain and host cultivar, *Arch. Microbiol.* 69, 171–179.
- Roughley R., Dart P. (1970) Root temperature and root-hair infection of *Trifolium subterraneum* L. cv. Cranmore, *Plant Soil* 32, 518–520.
- Ryle G.J.A., Powell C.E., Gordon A.J. (1979) The respiratory costs of nitrogen fixation in soybean, cowpea, and white clover I. nitrogen fixation and the respiration of the nodulated root, *J. Exp. Bot.* 30, 135–144.
- Sall K., Sinclair T.R. (1991) Soybean genotypic differences in sensitivity of symbiotic nitrogen fixation to soil dehydration, *Plant Soil* 133, 31–37.
- Sau F., Boote K.J., Ruíz-Nogueira B. (1999) Evaluation and improvement of CROPGRO-soybean model for a cool environment in Galicia, northwest Spain, *Field Crops Res.* 61, 273–291.
- Schiltz S., Munier-Jolain N., Jeudy C., Burstin J., Salon C. (2005) Dynamics of exogenous nitrogen partitioning and nitrogen remobilization from vegetative organs in pea revealed by ^{15}N in vivo labeling throughout seed filling, *Plant Physiol.* 137, 1463–1473.
- Schmid M., Neftel A., Riedo M., Fuhrer J. (2001) Process-based modelling of nitrous oxide emissions from different nitrogen sources in mown grassland, *Nutr. Cycl. Agroecosyst.* 60, 177–187.
- Schomberg H.H., Weaver R.W. (1992) Nodulation, nitrogen fixation, and early growth of arrowleaf clover in response to root temperature and starter nitrogen, *Agron. J.* 84, 1046–1050.
- Schulze J., Adgo E., Merbach W. (1999) Carbon costs associated with N_2 fixation in *Vicia faba* L. and *Pisum sativum* L. over a 14-day period, *Plant Biol.* 1, 625–631.
- Schwinning S., Parsons A.J. (1996) Analysis of the coexistence mechanisms for grasses and legumes in grazing systems, *J. Ecol.* 84, 799–813.
- Serraj R., Drevon J.J. (1998) Effects of salinity and nitrogen source on growth and nitrogen fixation in alfalfa, *J. Plant Nutr.* 21, 1805–1818.
- Serraj R., Sinclair T.R. (1998) N_2 fixation response to drought in common bean (*Phaseolus vulgaris* L.), *Ann. Bot.* 82, 229–234.
- Serraj R., Bona S., Purcell L.C., Sinclair T.R. (2001) Role of soil-oxygen enrichment in the response of nitrogen fixation to drought stress in field-grown soybean, *Soil Crop Sci. Soc. Fla. Proc.* 60, 88–93.
- Sharpley A.N., Williams J.R. (1990) EPIC-erosion/productivity impact calculator 1. Model documentation, USDA Tech. Bull. No. 1768, pp. 1–235.
- Sheehy J.E. (1987) Photosynthesis and nitrogen fixation in legume plants, *Crit. Rev. Plant Sci.* 5, 121–159.
- Sheehy J.E., Woodward F.I., Gosse G. (1991) Measurements of nitrogen fixation (C_2H_2), photosynthesis and respiration using an open system in the natural environment, *Ann. Bot.* 67, 131–136.
- Sinclair T.R. (1986) Water and nitrogen limitations in soybean grain production I. model development, *Field Crops Res.* 15, 125–141.
- Sinclair T.R., Muchow R.C., Ludlow M.M., Leach G.J., Lawn R.J., Foale M.A. (1987) Field and model analysis of the effect of water deficits on carbon and nitrogen accumulation by soybean, cowpea and black gram, *Field Crops Res.* 17, 121–140.
- Small J.G.C., Joffe A. (1968) Physiological studies on the genus *Trifolium* with special reference to the South African species. II. Influence of root temperature on growth, nodulation and symbiotic nitrogen fixation, *S. African J. Agric. Sci.* 11, 41–56.
- Soussana J.F., Minchin F.R., Macduff J.H., Raistrick N., Abberton M.T., Michaelson-Yeates T.P.T. (2002) A simple model of feedback regulation for nitrate uptake and N_2 fixation in contrasting phenotypes of white clover, *Ann. Bot.* 90, 139–147.
- Sprent J.I. (1972) Effects of water stress on nitrogen-fixing root nodules. IV. Effects on whole plants of *Vicia Faba* and *Glycine max*, *New Phytol.* 71, 603–611.
- Sprent J.I. (1976) Water deficits and nitrogen-fixing root nodules, in: Kozłowski T.T. (Ed.), *Water Deficits and Plant Growth Vol IV. Soil water measurement, plant responses, and breeding for drought resistance*. Academic Press, New York, pp. 291–315.
- Stout W.L., Fales S.L., Muller L.D., Schnabel R.R., Weaver S.R. (2000) Water quality implications of nitrate leaching from intensively grazed pasture swards in the northeast US, *Agric. Ecosyst. Environ.* 77, 203–210.
- Streeter J. (1988) Inhibition of legume nodule formation and N_2 fixation by nitrate, *Crit. Rev. Plant Sci.* 7, 1–23.
- Svenning M.M., Junntila O., Macduff J.H. (1996) Differential rates of inhibition of N_2 fixation by sustained low concentrations of NH_4^+ and NO_3^- in northern ecotypes of white clover (*Trifolium repens* L.), *J. Exp. Bot.* 47, 729–738.
- Thornley J.H.M. (1998) Plant Submodel, in: Thornley J.H.M. (Ed.), *Grassland Dynamics: An Ecosystem Simulation Model*, CAB International, Wallingford, Oxon, UK, pp. 53–57.
- Thornley J.H.M. (2001) Simulating grass-legume dynamics: a phenomenological submodel, *Ann. Bot.* 88, 905–913.
- Thornley J.H.M., Cannell M.G.R. (2000) Dynamics of mineral N availability in grassland ecosystems under increased $[\text{CO}_2]$: hypotheses evaluated using the Hurley Pasture Model, *Plant Soil* 224, 153–170.
- Thornley J.H.M., Bergelson J., Parsons A.J. (1995) Complex dynamics in a carbon-nitrogen model of a grass-legume pastures, *Ann. Bot.* 75, 79–94.
- Trindade H., Coutinho J., Jarvis S., Moreira N. (2001) Nitrogen mineralization in sandy loam soils under an intensive double-cropping forage system with dairy-cattle slurry applications, *Eur. J. Agron.* 15, 281–293.
- Twary S.N., Heichel G.H. (1991) Carbon costs of dinitrogen fixation associated with dry matter accumulation in alfalfa, *Crop Sci.* 31, 985–992.
- Venkateswarlu B., Saharan N., Maheswari M. (1990) Nodulation and N_2 (C_2H_2) fixation in cowpea and groundnut during water stress and recovery, *Field Crops Res.* 25, 223–232.
- Voisin A.-S., Bourion V., Duc G., Salon C. (2007) Using an ecophysiological analysis to dissect genetic variability and to propose an ideotype for nitrogen nutrition in pea, *Ann. Bot.* 100, 1525–1536.
- Voisin A.-S., Salon C., Jeudy C., Warembourg F.R. (2003) Symbiotic N_2 fixation activity in relation to C economy of *Pisum sativum* L. as a function of plant phenology, *J. Exp. Bot.* 54, 2733–2744.
- Voisin A.-S., Salon C., Munier-Jolain N.G., Ney B. (2002) Effect of mineral nitrogen on nitrogen nutrition and biomass partitioning between the shoot and roots of pea (*Pisum sativum* L.), *Plant Soil* 242, 251–262.

- Warembourg F.R., Roumet C. (1989) Why and how to estimate the cost of symbiotic N_2 fixation? A progressive approach based on the use of ^{14}C and ^{15}N isotopes, *Plant Soil* 115, 167–177.
- Waterer J.G., Vessey J.K. (1993a) Effect of low static nitrate concentrations on mineral nitrogen uptake, nodulation, and nitrogen fixation in field pea, *J. Plant Nutr.* 16, 1775–1789.
- Waterer J.G., Vessey J.K. (1993b) Nodulation response of autoregulated or NH_4^+ -inhibited pea (*Pisum sativum*) after transfer to stimulatory (low) concentrations of NH_4^+ , *Physiol. Plant.* 88, 460–466.
- Waterer J.G., Vessey J.K., David Raper J.C. (1992) Stimulation of nodulation in field peas (*Pisum sativum*) by low concentrations of ammonium in hydroponic culture, *Physiol. Plant.* 86, 215–220.
- Watson C.A., Goss M.J. (1997) Estimation of N_2 -fixation by grass-white clover mixtures in cut or grazed swards, *Soil Use Manage.* 13, 165–167.
- Waughman G.J. (1977) The effect of temperature on nitrogenase activity, *J. Exp. Bot.* 28, 949–960.
- Weisz P.R., Sinclair T.R. (1987) Regulation of soybean nitrogen fixation in response to rhizosphere oxygen. II. Quantification of nodule gas permeability, *Plant Physiol.* 84, 906–910.
- Weisz P.R., Denison R.F., Sinclair T.R. (1985) Response to drought stress of nitrogen fixation (acetylene reduction) rates by field-grown soybeans, *Plant Physiol.* 78, 525–530.
- Whitehead D.C. (1995) Legumes: Biological Nitrogen Fixation and Interaction with Grasses, in: Whitehead D.C. (Ed.), *Grassland Nitrogen*, CAB International, Wallingford, UK, pp. 36–57.
- Wood M. (1996) Nitrogen Fixation: How much and at what cost? in: Younie D. (Ed.), *Legumes in Sustainable Farming Systems*, Occasional Symposim No. 30, British Grassland Society, Reading, UK, pp. 26–35.
- Wu L., McGechan M.B. (1999) Simulation of nitrogen uptake, fixation and leaching in a grass/white clover mixture, *Grass Forage Sci.* 54, 30–41.
- Yu M., Gao Q., Shaffer M.J. (2002) Simulating interactive effects of symbiotic nitrogen fixation, carbon dioxide elevation, and climatic change on legume growth, *J. Environ. Qual.* 31, 634–641.