

Article

Assessment of Nitrogen Uptake and Biological Nitrogen Fixation Responses of Soybean to Nitrogen Fertiliser with SPACSYS

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Abstract: Chemical fertiliser nitrogen addition will inhibit biological nitrogen fixation (BNF) for soybean (*Glycine max* [L.] Merr) growth. The optimal balance of these two nitrogen input sources has been a key issue for sustainable development in Northeast China. We used the data collected from a four-year experiment with varied irrigation and fertiliser treatments from 2007 to 2010 to evaluate the SPACSYS (Soil-Plant-Atmosphere Continuum SYStem) model. The validated model was run to investigate the responses to different management practices in seed yield, BNF, protein yield and soil nitrogen budgets. Scenario testing showed average yield increase of 2.4–5.2% with additional 50–100 kg N/ha application. Irrigation at the reproductive stage improved seed yield in drier years with an increase of 12–33% compared with the rain-fed treatment. BNF was suppressed by fertiliser nitrogen application and drought stress with a decrease of 6–33% and 8–34%, respectively. The average nitrogen budget without fertilization indicated a deficit of 39 kg N/ha. To attain higher seed yield, applying fertiliser at 25–30 and 15–20 kg N/ha before sowing is advised in drier and wetter years, respectively. To achieve a higher seed nitrogen content, an application rate of 55–60 and 45–50 kg N/ha is recommended for drier and wetter years, respectively.

Keywords: soybean; Northeast China; yield; biological nitrogen fixation; seed N; N budget

1. Introduction

The soybean (*Glycine max* [L.] Merr) is the fourth largest crop in China and one of the main crops in the Northeast China plain (NEP). The local farmers' interest in growing soybean has steadily increased since the 1980s [1,2], and total sown area and production in the NEP accounted for more than 30% of the national total in 2015 [2]. Average yields were similar for both the NEP and the whole country before the 1980s but since then have been 30% higher for the NEP [2,3]. This increase is mainly attributed to cultivar improvement and better management practices, including nitrogen (N) fertilization and tillage [3,4].

To maintain higher soybean yields, N should be supplied at the right time with the right amount. Other than N provided by soil mineralization, biological N₂ fixation (BNF) and N fertilizer (either chemical or manure) are the two important sources to meet soybean N demand. BNF normally starts at the third node (V3, three nodes on the main stem with fully developed leaves beginning with the unifoliolate nodes) stage [5–7] with a lower rate until the sixth node (V6) stage [5–7]. It reaches a maximum from pod setting to the beginning of the seed filling stage, which coincides with the peak N demand for protein synthesis and then decreases [7–10]. If N supply by BNF and soil mineralization is insufficient to satisfy crop demand, additional N should be supplied, otherwise photosynthesis could

be limited. Unfavourable climatic conditions also constrain soybean yield and BNF [1]. For example, drought stress limits not only the above-ground biomass accumulation, but also root exploration and nodule activity, resulting in a decrease in BNF rate and N uptake [9,11,12]. If this occurs at the reproductive stage, then yield depression will be more severe [9].

It is still controversial as to whether fertiliser N addition will increase seed yield and whether it is economically worthwhile [13–15]. Soybean yield has shown a positive response to N fertiliser when BNF could not meet the N demand [15], but no consistent conclusion has been made regarding net profit accounting for the increased fertiliser cost and additional income for the soybean producers [13,15]. Salvagiotti et al. [15] in their review, observed a negative exponential relationship between N fertiliser addition and BNF for applications up to 400 kg N/ha, although no clear conclusion was made for lower application rates (<60 kg N/ha), which is common practice in the NEP. For example, some authors have reported that nodule weight and number were suppressed by fertiliser application regardless of fertiliser time or rate [16], but others found no significant reduction in nodule number at low fertiliser rates of <60 kg N/ha [17,18]. Therefore, it is essential to investigate soybean growth and yield responses to different N fertilization rates with the aim of achieving higher yield without compromising BNF.

A soil N budget is a useful tool to indicate system level N loss or gain, N use efficiency, or long-term soil health and sustainability [12,19]. In a cropping system that includes a legume crop, a crop-based N budget can be calculated as the difference between the sum of BNF and fertiliser N input and the harvest N offtake, providing a useful indicator for maximizing yield and minimizing input [14,15,20]. However, this simple approach has the potential to mischaracterize environmental impacts given the uncertainties related to the fate of N (e.g., whether surplus N is stored in the soil or lost to the environment when fertiliser is applied) [19]. Thus, a soil-based N budget derived by summing all N inputs and subtracting all N outputs for a crop-soil system can reduce the uncertainties because both leaching and gaseous N emissions are considered.

Crop simulation models can be used to efficiently explore how different field management practices influence crop growth, nutrient cycling and soil water redistribution in agroecosystems. Previous studies have focused on soybean yield and soil water dynamics in the NEP using models in the DSSAT (Decision Support System for Agro-technology Transfer) modelling family [21], but with very little attention to BNF or soil-crop N budgets. The SPACSYS (Soil-Plant-Atmosphere Continuum SYSTEM) model [22] is able to quantify the dynamics of the BNF rate while simulating soil carbon (C) and N cycling and water movement, taking account of various agronomic management practices including fertiliser N application, irrigation and tillage.

In this study, previously unpublished datasets from 2007 to 2010 from an experimental site in Northeast China with different fertiliser and irrigation schedules were used to evaluate the ability of SPACSYS to simulate soybean growth in that region. The validated model was then used to explore how different field management practices could affect BNF, yield, seed N content and the soil N budget and provide recommendations for optimum fertiliser N use under different climatic conditions in the region.

2. Materials and Methods

2.1. Study Site

The experiment was carried out from 2007 to 2010 at the Gongzhuling experiment site (43°29' N, 124°48' E), Jilin province, within a temperate continental monsoon climate. The long-term (1951–2010) mean temperature over the growing period (from late April to early September) at the site was 19.4 °C, with the highest temperature in July. Long-term average precipitation over the soybean growing period was 482 mm, with the maximum amount of precipitation in July and August. The mean total sunshine hours were 1069 h over the same period (Figure 1). The dominant soil texture is clay loam (FAO classification), and soil properties are shown in Table 1.

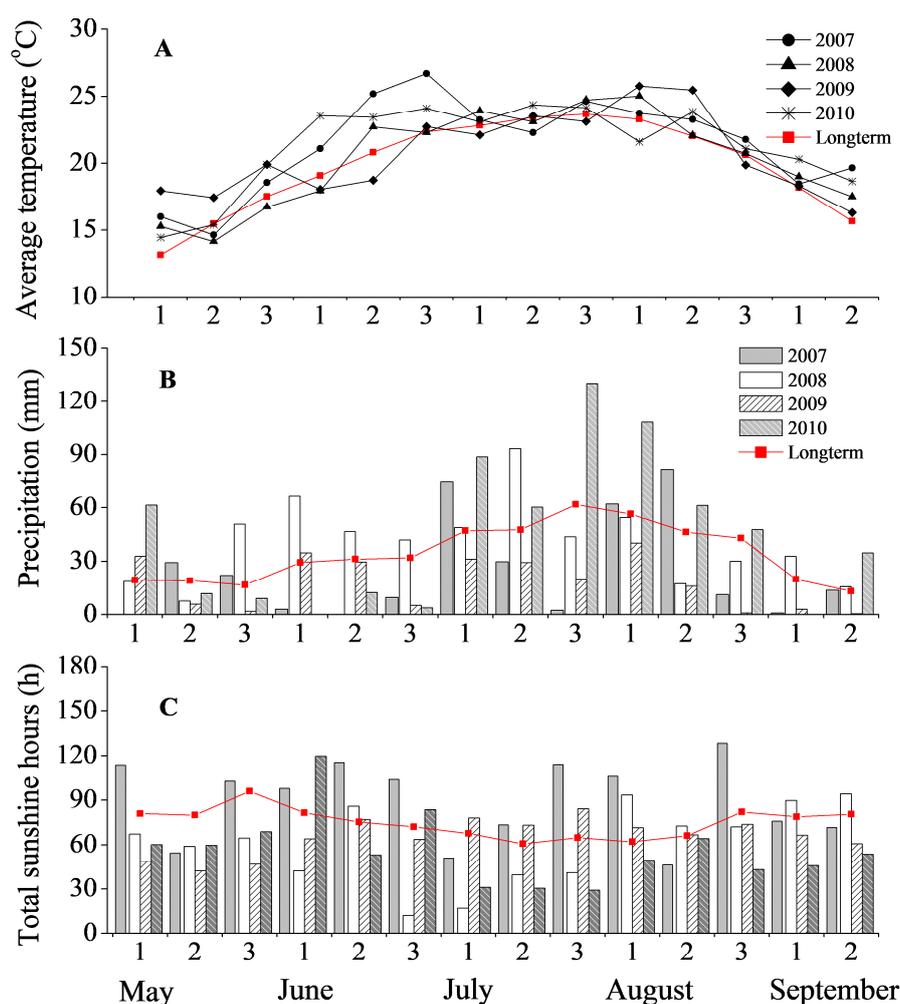


Figure 1. Ten-day average temperature (A), accumulated precipitation (B) and total sunshine hours (C) during the soybean growing season from 2007 to 2010 and long-term average at the Gongzhuling experimental site. 1 represents the first ten days, 2 represents the middle ten days and 3 represents the last ten (eleven) days.

Table 1. Soil physical and chemical properties at the experiment site.

Depth	Bulk Density	Volumetric Water Content at 15 kPa	Field Capacity	Soil Organic Matter	Total N	NH ₄ -N	NO ₃ -N	pH
(cm)	(g/cm ³)	(%)	(%)	(g/kg)	(g/kg)	(mg/kg)	(mg/kg)	(—)
0–10	1.30	14.3	35.9	25.1	1.59	6.65	16.77	6.34
10–20	1.32	14.2	34.9	24.8	1.55	6.82	10.48	6.27
20–40	1.34	14.0	33.8	23.6	1.02	4.46	9.28	6.56
40–60	1.52	19.0	43.8	6.7	0.33	—	—	6.52
60–80	1.63	18.9	37.5	5.8	0.26	—	—	6.56

The weather conditions across the growing season were quite different in each year. Average temperatures during the growing season for each year were slightly higher than the long-term average, especially for the vegetative phase (from May to the end of June). The growing seasons of 2007 and 2009 were drier, with total precipitation of 341 and 252 mm, respectively, compared with 569 and 630 mm in 2008 and 2010, respectively. The uneven distribution of precipitation resulted in a slight drought stress during August in 2008 and during June in 2010. The sunshine hours differed greatly for the four growing seasons. The total sunshine hours in the 2007 growing season (1255.7 h) were higher

than the long-term average, while those for the 2008, 2009 and 2010 growing seasons were lower, with values of 852, 918 and 794 h, respectively. However, sunshine hours for the seed filling period in 2007 (281 h), 2008 (238 h) and 2009 (212 h) were higher than the long-term average (210 h), and lower in 2010 (157 h). In summary, 2007 was drier with the most sunshine hours, 2008 wetter but with a slight drought stress at the reproductive stage and fewer sunshine hours, 2009 drier with fewer sunshine hours and 2010 wetter with the least sunshine hours (Figure 1).

2.2. Experimental Design and Data Collection

The experiment included two factors (fertiliser and irrigation) using a split-plot design with a row spacing of 0.6 m and planting density of 20 plants/m². The split-plot size was 51.3 m² (9.5 × 5.4 m) in 2007 and 2008 and 27 m² (6 × 4.5 m) in 2009 and 2010. Soybean seeds, of the indeterminate variety Jiunong21, were sown in late April (27th or 28th) each year and harvested in mid-September. Two levels of irrigation in each growing season were designed: (1) no-irrigation, i.e., rain-fed treatment (W0) and (2) irrigation at a 20 mm rate with the timing based on the soil moisture status (W1), at V6 (June 19th) in 2007, at R4 (pod setting stage) in 2008 and 2010 and at R1 (early flowering stage) in 2009. However, as soil moisture content was too low, an additional irrigation of 25 mm at R4 was applied in 2009. We, therefore, defined the irrigation treatment in 2009 as a new treatment (W2) to differentiate from W1. A basal application of compound fertiliser at the rate of 250 kg/ha (N:P:K = 15:20:15) was made in 2007. Fertiliser treatments during 2008–2010 were: no N fertiliser application (F0); application before sowing with an N rate of 37.5 kg/ha (F1); and application before sowing (37.5 kg/ha) plus top dressing at the flowering stage (22.5 kg/ha) (F2). There was a total of 20 combinations of factors (year × irrigation × fertilization) across the four years, and each replicated 3 (the 2008–2010 seasons) or 4 (the 2007 season) times.

The dates for the main phenological stages including emergence, initiation of flowering (R1), full flowering (R2), pod setting (R4), seed filling (R6) and maturity (R8) were recorded following the description by Fehr et al. [23] Three plants were randomly selected in each plot at these stages, and the leaf area index (LAI) and dry biomass of roots, leaves, stems and pods if present was measured in each growing season. Seed yield (dry matter) at harvest was calculated based on a 2 m² quadrat sample. LAI was estimated by the disc method [24]. Ten leaves were randomly chosen from the three plants and all of them were perforated once in the central nervure. Root biomass was measured by the root core method to a depth of 100 cm, with a 10 cm core diameter [25]. Roots were picked out and both leaf discs and roots were dried in a forced aeration oven at 70 °C until constant mass was achieved. Soil volumetric moisture content was measured using soil cores to the depth of 60 cm, divided into 4 layers with the top two layers of 10 cm each and the lower two of 20 cm each. Three cores per plot were taken randomly. The measurements were taken seven to eleven times over the growing season. The N content in each organ (roots, leaves, stems and seeds) was measured using the Kjeldahl method at each growth stage in 2008 (only for treatments with irrigation at different fertiliser application rates) and 2009 (for all the treatments).

Daily meteorological data for the site (maximum and minimum temperatures, sunshine hours, precipitation, relative humidity and wind speed) were downloaded from the China Meteorological Administration [26]. Daily solar radiation was estimated from daily sunshine hours [27].

2.3. The SPACSYS Model

The SPACSYS (Soil-Plant-Atmosphere Continuum SYStem) model is described fully by Wu et al. [22] The generic crop growth and development component describes phenological development, daily photosynthesis rate, root penetration and growth, N uptake, partitioning and translocation of photosynthate and absorbed N and the senescence of leaves, stems and roots. A BNF rate is calculated according to one of two algorithms, based on either root biomass [28] or above-ground biomass [29]. Nitrogen cycling is coupled with C cycling and comprises external N input (deposition, chemical fertiliser and manure), organic matter decomposition, nitrification, denitrification and N

losses through leaching and surface runoff. Organic matter (OM) decomposition flow includes fresh litter, dissolved OM, humus and microbial OM. The model algorithms are described in detail elsewhere [22,30,31], but we present here the specific calculations for the BNF rate (F_{fix}) based on root biomass:

$$F_{fix} = f_{nodule} \times W_{root} (106.6232 - 9.0213 \times \lg N_{con}) \times f_t \times f_w \quad (1)$$

where W_{root} is root biomass; f_{nodule} is the fraction of nodule in root biomass; N_{con} is soil mineral N content, which may inhibit the process of N_2 fixation at higher values [27,32,33]; and f_t and f_w are the temperature and water impact factors, respectively:

$$f_t = \begin{cases} 0.0 & (T < T_{fixmin}, T > T_{fixmax}) \\ \frac{T - T_{fixmin}}{4} & (T_{fixmin} \leq T \leq T_{fixoptl}) \\ 1.0 & (T_{fixoptl} < T < T_{fixoptu}) \\ \frac{T_{fixmax} - T}{4} & (T_{fixoptu} < T \leq T_{fixmax}) \end{cases} \quad (2)$$

$$f_w = \begin{cases} 1 & (\frac{W_a}{W_{max}} \geq 0.55) \\ (\frac{20}{11}) \times \frac{W_a}{W_{max}} & (\frac{W_a}{W_{max}} < 0.55) \end{cases} \quad (3)$$

where T_{fixmin} is the minimum temperature below which fixation ceases; $T_{fixoptl}$ and $T_{fixoptu}$ define the optimum temperature range; T_{fixmax} is the maximum temperature above which fixation stops; W_{max} is maximum available water capacity; and T and W_a are soil temperature and available water content, respectively.

2.4. Model Calibration and Validation

A subset of data for seed yield, dynamics of LAI, dry matter in different plant organs and N content from the F0 × W0 treatment over the period between 2008 and 2010 was used to calibrate the SPACSYS model. The remaining data were used for model validation. The plant genetic parameter values that lead to minimum bias at the calibration stage were used for validation and scenario simulation (see next sub-section). The model was run from 2005 to reduce the side-effects of the configured initial conditions. The calibrated parameters used in this study are shown in Table 2. A combination of 4 statistical indicators was used to evaluate model performance: (1) the correlation for determination (R^2)—evaluating the percentage of variation in observations explained by the model; (2) the relative root mean square error (nRMSE%)—quantifying the relative magnitude of error; (3) the modelling efficiency (EF)—determining whether the modelling output matches the observed data ($EF > 0$ is the condition to achieve “good fitting results” between simulated and observed data); and (4) the index of agreement (D)—reflecting the degree to which the observed variate is accurately estimated by the simulated variate [34]. EF and D are calculated as:

$$EF = \frac{\sum_{i=1}^n (O_i - O)^2 - \sum_{i=1}^n (S_i - S)^2}{\sum_{i=1}^n (O_i - O)^2} \quad (4)$$

$$D = 1 - \frac{\sum_{i=1}^n (O_i - S_i)^2}{\sum_{i=1}^n (|O_i - O| + |S_i - S|)^2} \quad (5)$$

where n is the total number of samplings; O_i and O are the i th observation value at sampling i and the average of the observation values, respectively; S_i and S are the i th simulation value and the average of the simulation values, respectively and “|” is an absolute calculation.

Table 2. Calibrated crop parameters used in simulation.

Parameter	Unit	Value
<i>Crop growth and development</i>		
Accumulated temperature required from sowing to emergence	°C·d	160
Accumulated temperature required from sowing to pod setting	°C·d	1550
Accumulated temperature required from pod setting to maturity	°C·d	1120
Critical photoperiod for vegetative stage over which plant development will stop	h	14.0
Threshold temperature for vegetative stage	°C	9.0
Threshold temperature for reproductive stage	°C	10.0
Optimum temperature for photosynthesis	°C	20.0
Extinct coefficient	—	1.0
Leaf transmission coefficient	—	0.15
Minimum leaf nitrogen concentration below which photosynthesis ceases	gN·g ⁻¹ ·DM	0.01
Optimum leaf nitrogen concentration above which photosynthesis is in unify	gN·g ⁻¹ ·DM	0.06
Q10 value for plant maintenance respiration	—	3.0
Specific leaf area	m ² ·g ⁻¹ ·DM	0.0325
Root death rate as litter	d ⁻¹	0.04
Rate of stem-carbon lost as litter	d ⁻¹	0.02
<i>N₂ fixation</i>		
Fraction of nodule in root biomass	—	0.20
The lower threshold of optimum temperature for N fixation	°C	13.0
The upper threshold of optimum temperature for N fixation	°C	26.0
The minimum temperature below which N fixation ceases	°C	9.0
The maximum temperature over which N fixation stops	°C	30.0

2.5. Simulation Scenarios Design

In order to search for optimum agronomic management practices for maintaining higher soybean yields while maximizing BNF, we designed various scenarios regarding fertiliser N application and irrigation to simulate their effects on soybean yield, BNF and seed N content. Five fertiliser application strategies (application before sowing, BS; application at initial flowering stage, R1; application at pod initiation stage, R3; application before sowing and N top dressing at initial flowering stage with split amount of 7:3, BR1; application before sowing and N top dressing at pod initiation stage with split amount of 7:3, BR3) with various application rates (0, 50 and 100 kg N/ha) and four irrigation schedules (rain-fed, W0; irrigating before flowering stage, Wr; irrigating at the R3 stage, W1; irrigating at both R3 and R5 stage with a doubled total irrigation amount, W2) were included, giving 44 scenarios in total. For each irrigation event, 40 mm of water was applied [35]. Further simulations were designed for N fertiliser rates between 0 and 100 kg N/ha with an increment of 10 kg N/ha applied before sowing to study the optimum fertiliser N rate for achieving a higher seed yield, and the same rates applied at the reproductive stage to achieve a higher seed N in the NEP.

2.6. Nitrogen Budget Calculation

A soil N budget during the growing season was calculated based on the outputs from the simulations for the different scenarios:

$$N_{bud-soil} = N_{fert} + N_{depo} + N_{fix} - N_{offtake} - N_{loss} - N_{deni} \quad (6)$$

where N_{fert} is N input from chemical fertiliser and manure; N_{depo} is N input from atmospheric deposition; N_{fix} is N input from BNF; $N_{offtake}$ is N removed through harvested seeds and residue; and N_{loss} and N_{deni} are N losses through leaching (below 1.5 m soil depth) and surface runoff, and denitrification,

respectively. When $N_{bud-soil} < 0$, it can be inferred that the soil is a net source of N, otherwise it is a net sink.

3. Results

3.1. Model Calibration and Validation for Plant Growth, Soil Water Content and Soil Temperature

Validation of the calibrated model output for crop dry matter accumulation, N content in various organs and LAI at different stages, soil temperature and moisture during the growing seasons for the F0 × W0 treatment is shown in Figures 2–4. Overall, the simulated results were at an acceptable level, with simulated trends and patterns the same as the observed ones (Table 3). The model captured the trend in leaf biomass and LAI well, with $R^2 > 0.9$ and $EF > 0.8$ (Figure 2B–F, Table 3). The simulation bias for stem biomass was larger in the first three stages, with a better fit between the simulated and observed values in later stages (Figure 2G–I). Root biomass was generally well simulated with a little overestimation in the early stages (Figure 2J–L). Simulated N content in different organs agreed with measured values in most cases, especially for leaf and seed, with $nRMSE\% < 20\%$ (Figure 3, Table 3).

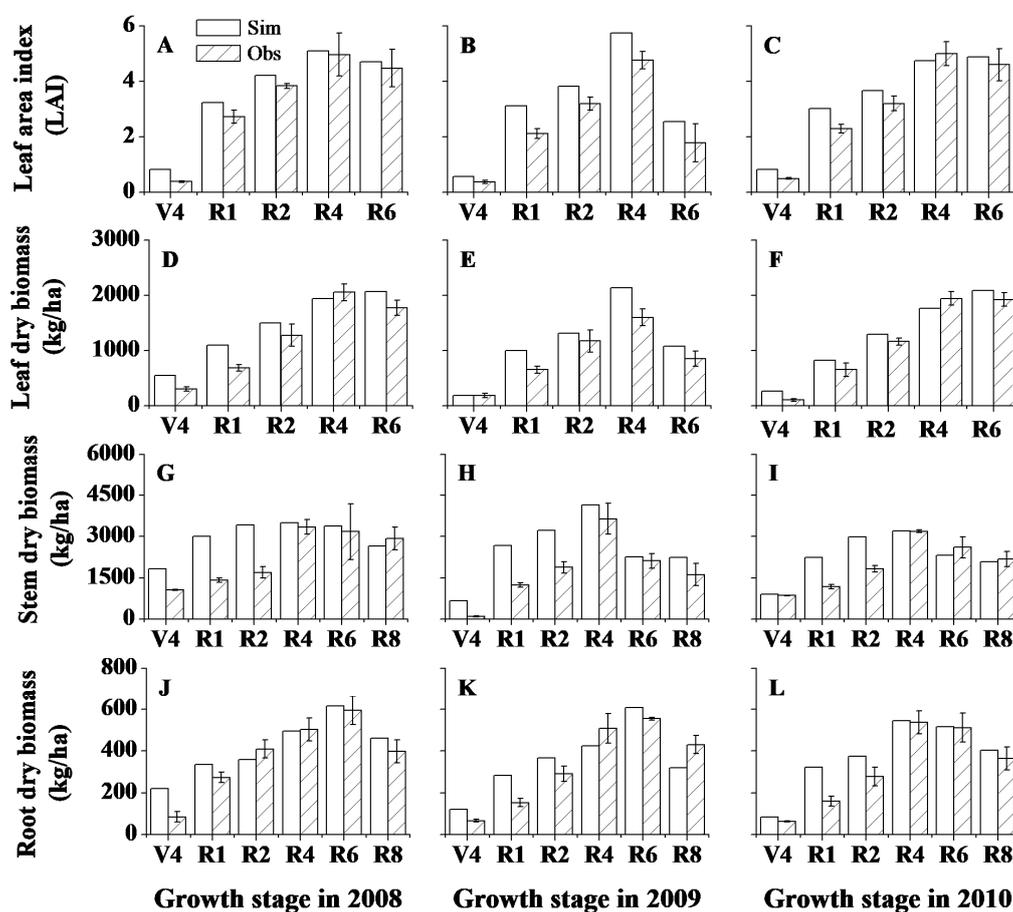


Figure 2. Comparison between simulated and observed data on Model simulation for dynamics change of leaf area index (LAI) (A–C), leaf dry biomass (D–F), stem dry biomass (G–I) and root dry biomass (J–L) at various growing stages during different growing seasons for the F0 × W0 treatment.

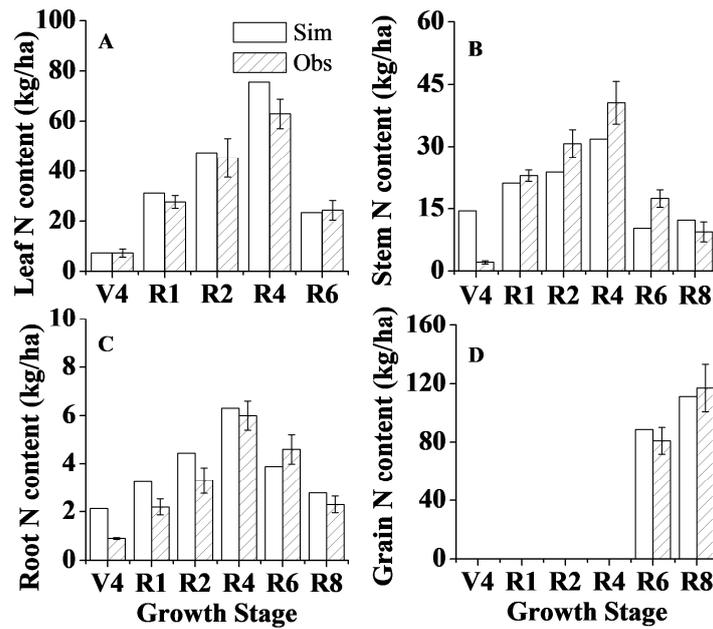


Figure 3. Comparison between simulated and observed N content in leaves (A), stems (B), roots (C) and seeds (D) at various growing stage in 2009 for the F0 × W0 treatment.

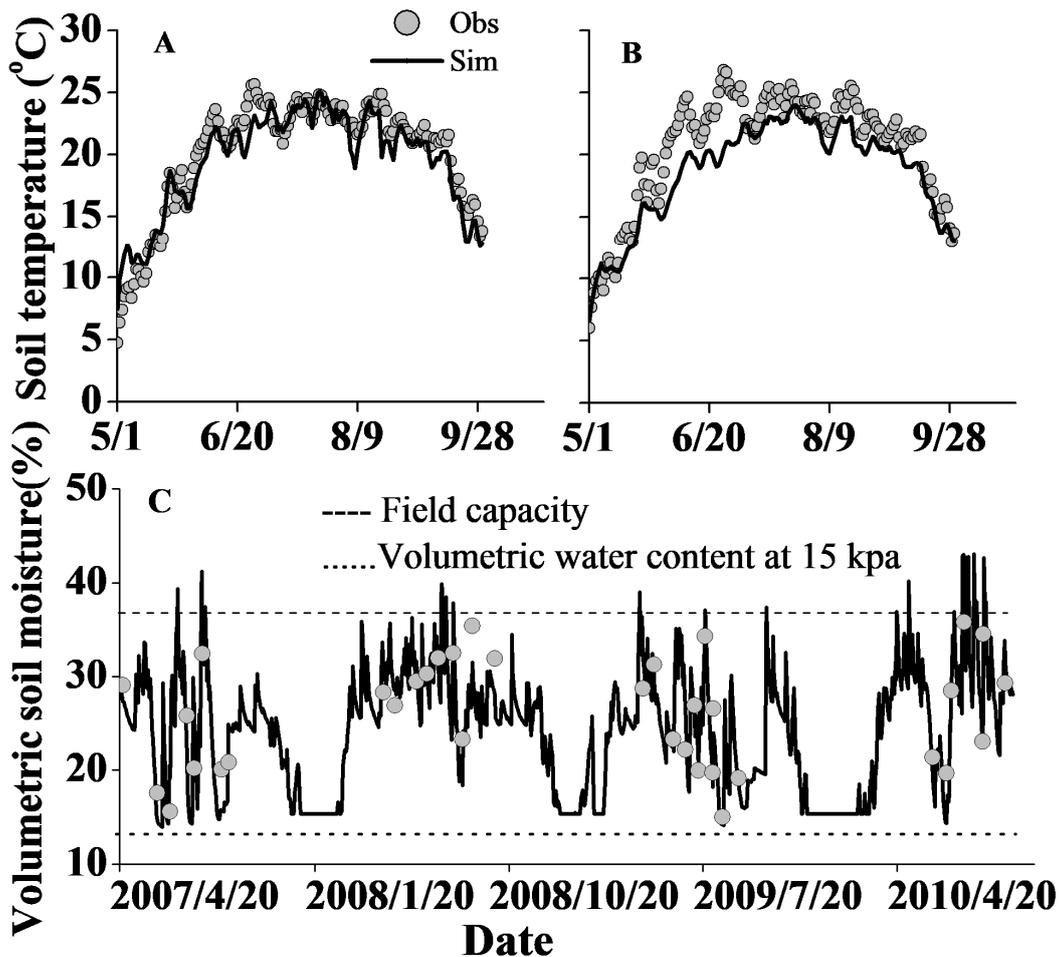


Figure 4. Comparison between simulated and observed soil temperature at 10 cm (A) and 20 cm (B) soil depth for 2010 and soil water content in the 0–10 cm soil depth (C) for the rainfed treatment.

Table 3. Statistical analysis of simulated soil water content, leaf area index, dry matter and nitrogen (N) content of leaves, stems roots and seeds and seed yield. (R^2) the correlation for determination; (nRMSE%) the relative root mean square error; (EF) the modelling efficiency; (D) the index of agreement.

	Calibration					Validation				
	No	R ²	nRMSE%	EF	D	No	R ²	nRMSE%	EF	D
Soil Temperature (°C)	306	0.88 *	10.1	0.81	0.97					
Soil water content (%)	110	0.72 *	14.4	0.57	0.90	171	0.65 *	15.5	0.48	0.90
Leaf area index (LAI)	15	0.96 *	18.8	0.88	0.97	83	0.86 *	22.1	0.83	0.90
Leaf dry matter (kg/ha)	15	0.92 *	23.4	0.84	0.96	83	0.90 *	17.6	0.90	0.97
Stem dry matter (kg/ha)	18	0.52 *	44.9	0.22	0.72	100	0.52 *	38.8	0.25	0.77
Root dry matter (kg/ha)	18	0.84 *	23.3	0.79	0.94	100	0.83 *	20.1	0.82	0.94
Seed Yield (kg/ha)	6	0.99 *	6.1	0.96	0.99	38	0.86 *	12.1	0.85	0.95
Leaf N (kg/ha)	5	0.99 *	17.8	0.90	0.98	40	0.92 *	15.7	0.91	0.98
Stem N (kg/ha)	6	0.75 *	36.6	0.66	0.85	48	0.61 *	29.0	0.61	0.88
Root N (kg/ha)	6	0.85 *	27.7	0.72	0.91	48	0.71 *	29.5	0.61	0.89
Grain N (kg/ha)						16	0.94 *	7.7	0.92	0.97

* Significant association at the 5% level.

The model captured the peaks and troughs in soil temperatures reasonably well (Figure 4A,B). Specifically, the model reproduced the fluctuations in the 10 cm soil temperature better than those for 20 cm, for which it slightly under-predicted during the vegetative stage. Dynamics of soil water content in the soil profile were captured by the model with nRMSE% < 15%. The model performed better for deeper soil layers (data not shown) than for the top soil layers. The simulated values for the top 10 cm soil layer matched the observed ones well, but slightly under-predicted against 2008 observations (Figure 4C).

Statistical analysis for the comparison between simulated and measured values for validation is presented in Table 3. A comparison between simulated and measured seed yield for all treatments is shown in Figure 5. Both estimated yield and seed N content matched observations very well, with nRMSE% of 12.1% and 7.7%, respectively. Although leaf dry matter, leaf N content and LAI showed large relative errors, the simulated output matched observed data very well, with EF and R² close to 1. Similarly, the general trend and pattern of the simulated root biomass matched the observations. Simulation for stem biomass and stem N content was less good, with nRMSE% > 30%, but R² > 0.5 and EF > 0. From these comparisons, we concluded that the simulated outputs were acceptable and that the SPACSYS model could be used for further scenario analysis.

3.2. Response of Seed Yield to Simulated Field Management Strategies

Soybean seed yield varied from 1970 to 3400 kg/ha under the different simulated field management strategies (Table 4). Yield increased with N fertiliser application rate and irrigation, but the rate of increase differed under dry and wet growing conditions. The highest irrigation use efficiency and higher yield increase occurred in drier years. For example, yield increased by 3.9–15.2% and 20.9–33.3%, compared with yield from W0 in 2007 and 2009, respectively, but there were no differences between the irrigated and rain-fed treatments for 2008 and 2010. Notably, compared with the rain-fed treatment, yield increased substantially by 12% for treatment W1 but only by 4% for treatment Wr in 2007, with a further 2% increase for treatment W2 compared with that for treatment W1 in the same year. However, yield increased by 26–29% for treatment Wr in 2009, and a further 4–5% improvement for treatment W2. Yield increased by up to 9% for an increase in N application from 50 to 100 kg N/ha, a smaller response to that from increased irrigation. When fertiliser was applied as a single application, seed yield was higher with basal application than for application during the reproductive stages. These results demonstrated that N supply was not the main constraint to yield increase in this region but that climatic conditions were. Irrigation applied during the reproductive stage resulted in higher yield regardless of whether there was drought stress.

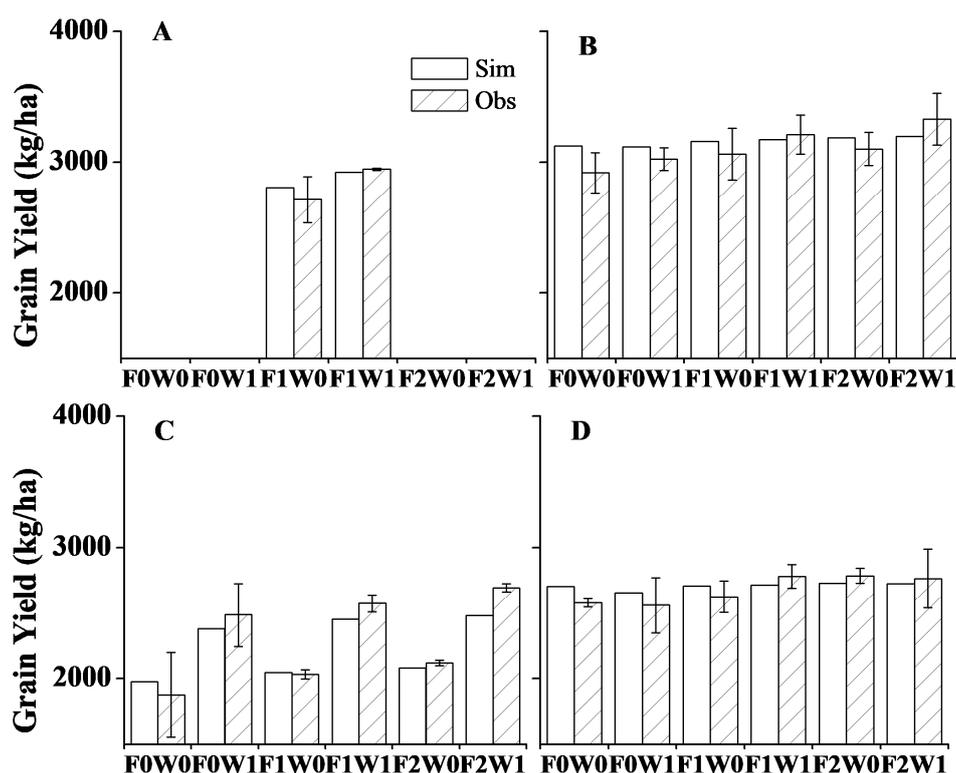


Figure 5. Model evaluation of seed yield for different treatments during 2007–2010. (A) 2007, (B) 2008, (C) 2009, (D) 2010.

3.3. Response of BNF to Field Management Strategies

Nitrogen fixation responded positively to the increase in irrigation but negatively to the increase in fertiliser N application and delays in application timing. From the model simulations, soybean fixed between 97 and 143.5 kg N/ha in 2007, between 88 and 125 kg N/ha in 2008, 68 and 134 kg N/ha in 2009 and 69 and 105 kg N/ha in 2010 (Table 4). The responses of BNF to the irrigation schedules were different from year to year over the simulation period, indicating the importance of weather conditions during a growing season. For treatment Wr (i.e., irrigation before flowering), BNF increased by 9–17% for 2007, 0.6–3.6% for 2008, 18–29% for 2009 and 4–9% for 2010 compared with the rain-fed treatment in the same year. However, for treatment W1, BNF increased by 7–9% for 2007, 0.5–4.0% for 2008, 19–30% for 2009 and 4.5–6.5% for 2010. If the crop was irrigated twice (W2), BNF increased by 20–25% for 2007, 9–13% for 2008, 29–33% for 2009 and 2.5–4.0% for 2010. From our simulations, basal N application (BS) at rates of 50 and 100 kg N/ha resulted in a decrease of 6–16%, 8–17%, 7.5–17% and 10–20% in BNF for 2007, 2008, 2009 and 2010, respectively. However, BNF decreased by 15–33% when fertiliser was applied at a late stage of soybean development compared with no fertiliser application.

3.4. Response of N Content in Seed to Field Management Strategies

Seed N content increased in response to N application, and between 179–211 kg N/ha was accumulated in seed in the wetter years, but only 60–70% of this amount was achieved in the drier year 2009 without irrigation (Table 4). Irrigation increased seed N content by over 10% in the drier years but only by a small amount, if at all (–2.2–4.3%), in the wetter years compared to that for the rain-fed treatments. Response of seed N content to fertiliser N application rate might not be economical, with an increase of 7.6–23.7 kg N/ha when doubling the application rate from 50 to 100 kg N/ha. Seed N content was the least when fertiliser was applied as a single dose at the reproductive stage, especially at R3. Basal application only resulted in a higher seed N content compared with that applied at R3 alone, with an average increase of 2.8, 1.2, 5.3 and 10.8 kg N/ha for 2007, 2008, 2009 and 2010, respectively.

Table 4. Seed yield, biological N fixation and N content in seed from 2007 to 2010 with different irrigation and fertiliser management strategies.

Time (Stage)		BS ¹			BR1 ²		BR3 ³		R1 ⁴		R3 ⁵	
Rate (kg/ha)		0	50	100	50	100	50	100	50	100	50	100
Yield (kg/ha)												
2007	W0 ⁶	2716	2839	2953	2823	2934	2825	2937	2793	2880	2801	2899
	Wr ⁷	2833	2957	3074	2935	3050	2941	3055	2902	2988	2914	3011
	W1 ⁸	3084	3192	3301	3182	3286	3185	3292	3162	3247	3174	3277
	W2 ⁹	3129	3237	3346	3227	3331	3230	3335	3207	3291	3218	3321
2008	W0	3125	3126	3202	3121	3193	3122	3196	3108	3172	3114	3187
	Wr	3052	3127	3200	3122	3191	3124	3195	3109	3168	3118	3189
	W1	3059	3133	3207	3127	3195	3130	3201	3112	3171	3123	3195
	W2	3083	3154	3210	3147	3214	3149	3219	3108	3188	3141	3209
2009	W0	1977	2068	2161	2065	2155	2061	2146	2052	2139	2043	2118
	Wr	2551	2611	2746	2640	2730	2633	2716	2629	2725	2619	2702
	W1	2432	2522	2616	2520	2608	2514	2596	2504	2587	2493	2573
	W2	2635	2727	2823	2725	2816	2719	2808	2711	2799	2703	2793
2010	W0	2635	2710	2786	2699	2770	2693	2758	2675	2729	2658	2693
	Wr	2694	2771	2849	2758	2828	2754	2819	2727	2778	2717	2752
	W1	2684	2744	2818	2731	2799	2725	2789	2704	2754	2688	2724
	W2	2680	2759	2831	2744	2812	2738	2801	2717	2734	2701	2734
N ₂ fixation (kg N/ha)												
2007	W0	130	119	109	116	105	116	103	110	98	109	97
	Wr	144	135	128	129	120	130	120	121	107	122	107
	W1	139	127	119	125	114	124	112	120	106	119	104
	W2	142	130	121	127	116	127	114	122	108	121	106
2008	W0	124	111	103	109	99	108	96	105	92	101	88
	Wr	125	115	106	112	102	111	99	108	96	104	89
	W1	125	114	106	112	102	111	99	108	96	104	89
	W2	124	113	104	110	101	109	97	105	95	102	89
2009	W0	96	87	79	85	76	83	72	80	70	76	68
	Wr	121	110	102	109	99	105	92	101	90	96	81
	W1	120	110	102	108	99	105	93	101	88	95	81
	W2	134	124	116	123	114	120	109	115	104	110	97
2010	W0	99	87	79	84	75	83	74	80	70	79	25
	Wr	105	94	86	89	80	88	79	84	73	83	73
	W1	103	91	82	87	78	86	76	83	72	81	71
	W2	105	93	84	89	80	88	78	82	72	82	72
Seed N content (kg N/ha)												
2007	W0	155	178	201	176	198	177	199	173	192	175	197
	Wr	167	190	214	187	210	188	211	182	202	185	207
	W1	174	197	220	195	217	196	218	192	212	195	219
	W2	175	197	221	196	218	197	219	193	213	196	220
2008	W0	183	196	212	195	210	195	211	193	208	194	211
	Wr	179	194	210	193	208	194	209	191	205	193	210
	W1	179	195	211	194	209	195	210	192	205	194	211
	W2	181	196	208	195	209	195	210	193	205	194	210
2009	W0	115	130	150	129	149	129	147	128	147	126	143
	Wr	156	172	196	176	196	174	193	172	193	169	188
	W1	151	171	192	170	190	169	187	167	186	165	183
	W2	164	188	209	187	208	186	206	184	204	183	203
2010	W0	160	176	192	174	189	172	186	169	182	166	174
	Wr	167	183	200	180	196	179	194	175	186	172	180
	W1	163	177	193	175	190	174	187	173	182	167	175
	W2	162	178	193	175	190	174	187	170	181	167	174

¹ Basal application only. ² Basal application (70%) and top dressing at R1 (30%). ³ Basal application (70%) and top dressing at R3 (30%). ⁴ Fertiliser applied at R1 stage only. ⁵ Fertiliser applied at R3 stage only. ⁶ Rain-fed treatment. ⁷ Irrigation applied before flowering. ⁸ Irrigation applied at pod setting stage. ⁹ Irrigation applied at pod setting stage and seed filling stage with doubled irrigation amount.

3.5. Soil Mineral N Budget under Various Field Management Strategies

The soil mineral N budget over the soybean growing season was negative when no fertiliser was applied, with a range between -3.5 and -58.2 kg N/ha and an average value of -39.0 kg N/ha (Figure 6). It increased with N fertiliser application; a treatment of 100 kg N/ha leads to an average increase of 28.3, 33.4, 33.8 and 38.2 kg N/ha in 2007, 2008, 2009 and 2010, respectively. The higher value of the soil N budgets in 2007 and 2009 indicated a lower plant N uptake efficiency in these drier years. Atmospheric deposition over the growing season ranged from 20.6 to 28.9 kg N/ha, responsible for 9–22% of the total N input. From the simulations, N loss through denitrification ranged between 1.5 and 7.9 kg N/ha. The loss through leaching and surface runoff went from nil to 37 kg N/ha and became larger at the higher application rate with additional irrigation application, especially in 2010, when N losses accounted for 10.9–13.2% of the total input under the W0 treatment and for 15.1–18.3% under the W2 treatment. Meanwhile, it was between 10.9–16.8% with incorporation of basal fertilization, with an additional 1.0–1.5% loss if a single fertiliser application was made at the reproductive stages.

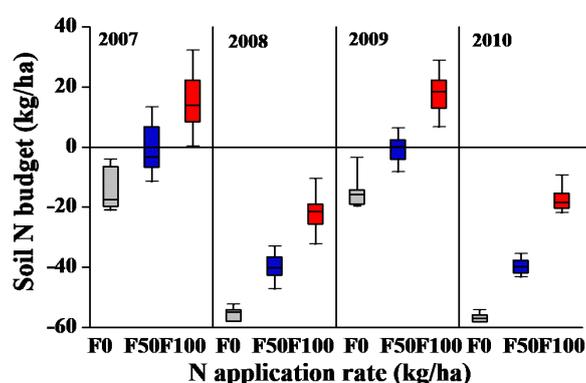


Figure 6. N budgets at different fertiliser N application rates and irrigation treatments. Grey columns represent those without N application, blue with an application rate of 50 kg N/ha and red for 100 kg N/ha application. The box plots show the 0, 25, 75 and 100 percentile budget. The lines in the box represent the mean budget.

3.6. Recommendations for Fertiliser N Application Rate in Northeast China

In general, irrigation at the reproductive stage increases seed yield, BNF and seed N in drier years, while the rain-fed system is better in wetter years. Fertiliser applied before sowing (BS, BR1, BR3) can improve seed yield and N content, but when applied at the reproductive phase resulted in an additional reduction in BNF (Table 4). Biological N fixation is generally regarded as an environmentally friendly source of N. However, soil N budgets were negative if no fertiliser was applied during the growing season (Figure 6), indicating that the system was unsustainable. A certain amount of fertiliser N as basal application could, therefore, be beneficial for BNF compared with other application schedules. However, higher rates constrained BNF. The simulation results indicated that the appropriate amount (the intersection of the two lines in Figure 7) of N fertiliser for the rain-fed treatment could lead to yield increase without reducing BNF by too much (left panels in Figure 7). A fertiliser application rate of 31.5, 19.4, 30.5 and 16.3 kg N/ha was appropriate for 2007, 2008, 2009 and 2010, respectively. With irrigation at the reproductive stage, the appropriate fertiliser application rate was 26.2, 21, 29.6 and 16.6 kg N/ha for 2007 to 2010, respectively (data not shown). Combining all simulations, it is suggested that 25–30 kg N/ha can be applied before sowing during drier years with/without irrigation, and 15–20 kg N/ha can be applied in wetter years.

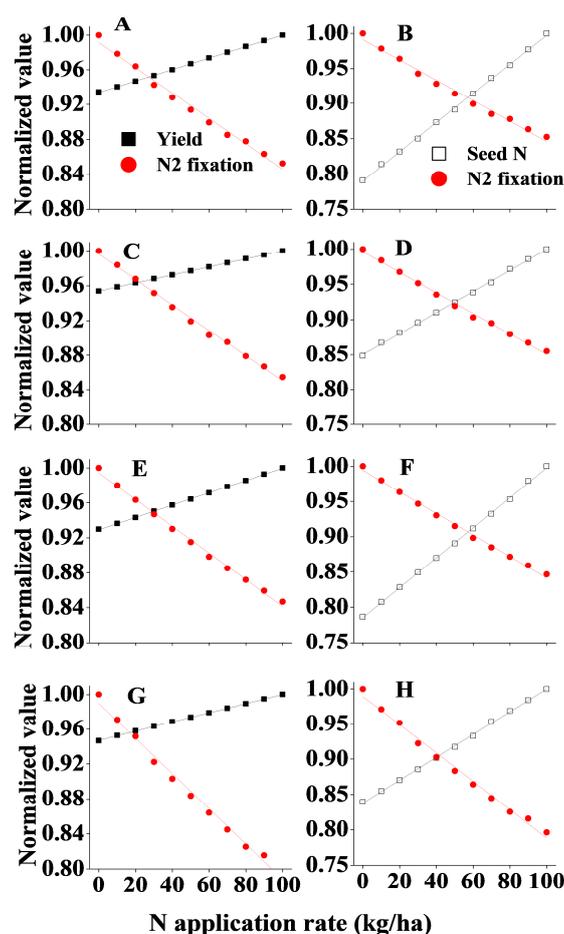


Figure 7. Responses of seed yield, N content in seeds and BNF to fertiliser N rates applied before sowing for the rain-fed treatment during the experimental years of 2007 (A,B), 2008 (C,D), 2009 (E,F) and 2010 (G,H).

Higher seed N content is used as an indicator of seed quality. From the simulations, fertiliser applied at the reproductive stage only resulted in lower seed N content compared to the strategies in which a basal application was included (Table 4). An appropriate fertiliser application rate of 57.5, 47.9, 58.5 and 41.9 kg N/ha before sowing was advised for 2007, 2008, 2009 and 2010, respectively, under rain-fed conditions (right panel in Figure 7) and 55.9, 48.7, 58.0 and 42.1 kg N/ha under irrigation (data not shown). In summary, the simulated results indicated that an application rate of 55–60 kg N/ha was suitable to achieve a higher seed N content in drier years, and 40–50 kg N/ha in wetter years. A higher N application rate is more important to maintain higher seed N content than for yield.

4. Discussion

The SPACSYS model was validated against the field experiment data from 2007–2010 in Northeast China. Results showed that after calibration it could effectively reproduce the observed dynamics of soybean growth in terms of biomass and N content in leaves, roots and seeds, final yield, soil temperature and soil water content (Table 3). However, larger discrepancies still existed for stem biomass and N content, which might be caused by simplified photosynthate partitioning coefficients to various organs in the model, but these discrepancies were less important in the context of our study. Such discrepancies are not uncommon [36], and in an inter-comparison of five soybean simulation models, Battisti et al. (2017) reported that results for crop growth under different climate conditions were often unsatisfying [37]. As a legume crop, it is particularly important to consider the ability to model BNF in soybean. Quantitative assessment of this was not included in recent published simulation

results for North China that mainly focused on final yield, soil water and evapotranspiration [21,38]. The inclusion of the simulation of the BNF rate in SPACSYS, using the root-based algorithm (with the model giving reliable estimation of root biomass, Table 3), therefore, represents an important progression in our ability to model soybean production and, particularly, how it is influenced by different irrigation and N fertiliser application strategies.

From the model simulations, soybean yield significantly increased with irrigation during the reproductive stage under dry conditions, consistent with previous observations that a single irrigation event at the reproductive stage increased yield by 30% [4]. However, the effect of N fertiliser application on yield was not as obvious compared to irrigation for all the experimental years (yield increase < 10% with a fertiliser application rate up to 100 kg N/ha). Previous experimental results have shown a similar yield increase with fertilization [18,33]. Seed N content increased in response to N application, consistent with field observations made using the ¹⁵N natural abundance method [39], and between 179–211 kg N/ha was accumulated in seed in the wetter years, but only 60–70% of this amount was achieved in 2009 without irrigation (Table 4). The effect of N fertiliser rate on soybean yield may vary with soil fertility, where differing amounts of mineral N can become available through mineralization [15,33]. In our study, we suggest that climate conditions were the major constraint on yield. The soil in the study area was fertile with a high organic matter content, where N supply from mineralization (with a range from 88 to 157 kg N/ha from the simulations) and BNF would be sufficient for soybean growth with low yield potential [10], meaning that weather conditions, especially photosynthetic active radiation (PAR) and rainfall, are the main driving variables for seed yield [3]. Soybean has a high water requirement between growth stages R1 to R5 [35]. By alleviating drought stress during this period, a 20–30% yield improvement can be achieved [35]. From our study, irrigation at R3 was the most effective.

Drier growing conditions obviously constrained BNF. Drought in 2009 resulted in lower BNF compared with that in 2007. It has been reported that BNF is more sensitive to drought than other physiological processes [9,40] because plants have to justify the partitioning of fixed C to cope with the drought impact, and nodule formation and fixation activities are most affected [3]. Soybean has the highest BNF ability at R5 and maximum N demand occurs between stages R3 and R5 [5,7,9]. BNF is also closely related to the crop growth rate i.e., C supply [5]; less photosynthesis through the growing season in 2010 resulted in a failure in insufficient energy for BNF compared with other experimental years. From our model simulations, BNF was 41% less in 2008 compared to that in 2007 during R3–R5 because photosynthesis rates during this stage were much lower in 2008.

Applied fertiliser N is reported to inhibit BNF [14,33,41,42] because a high soil mineral N content can constrain nodule numbers and biomass [16,40]. From our simulations, these constraints became larger with delayed fertiliser application timing. This is consistent with a previous field study in which fertiliser N application at the reproductive stage gave 3.3–5.5% reduction in the contribution of BNF compared with fertiliser application at sowing [8]. A possible reason is that legume plants may suffer from N hunger for a period of 15–20 days after sowing prior to nodule development and N fixation beginning, so a small amount of fertiliser at sowing can help root formation [11] and alleviate any N shortage over this period. On the other hand, BNF is an energy intensive process and legume crops will favour mineral N whenever it is available in preference to BNF. In our study, BNF was calculated based on a root-based algorithm generated from a perennial temperate forage legume, because robust data on soybean nitrogen fixation for this region are lacking; improvements to these estimates would benefit from controlled experiments on soybean N fixation rate and the influencing factors.

However, fertilization at the beginning of the growing season (BS, BR1 and BR3) can enhance seed N content, compared with a single application during the reproductive phase, which is supported by previous studies [13,41]. In cool climate zones, starter N fertiliser can have a positive effect on early root and above-ground growth, thus enabling more soil mineral N to be taken up, which would provide more N remobilized from leaves and stems during the reproductive phase [18,43].

In order to have sustainable agricultural systems, there should ideally be a balance between N inputs and outputs, without too much N either drawn from or left over in the soil [42]. Synchronization of higher temperatures and more precipitation in July (R3-R5 phase) resulted in a higher N mineralization rate to meet the N demand of the crop. With soil total N at this site of about 8.7 Mg/ha in the top 80 cm soil depth (Table 1), the deficit (39 kg N/ha, and compensated by the soil mineralization process) under the condition of no fertiliser application implies that soil total N would be used up in around 255 years at a mean annual turnover rate of 0.39% (0.04–0.67%). The soil N budget will always be negative without external N input and would become increasingly negative with increasing seed yield, indicating a soil N deficit [14]. The suggested fertiliser N application rates from this simulation were within the range of local practices (fertiliser applied before sowing). Nitrogen losses through denitrification, leaching and surface runoff were not only determined by external N inputs and plant uptake, but also by N mineralization. It has been reported that 55% of N losses may be attributed to the release of native soil N into the environment, due to the asynchrony between soil mineralization and crop uptake in a maize-soybean rotation system in the USA [19]. In our simulations, the derived N budget included N losses through denitrification and via leaching, accounting for the impact of N mineralization and nitrification which previous studies have failed to consider due to difficulties in measurement [20,42].

5. Conclusions

The process-based SPACSYS model was shown to provide good simulations of the dynamics of dry matter and N content in leaves, roots and seeds, and soil water content and temperature compared with field observations from Northeast China. Scenario testing suggested that yield did not substantially increase with additional N fertiliser, with a trade-off between added fertiliser N and BNF, while irrigation at the reproductive stage improved yield in the drier years but was not necessary in the wetter years. The soil-plant N budget was negative without N fertilization. However, N losses through runoff and leaching in wetter years can account for up to 18% of input N. From the perspective of maintaining the soil N balance and achieving higher yield, applying 25–30 kg N/ha before sowing was advised for drier years and 15–20 kg N/ha for wetter years. To achieve a higher seed N content, an application rate of 55–60 kg N/ha was recommended for drier year and 45–50 kg N/ha for wetter years.

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