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Is soybean yield limited by nitrogen supply?

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ABSTRACT

As soybean yield continues to increase, it seems critical to know if there is a yield level at which potential contribution of indigenous nitrogen (N) sources (N fixation and soil mineralization) becomes insufficient to meet crop N requirements for high yields, while still maintaining or increasing protein and oil concentration. We have hypothesized that, in absence of other limiting factors, degree of N limitation increases with increasing yield potential (Yp) of the production environment. To test this hypothesis, we developed a novel protocol to ensure an ample N supply during the entire crop season (full-N treatment). That protocol was applied to field-grown irrigated soybean in Balcarce (Argentina) and Nebraska (USA), where measured full-N seed yields were $\pm 15\%$ of their simulated Yp in 92% of the cases. The combination of locations, years, sowing dates, and N treatments resulted in a wide range of seed yields, from 2.5 to 6.5 Mg ha⁻¹. Overall, full-N seed yield averaged 11% higher than seed yield without N addition (zero-N). However, magnitude of yield difference between full-N and zero-N depended upon Yp, ranging from no detectable yield difference in low-Yp (ca. 2.5 Mg ha⁻¹) to up to 900 kg ha⁻¹ in high-Yp environments (ca. 6 Mg ha⁻¹). Seed yield differences were associated with higher aboveground dry matter, seed number, and seed weight in the full-N versus zero-N treatments. Seed protein (but not oil) concentration was higher in the full-N treatment, and both protein and oil yields were higher in the full-N versus zero-N treatments. Findings from this study indicate that (i) N limits soybean seed yield (as well as protein yield, and oil yield) in environments with high Yp, where indigenous N sources seem insufficient to fully satisfy crop N requirements, and (ii) yield response to N fertilizer can occur above a 2.5 Mg ha⁻¹ Yp threshold and has an upper limit of 250 kg seed per Mg increase in Yp.

1. Introduction

Soybean [*Glycine max* (L.) Merr.] is the most important legume crop globally, with a respective harvested area and total production of 118 million ha and 307 million Mg (FAOSTAT 2017, <http://faostat3.fao.org>), accounting for 56% of total global oilseed production (Wilson, 2008). Soybean is a key component of global food security as a source of protein for human food and animal feed, and oil for cooking and biofuel. Meeting soybean demand on existing cropland area for a global population of 9.7 billion people by year 2050 will put pressure on narrowing the existing gap between average producer yield and yield potential (Cassman et al., 2003; van Ittersum et al., 2013). Yield potential (Yp) is defined as the yield of a well-adapted cultivar when grown without limitations in water and nutrient supply and kept free of biotic stresses (weeds, diseases, and insect pests) (Evans, 1993). Hence, for a given site-year, soybean Yp is determined by solar radiation, temperature, and other factors that influence the length of time during which the crop was grown, such as variety maturity group and sowing

date. For example, soybean Yp has been postulated to range between 6 and 8 Mg ha⁻¹ in favorable environments of the US Corn Belt (Specht et al., 1999; Sinclair and Rufty, 2012).

Relatively high amounts of nitrogen (N) must be taken up by all crops to achieve high seed yields, particularly legumes, because of their high seed protein content (Sinclair and De Wit, 1976; Giller and Cadisch, 1995). On average, a soybean crop accumulates ca. 79 kg N ha⁻¹ in its aboveground biomass per each additional Mg seed yield, with the latter expressed at standard seed moisture of 0.130 kg H₂O kg⁻¹ seed (Salvagiotti et al., 2008; Tamagno et al., 2017). This ratio can be used to estimate the N uptake requirement over a range of soybean seed yields. For example, seed yields ranging from 6 to 8 Mg ha⁻¹ would be expected to have an associated N uptake requirement range of 480–640 kg N ha⁻¹. In contrast, only 240 kg N uptake ha⁻¹ would be required for a soybean yield of 3 Mg ha⁻¹, which is equivalent to the average soybean yield during the last 5 years in the United States (US) and Argentina (USDA-NASS, 2010–2014; https://www.nass.usda.gov/Quick_Stats/; <https://datos.magyp.gob.ar/>).

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Soybean rarely receives N fertilizer in producer fields (though a small application as ‘starter’ fertilizer is sometimes applied at sowing time). Still, there is currently much interest in the degree to which well-managed soybean crops, grown in favorable production environments, can meet the large N requirement for 6–8 Mg ha⁻¹ seed yields by relying exclusively on soil N mineralization and N fixation.

Soybean yield continues to increase over time due to genetic and agronomic improvement (Grassini et al., 2014a,b; Specht et al., 2014). Hence, it would be useful to discern the seed yield level at which the potential contributions of N from fixation and N from soil organic matter mineralization might jointly become insufficient to satisfy crop N requirement. Acquisition of that information requires data collection from soybean crops grown in stress-free conditions, where measured yields approach the site-year-specific Y_p, as determined by weather, sowing date, and variety. In such scenarios, experimental comparison of a ‘zero-N’ treatment receiving no N fertilizer (i.e., crop must rely exclusively on the indigenous N sources including N-fixation) versus a full-N treatment receiving N fertilizer applied as needed to sequentially ensure ample N supply throughout the crop-growing season. This comparison would need to be repetitively conducted across a wide range of Y_p production systems to generate sufficient data for determining whether there is a yield level at which those indigenous N sources are insufficient to meet crop N requirements.

Meeting crop N requirement is challenging because it requires temporal synchronization between the seasonal supply of N from indigenous N sources and seasonal crop N demand, and addition of N fertilizer when the latter exceeds the former at any time during the crop season (Cassman et al., 2002). No previous study has explicitly attempted to grow soybean in production settings of very high yields while ensuring non N-limiting conditions (Salvagiotti et al., 2008 and references cited therein). While a few studies have reported using large N fertilizer amounts in soybean (> 300 kg ha⁻¹), N fertilizer in these studies was applied as a single large dose near the sowing date, or in split applications during early vegetative stages (e.g., Brevedan et al., 1978; Herridge and Brockwell, 1988; Ray et al., 2006; Wilson et al., 2014). Because soybean absorbs ca. 60% of total N uptake during the pod setting and seed filling phases (Thies et al., 1995; Bender et al., 2015; Gaspar et al., 2017), it is difficult to determine the degree to which these previous studies have ensured non-N limiting conditions during those phases. Moreover, the yield response (or lack of response) to N fertilizer, reported by these previous studies, was likely confounded by other non-N growth-limiting factors. For example, in water-limited conditions, the yield response to N fertilizer can be amplified by the negative effect of temporary water shortages on N fixation (Purcell et al., 2004; Ray et al., 2006). Moreover, even in absence of water limitation, other growth-reducing factors may have limited crop growth in these experiments, given that measured yields consistently fell short of the high range of 6–8 Mg ha⁻¹ soybean Y_p (Specht et al., 1999; Sinclair and Ruffy, 2012), and also were less than measured yields (5–6 Mg ha⁻¹) that are routinely attained by progressive soybean producers (Grassini et al., 2014a,b, 2015).

In this study, we hypothesized that, in absence of other limiting factors, the degree of N limitation increases with higher Y_p. To test this hypothesis, we developed a protocol to ensure ample N supply during each phase of the soybean crop season. The protocol was applied to field-grown irrigated crops in Balcarce (Argentina) and Nebraska (USA) that were within ± 15% of their simulated Y_p based on site-year specific weather, sowing date, and variety. Results were interpreted using simple eco-physiological frameworks.

2. Materials and methods

2.1. Field experiments

Field experiments were conducted in Balcarce (BA), Argentina, during two crop seasons (BA-Y1: 2014/2015 and BA-Y2: 2015/2016),

Table 1

Description of field experiments conducted in Balcarce (Argentina) and Nebraska, NE (USA).

Experiment	Crop season	Location	Variety name and maturity group (MG)	Sowing date
Balcarce (BA-Y1)	2014/2015	37.7647 S 58.3125 W 118 m a.s.l.	DM2200 (MG:2.1), DM3810 (MG:3.8), DM4612 (MG:4.6)	Nov 1, Nov 27, Dec 18, Jan 6
Balcarce (BA-Y2)	2015/2016	37.7652 S 58.3117 W 118 m a.s.l.	DM2200 (MG:2.1), DM3312 (MG:3.3), DM3810 (MG:3.8)	Nov 3, Dec 12, Jan 11
Atkinson, NE	2016	42.6372 N 98.9561 W 635 m a.s.l.	AG2723 (MG:2.7)	April 25
Mead, NE	2016	41.2441 N 96.5016 W 368 m a.s.l.	AG2723 (MG:2.7)	May 8
Saronville, NE	2016	40.6005 N 97.9658 W 538 m a.s.l.	AG2431 (MG:2.4)	April 26
Smithfield, NE	2016	40.5380 N 99.6833 W 769 m a.s.l.	P24T19 (MG:2.4)	May 13

and at four sites in Nebraska (NE), USA, during one crop season (2016). The experiments in BA consisted of a combination of sowing date, variety maturity group, and N treatments (zero-N and full-N, see Section 2.2), whereas experiments in NE were replicated at four producer irrigated high-yield fields that included the same two N treatments (Table 1). For simplicity, the combinations of crop season x sowing date x variety in BA, or the producer fields in NE, are hereafter called ‘environments’. In all experiments, crops were irrigated and managed to ensure optimal water and nutrient supply (except, of course, for N in the zero-N treatment, see Section 2.2) and to avoid stress from weeds, insects and pathogens. Irrigation was applied throughout the crop season with application event amounts adjusted periodically to match seasonal changes in crop water demand. In NE producer fields, soil water content in the upper 90 cm of soil was monitored using Watermark[®] sensors, which indicated that soil water status was consistently above 65% of total soil plant available water between emergence and physiological maturity. Several prophylactic foliar applications of herbicide, fungicide, and insecticide kept the crops free from biotic stresses in all experiments. A meteorological station located at each site provided daily weather data.

2.1.1. Balcarce, Argentina

Field experiments were conducted on a deep fine-loamy Typic Argiudol. Topsoil (0–20 cm) organic matter, extractable phosphorous (P Bray-1 method), and soil pH were 27 g C kg⁻¹, 22 mg kg⁻¹, and 6.4 in BA-Y1, and 28 g C kg⁻¹, 25 mg kg⁻¹, and 6.6 in BA-Y2. Soil N-NO₃⁻ in the upper 60 cm at sowing ranged from 59 to 114 kg ha⁻¹ in BA-Y1 and from 61 to 105 kg ha⁻¹ in BA-Y2. Previous crop was wheat and hairy vetch [*Vicia villosa* Roth.] in BA-Y1 and BA-Y2, respectively. The field was disked before sowing only in BA-Y1. Seeds were treated with fungicide and inoculated in both years using the best available product in the market and following recommended inoculation practices (Nitragin Optimize II[®] Pack Apron[®] & Jumpstart). Row spacing was 0.35 m and plants were thinned at V1 stage to ca. 35 plants m⁻². Phosphorous, sulfur, and calcium fertilizer amounts applied before sowing were 19, 21, 26 kg ha⁻¹ in BA-Y1 and 20, 22, 27 kg ha⁻¹ in BA-Y2, respectively.

The factorial experiments in Argentina were arranged in a split-split plot design with four replicates in both years. Sowing dates were main plots, three varieties of contrasting maturity group were subplots, and the two N treatments were sub-subplots (Table 1). Sub-subplot size was 3.8 × 10 m and 6.3 × 6 m for BA-Y1 and BA-Y2, respectively. Sowing

dates were equally spaced by 200 °Cd (BA-Y1) and 300 °Cd (BA-Y2) based on measured daily temperature and using a base temperature (T_b) of 10 °C (Tenorio et al., 2017). The purpose of combining different maturity groups and sowing dates was to generate a wide range of Y_p along which the two N treatments could be compared. On average, there was a yield decrease of 38 kg ha⁻¹ per day of delay in sowing, resulting on a yield difference of ca. 2.6 Mg ha⁻¹ between earliest and latest sowing date treatments.

2.1.2. Nebraska, USA

Experiments were conducted in four pivot-irrigated producer fields in NE located near Atkinson, Mead, Saronville, and Smithfield during the 2016 crop season (Table 1). These fields had persistently produced high soybean yields in previous years (> 5.0 Mg ha⁻¹). Soils were deep, without physical or chemical constraints to root growth. Soil series were O'Neil sandy loam (Atkinson), Yutan silty clay loam (Mead), Hastings silt loam (Saronville), and Holdrege silt loam (Smithfield). Average initial soil tests in the upper 30 cm indicated a pH of 6.3, 6.1, 6.1, and 7.2; organic matter of 9, 20, 18, and 16 g C kg⁻¹; extractable P (Bray-1 method) of 49, 16, 126, and 16 mg kg⁻¹; and 1 M NH₄-acetate extractable K of 357, 335, 395, and 487 mg kg⁻¹. Soil N-NO₃⁻ in the upper 60 cm at sowing ranged from 33 to 54 kg ha⁻¹ across locations. The previous crop was maize in the four fields. Fields were disked (Atkinson and Saronville), strip-tilled (Mead), and no-till (Smithfield). In all four fields, row spacing was 0.76 m, and seeding rate ranged from 35 to 45 seeds m⁻², well above those recommended to maximize seed yield (de Bruin and Pedersen, 2009). Seeds were treated with fungicide and insecticide in all cases, but received no inoculant, which is the common producer practice for fields with a prior crop history of soybean in the US Corn Belt, primarily because of the lack of yield response to inoculation, especially in high-yield environments (de Bruin et al., 2015; Leggett et al., 2017 and references cited therein). Pre-sowing nutrient applications (in kg ha⁻¹) included N (20), P (22), K (50), Ca (62), Mg (8), S (17), Zn (2) at Atkinson, N (13) P (20) S (11) Zn (1) at Mead, N (17), P (35), S (10), Zn (1) at Saronville, and P (38), S (1) at Smithfield. Experiments in each field were arranged in a completely randomized design with two N treatments, and four replicates per treatment. The eight contiguous plots (16 × 11 m each) were purposely located in a high-yield area in each field, which was identified based on field yield maps from previous years.

2.2. Protocol for nitrogen fertilizer application

There were two N treatments (full-N and zero-N), which were randomly assigned to the sub-subplots (BA) or to the replicates (NE). The aim of the full-N treatment was to ensure non-N limiting conditions during the entire crop season by temporal additions of N fertilizer. In contrast, the zero-N treatment relied on indigenous N sources, including inorganic soil N at sowing, in-season N mineralization, and N fixation. Crops also received a small N 'starter' application at sowing (< 20 kg N ha⁻¹) in three of the four producer fields in NE. Similarly, crops in NE received additional (non-fertilizer) groundwater N input through irrigation (57, 1, 7, and 2 kg N ha⁻¹ in Atkinson, Mead, Saronville, and Smithfield, respectively). At each site, contribution from indigenous N sources, excluding N fixation, was estimated by measuring total N uptake in the aboveground dry matter at physiological maturity of a maize crop grown in a zero-N plot located adjacent to each experiment.

The protocol developed to apply N fertilizer in the full-N treatment was designed to fully satisfy the crop N requirements needed to support the Y_p calculated for each environment. Soybean CROPGRO model (Boote et al., 1998), embedded in DSSAT v 4.5 (Jones et al., 2003; Hoogenboom et al., 2010), and SoySim model (Setiyono et al., 2010) model were used to simulate Y_p in BA and NE, respectively, using long-term (> 25 years) measured daily weather data. These two models have been satisfactorily evaluated on their ability to reproduce

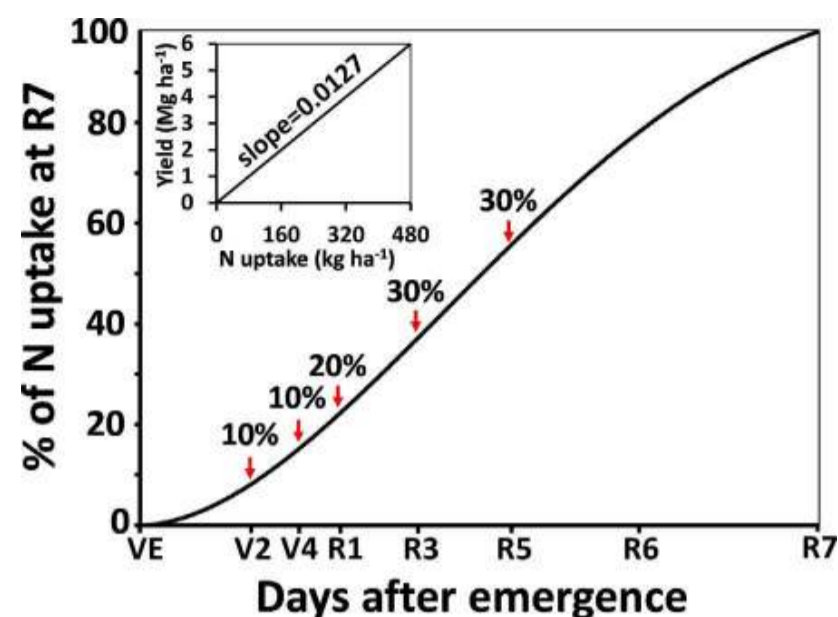


Fig. 1. Scheme showing the nitrogen (N) fertilization protocol followed in the experiments. Total N fertilizer amount was estimated for each environment based on site-specific yield potential and the relationship between seed yield and total N uptake (inset). Total N fertilizer amount was split into 5 applications (red arrows) based on the temporal (daily basis) dynamics of N uptake in soybean (solid line). As shown, the N fertilizer amount applied in each application corresponded to 10% (V2 and V4 stages), 20% (R1 stage), and 30% (R3 and R5 stages) of total N fertilizer applied. Crop stages are based on Fehr and Caviness (1977) staging system and are spaced on the x-axis according to the approximate calendar date of their occurrence in the experiments. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

measured yields in well-managed experiments (Setiyono et al., 2010; Aramburu Merlos et al., 2015). Maximum simulated Y_p across years was ca. 7.2 (early sowing) and 3.8 Mg ha⁻¹ (late sowing) in BA and ca. 7.5 Mg ha⁻¹ for all of the sites in NE. The latter was consistent with the Y_p for soybean of 6–8 Mg ha⁻¹ proposed by Specht et al. (1999) and Sinclair and Ruffey (2012) for favorable production environments in the US Corn Belt.

Total N fertilizer amount to use in the full-N treatment was calculated based on the maximum Y_p simulated for each environment (crop season × sowing date × variety in BA and producer field in NE), and a seed yield-N uptake ratio of 12.7 kg kg⁻¹ (Salvagiotti et al., 2008; Tamagno et al., 2017) (Fig. 1, inset). No attempt was made to account for belowground N (including N in roots, nodules, exudates, and rhizodeposition) because this parameter is likely to exhibit large variation across the wide range of environmental conditions evaluated in our study. Contribution from N fixation and mineralization were not accounted for the calculation of N fertilizer amount, because of the uncertainty in N supply from soil mineralization and trade-offs between N fixation and applied N fertilizer (Streeter and Wong, 1988). Total N fertilizer amount was increased by 40% in the four NE fields to compensate for potential N losses through volatilization and leaching resulting from mismatches between irrigation or rainfall events and fertilizer application and a few heavy rainfall events (> 50 mm) that occurred early in the season. In contrast, irrigation was applied right after the two largest N applications at R3 and R5 in BA, without occurrence of heavy rainfall events; hence, N fertilizer amount was increased only by 10%. Total N fertilizer amount applied in the full-N treatment ranged from 330 to 640 kg N ha⁻¹ across sowing dates in BA and averaged 870 kg N ha⁻¹ in NE producer fields.

Total N fertilizer was split in several applications during the crop season as a means of synchronizing the N supply and crop N demand (Fig. 1). Based on seasonal N uptake patterns reported by Thies et al. (1995) and Bender et al. (2015), total N fertilizer amount was split in five applications (V2, V4, R1, R3, and R5 stages), with respective N fertilizer amounts representing 10%, 10%, 20%, 30%, and 30% of the total applied N fertilizer. Granular urea fertilizer was applied and manually broadcasted between plant rows. The protocol was followed consistently in all experiments (BA) and producer fields (NE).

2.3. Measurements

Phenological events were tracked on a weekly basis in all experiments following the Fehr and Caviness (1977) staging system. Abscised leaves were collected weekly starting at R3 using nets placed in-between rows. At physiological maturity (R7 stage), 1-m row of consecutive plants in each sub-subplot in BA, or replicate in NE, surrounded by two rows receiving the same N treatment, was clipped at the soil surface and oven-dried to constant weight at 70 °C. Above-ground dry matter (ADM) at R7 was calculated as the sum of the standing dry matter plus the cumulative sum of abscised-leaf dry matter. The ADM samples were threshed by hand, then divided into the seed and non-seed fractions. Harvest index (HI) was determined as the ratio between seed biomass (on an oven-dry matter basis) and ADM. Larger plant samples (1.8, 2.8, and 4.6 m² in BA-Y1, BA-Y2, and NE, respectively) were collected from each sub-subplot in BA or main plot in NE to obtain an end-of-season estimate of seed yield. Two sub-samples of 200 seeds each were weighed to estimate mean individual seed mass (weight basis), which was then used with sample yield to derive the number of seed per harvested area. Seed yield and seed weight were adjusted at 0.130 kg H₂O kg⁻¹ seed. Soybean seed protein and oil concentration (in units of kg constituent kg⁻¹ seed) were determined for each N treatment seed sample using near-infrared analysis (FOSS Infratec™ 1241). Protein and oil yields (Mg ha⁻¹) were calculated by multiplying sample seed yield by the corresponding sample seed protein and oil concentrations. The yield and concentration of protein and oil were expressed on an oven seed dry-matter basis.

2.4. Data analysis

Agreement between measured yields and simulated Y_p based on site-year-specific weather, sowing date, and variety maturity group was evaluated by calculating the root mean square error (RMSE) and absolute mean error (ME) as follows:

$$RMSE = \sqrt{\frac{\sum (Y_p - Y_M)^2}{n}} \quad (1)$$

$$ME = \frac{\sum (Y_p - Y_M)}{n} \quad (2)$$

where Y_p is the simulated yield potential and Y_M is the measured yield (Mg ha⁻¹). Separate RMSE and ME were calculated for the two N treatments (full-N and zero-N). Regression analysis was used to detect departure from the null hypothesis of unity in charts comparing the two N treatments with respect to yield and other measured traits.

A combined analysis of variance (ANOVA) was conducted to determine the effect of N treatments on seed yield, ADM, HI, seed number and weight, and seed protein and oil concentration and yield (SAS[®] PROC MIXED v.9.3) (Moore and Dixon, 2015). The combined analysis assumes homogeneity of within-environment variances. Our experiments were not too different in relation with their variances (F_{max} < 6); hence, our combined ANOVA can be considered robust (Milliken and Johnson, 2009). In this analysis, each combination of crop season, sowing date, variety, and location was referred to as an environment. Each environment had two N treatments (full-N and zero-N), with four replicates. Environment and N treatments were treated as fixed effects. This analysis provided an estimate of the overall significance of the N treatment across a diverse range of Y_p. Although environments (site x crop season x variety x sowing date) cannot be directly compared, the interaction between environment and treatment can be used to evaluate the consistency of the N treatment response.

Differences in seed yield between full-N and zero-N treatments were investigated using two simple physiological frameworks:

$$\text{Seed yield (Mg ha}^{-1}\text{)} = \text{seed number (seed ha}^{-1}\text{)} \times \text{mean seed weight (Mg seed}^{-1}\text{)} \quad (3)$$

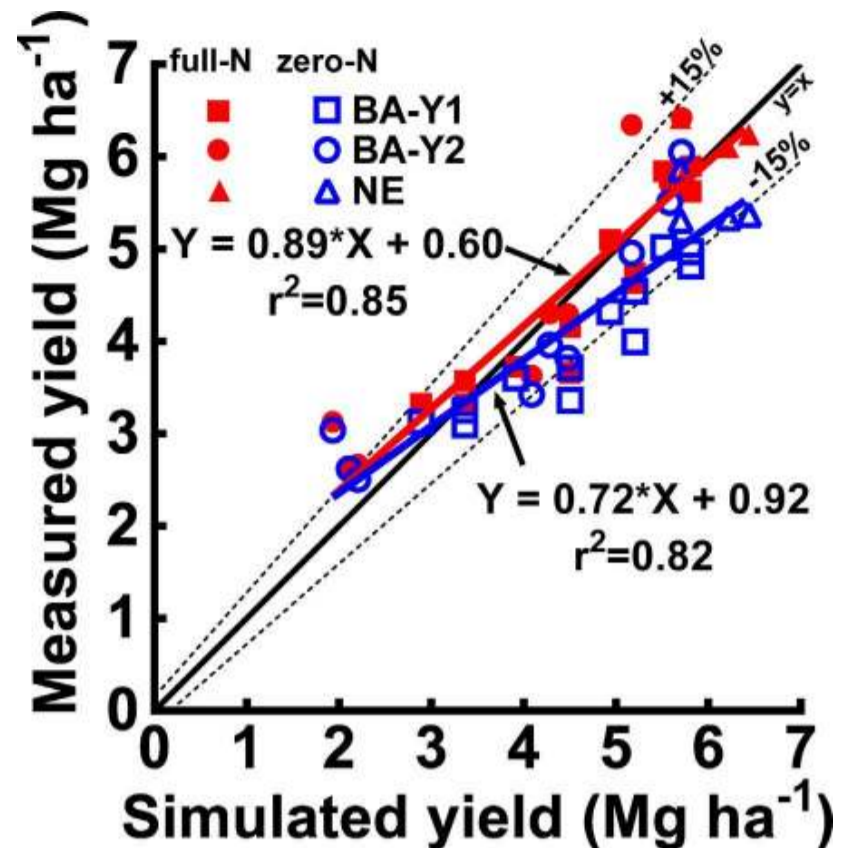


Fig. 2. Measured yields versus simulated yield potential for the two treatments of zero-N (empty symbols) and full-N (solid symbols). Each data point corresponds to the simulated yield potential (Y_p) and measured average yield for a given sowing date × variety × N treatment (Balcarce, BA) or for a producer field × N treatment (Nebraska, NE). Experiments were conducted in BA over two crop seasons (BA-Y1 and BA-Y2) and at four sites in NE during one crop season. Parameters of the fitted linear regression models (solid lines) are shown. Y_p was simulated using on-site daily measured weather and actual sowing date, plant density, and variety maturity group.

$$\text{Seed yield (Mg ha}^{-1}\text{)} = \text{ADM (Mg ha}^{-1}\text{)} \times \text{HI} \quad (4)$$

Mean values calculated for the different parameters (seed yield, seed number and weight, ADM, HI, and seed protein and oil yields and concentration) for the full-N treatment were plotted against averages computed for the zero-N treatment. Departures from the 1:1 line (which represents the null hypothesis of no response differential between full-N and zero-N) were investigated using linear and quantile regression (quantreg package, R Development Core Team, 2016). To assess changes in the seed constituents of protein and oil in relation with increasing yields, linear and quadratic equations were fitted to the relationships between protein and oil yield *versus* seed yield.

3. Results

3.1. Seed yield as influenced by nitrogen treatments

Diversity of locations, crop season, sowing dates, varieties, and N treatments resulted in a wide range of soybean yield across environments, from 2.52 to 6.46 Mg ha⁻¹ (Fig. 2). A yield threshold of 4.5 Mg ha⁻¹ has been used in the literature to define high-yield soybean environments (Salvagiotti et al., 2008). About half of measured yields in the present study were above this threshold. In fact, our highest yields fell well above the range reported in the literature for field-grown soybean in experiments with N fertilizer addition (see Salvagiotti et al., 2008 and references cited therein).

In 92% of the cases, measured full-N yields were within ± 15% of the simulated Y_p values generated with well-validated crop models that were supplied with inputs of local measured daily weather data and based on treatment-specific sowing date, plant density, and variety maturity group (Fig. 2). The two crop models (CROPGRO in BA and SoySim in NE) used here to simulate Y_p performed better at reproducing measured yields in the full-N than in zero-N treatment, as indicated by their respective RMSE (0.51 *versus* 0.67 Mg ha⁻¹) and ME values (0.10 *versus* -0.36 Mg ha⁻¹). Moreover, the test of observed slopes

Table 2

Analysis of variance for the effect of nitrogen (N) fertilizer on seed yield, seed number, and seed weight, aboveground dry matter at physiological maturity (ADM) and harvest index (HI), seed protein and oil concentration, and yields of those two seed constituents. Each variety \times sowing date \times year combination (Balcarce) or producer field (Nebraska) was considered to be a separate environment (E) relative to the N \times E interaction in this analysis. Rep: replicate.

Seed yield				Seed number		Seed weight		ADM		HI	
Fixed effects	d.f.	F	P > F	F	P > F	F	P > F	F	P > F	F	P > F
E [†]	24	32	< 0.001	34	< 0.001	27	< 0.001	19	< 0.001	4	< 0.001
N	1	87	< 0.001	27	< 0.001	117	< 0.001	33	< 0.001	1	0.411
N \times E	24	2	0.025	1	0.507	2	0.005	2	0.070	1	0.323
Random effects				MS		MS		MS		MS	
Rep (E)	75	330428		67219		77		2266459		0.003	
N \times Rep (E)	75	123244		30753		24		1339567		0.002	

Protein%				Oil%		Protein yield		Oil yield	
Fixed effects	d.f.	F	P > F	F	P > F	F	P > F	F	P > F
E [†]	24	16	< 0.001	56	< 0.001	31	< 0.001	42	< 0.001
N	1	275	< 0.001	41	< 0.001	159	< 0.001	63	< 0.001
N \times E	24	2	0.007	2	0.020	2	0.017	2	0.006
Random effects				MS		MS		MS	
Rep (E)	75	0.940		0.249		38850		11536	
N \times Rep (E)	75	0.419		0.106		15261		4478	

versus the null hypothesis of slope = 1 was non-significant for full-N ($P = 0.20$), but was significantly different for the zero-N treatment ($P < 0.001$) (*i.e.*, the respective red and blue regression lines in Fig. 2). Given that finding, one can reasonably infer that measured yield in the full-N treatment can serve as a proxy for Y_p for each environment (*i.e.*, Y_p with no N limitation). In contrast, the measured zero-N yields in production environments exhibiting a Y_p of $> 3.5 \text{ Mg ha}^{-1}$ fell below the 1:1 line, and the magnitude of this yield difference between fertilized and non-fertilized treatments increased as the Y_p of the production environment increased.

A statistically significant effect of N treatment on seed yield ($P < 0.001$) was observed in the ANOVA (Table 2). When averaged over all environments, seed yield in the full-N treatment was 0.46 Mg ha^{-1} greater (*i.e.*, 11% higher) compared to zero-N treatment (Fig. 3). The ANOVA also revealed a significant environment \times N interaction on seed yield ($P = 0.025$), which was consistent with the significant upward departure of the observed linear regression slope of 1.2 from the null hypothesis of unity ($P < 0.001$, Fig. 3). For each unit Mg ha^{-1} increase in zero-N seed yield above a threshold of Y_p of *ca.* 2.5 Mg ha^{-1} , there was a corresponding 1.2 Mg ha^{-1} increase in full-N seed yield. The yield difference between the full-N and zero-N treatments, increased with increasing Y_p of the production environments, from near zero in low- Y_p environments ($2\text{--}3 \text{ Mg ha}^{-1}$) up to *ca.* 0.90 Mg ha^{-1} in production environments with a high- Y_p of *ca.* 6 Mg ha^{-1} (Fig. 3, inset).

Measured N uptake in zero-N maize plots located adjacent to the experiments indicated that indigenous N supply (excluding N fixation) did not vary greatly across environments, ranging from 133 to 148 kg N ha^{-1} across experiments in BA and from 98 to 150 kg N ha^{-1} in NE producer fields, except for Atkinson (196 kg N ha^{-1}). Hence, our comparison of N treatments along the range of low- to high- Y_p environments was likely not confounded with co-variation in indigenous N supply.

3.2. Drivers of seed yield differences between N treatments

Changes in both seed number and individual seed mass (*i.e.*, weight) were consistent with the observed differences in seed yield between N treatments (Table 2, Fig. 4). Averaged over all environments, the full-N treatment resulted in more seeds produced per unit of harvested area (2534 versus $2406 \text{ seeds m}^{-2}$) and also resulted in greater mass per seed (183 versus 175 mg seed^{-1}). In contrast to seed number, the seed weight trend line resided above the 1-to-1 line over the entire range of

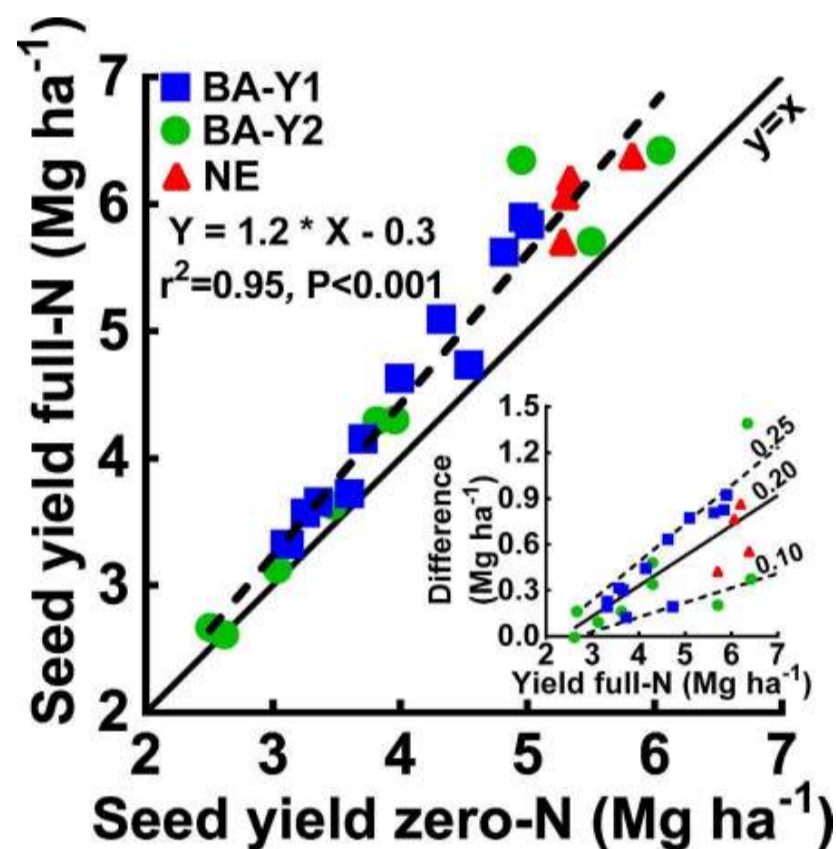


Fig. 3. Seed yield in the full-N versus zero-N treatments. Each data point represents the average yield for a given year \times sowing date \times variety (Balcarce; BA) or producer field (Nebraska; NE). Experiments were conducted in BA over two crop seasons (BA-Y1 and BA-Y2) and at four sites in NE during one crop season. Solid diagonal line indicates $y = x$. Parameters of the fitted linear regression (dashed line) and coefficient of determination (r^2) are also shown. Inset shows the seed yield difference between the full-N and zero-N treatments relative to the full-N treatment yield. Also shown is the slope of the fitted linear regression (solid line; $y = 0.2 \times X - 0.46$) and the slopes representative of the boundary functions for the 10th and 90th quantiles (dashed lines).

seed weight, suggesting that seed weight was persistently greater in full-N versus zero-N treatments. Interestingly, though N fertilization influenced both seed number and seed weight, a significant N \times E interaction was detected only for the latter (Table 2). Similarly, N treatments accounted for a much larger portion of the observed variation in seed weight in relation with seed number (F -value: 117 versus 27, respectively).

Greater ADM was evident in the full-N versus zero-N treatments (Table 2, Fig. 5). Across experiments, the full-N treatment generated an ADM that was 0.93 Mg ha^{-1} (9%) higher than that in the zero-N treatment. Notably, the ADM values measured in some of the high-yield environments in our experiment reached *ca.* 15 Mg ha^{-1} , and thus are

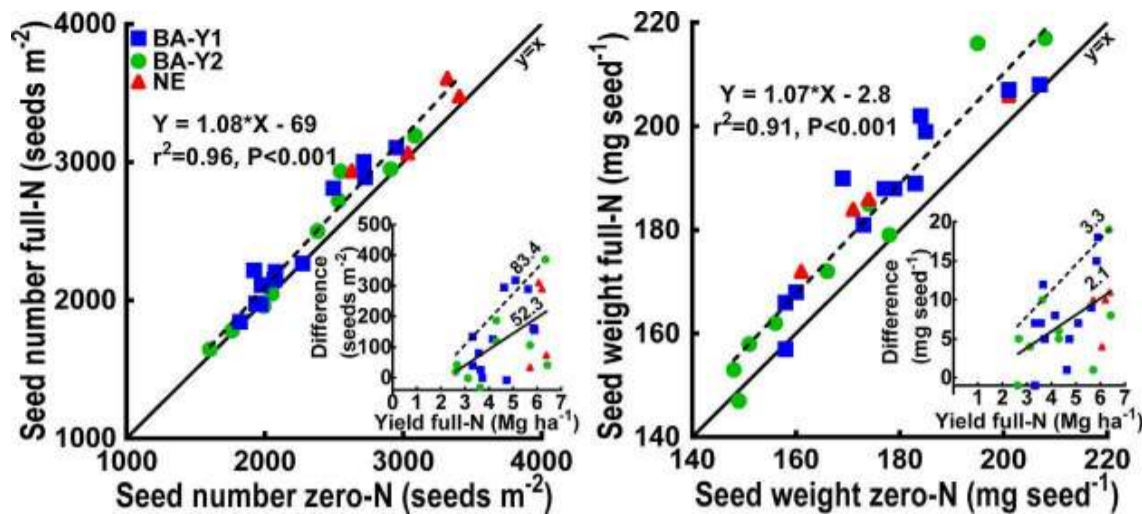


Fig. 4. Seed number per unit harvested area (left) and individual seed mass (right) in full-N versus zero-N treatments. Each data point represents the average yield for a given year \times sowing date \times variety (Balcarce; BA) or producer field (Nebraska; NE). Experiments were conducted in BA over two crop seasons (BA-Y1 and BA-Y2) and at four sites in NE during one crop season. Solid diagonal line indicates $y = x$. Parameters of the fitted linear regression (dashed line) and coefficient of determination (r^2) are also shown. Insets show the difference in seed number and weight between the full-N and zero-N treatments relative to the full-N treatment for those two measured variables. Also shown are the slopes of the fitted linear regressions (solid lines) and the slopes of the boundary function for the 90th quantile (dashed line). The boundary function for the 10th quantile is not shown due to the non-significance of that slope from zero.

close to the highest values reported to date for field-grown soybean in the literature (e.g., Setiyono et al., 2010; Van Roekel and Purcell, 2014). In contrast, sign and magnitude of differences in HI between full-N and zero-N treatments were not consistent across experiments, especially during the first crop season in Balcarce (Fig. 5). Measured HI in the full-N treatments was slightly smaller (0.33 versus 0.34) and higher (0.39 versus 0.37) than zero-N treatments in environments below 3.5 Mg ha^{-1} or above 5 Mg ha^{-1} of Y_p , respectively. Hence, changes in biomass partitioning along the range of Y_p may explain why the consistent difference in ADM between full-N and zero-N treatments (Fig. 5) did not translate in an equally consistent seed yield response across the entire range of Y_p (Fig. 3).

3.3. Soybean protein and oil as affected by N treatments

Averaged over all environments, the full-N treatment resulted in significantly greater yields of protein and oil ($P < 0.001$) (Table 2, Fig. 6). The protein and oil yield advantage arising from N fertilization averaged a respective 0.22 Mg ha^{-1} (15%) and 0.07 Mg ha^{-1} (10%). The steepness of the significant regression coefficients for the protein yield trend line (1.24) and oil yield trend line (1.18) relative to the null hypothesis of 1.0 (unity) is of interest. Clearly, when these two key soybean constituents were measured in units of dry matter harvestable per ha, N fertilization increased the (Mg ha^{-1}) values when going from low to high Y_p environments (Fig. 6). There was a small (0.3%), though statistically significant ($P < 0.001$, Table 2) decrease in seed oil concentration arising from N fertilization when averaged over all environments (i.e., 20.5 versus 20.8% for full-N and zero-N, respectively) (Fig. 6, inset). However, that small decrease in percentage oil concentration was substantively offset in the oil yield calculation because of the 11% increase in seed yield generated by N fertilization. Seed protein concentration was significantly increased by N fertilization ($P < 0.001$, Table 2). The increase, when averaged over all environments, was about 1.5 percentage points (i.e., 41.4 versus 39.9% for full-N and zero-N, respectively) (Fig. 6, inset). In contrast to oil yield, the large protein yield increase in the full-N treatment was attributable

to N-fertilizer induced increases in both seed yield (11%) and protein concentration (4%).

Soybean seed processors who purchase soybeans are not interested in protein or oil yields. Instead, their primary interest is the percentages of protein and oil in the seed, and they will selectively purchase soybeans from location-year sites with higher than average percentages in one or both constituents. In our experiment, the relationship between protein yield and seed yield was curvilinear, suggesting a ‘dilution’ in seed protein was occurring across the wide range of increasingly greater zero-N treatment seed yields (Fig. 7, left). Protein concentration decreased (from ca. 41 to 38%) as the yield level in the zero-N treatment increased (from ca. 2.5 to 6 Mg ha^{-1}). In contrast, full-N crops maintained the same protein concentration across the wide range of measured full-N treatment seed yields (Fig. 7, left). In other words, these observed patterns indicate that, when N fertilizer was used to enhance seed yield, the seed protein concentration at higher yield levels did not decline. The impact of N fertilization on seed oil was somewhat different in that the relationship of seed oil yield to seed yield was upwardly curvilinear for both the zero-N and full-N treatments (Fig. 7, right).

4. Discussion

Findings from the present study challenge the hypothesis that N fixation can fulfill plant N requirements irrespective of the Y_p of the production environment. Here we developed a seasonal N-fertilization protocol to ensure non-N limiting growing conditions for field-grown soybean. The protocol consisted of estimating total N crop requirements based on simulated site-specific Y_p levels, while ensuring a timely synchronization between N demand and crop phenology by scheduling of the fertilizer applications in fractional time-step amounts to match seasonal pattern in crop N uptake dynamics. The protocol was implemented at sites with a wide range of Y_p , ranging from ca. 2.5 to 6.5 Mg ha^{-1} , to evaluate the degree to which soybean yields may have been limited by N supply at those sites. We found a consistent increase in seed, protein, and oil yields in the full-N versus zero-N treatments,

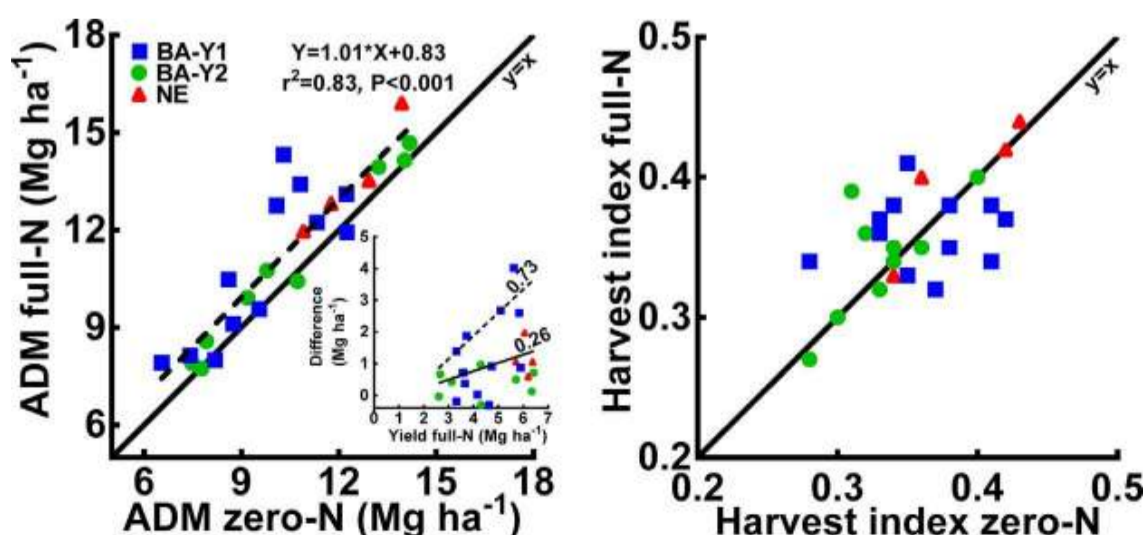


Fig. 5. Aboveground dry matter (ADM) at physiological maturity (left) and harvest index (right) in full-N versus zero-N treatments. Each data point represents the average yield for a given year \times sowing date \times variety (Balcarce; BA) or producer field (Nebraska; NE). Experiments were conducted in BA over two crop seasons (BA-Y1 and BA-Y2) and at four sites in NE during one crop season. Solid diagonal line indicates $y = x$. Parameters of the fitted linear regression (dashed line) and coefficient of determination (r^2) are shown when significant. Inset show the difference in ADM and HI between the full-N and zero-N treatments relative to full-N treatment for those two measured variables. Slopes of the fitted linear regression (solid line) and the boundary function for the 90th quantile (dashed line) are shown only for ADM. The boundary function for the 10th quantile is not shown due to the non-significance of that slope from zero.

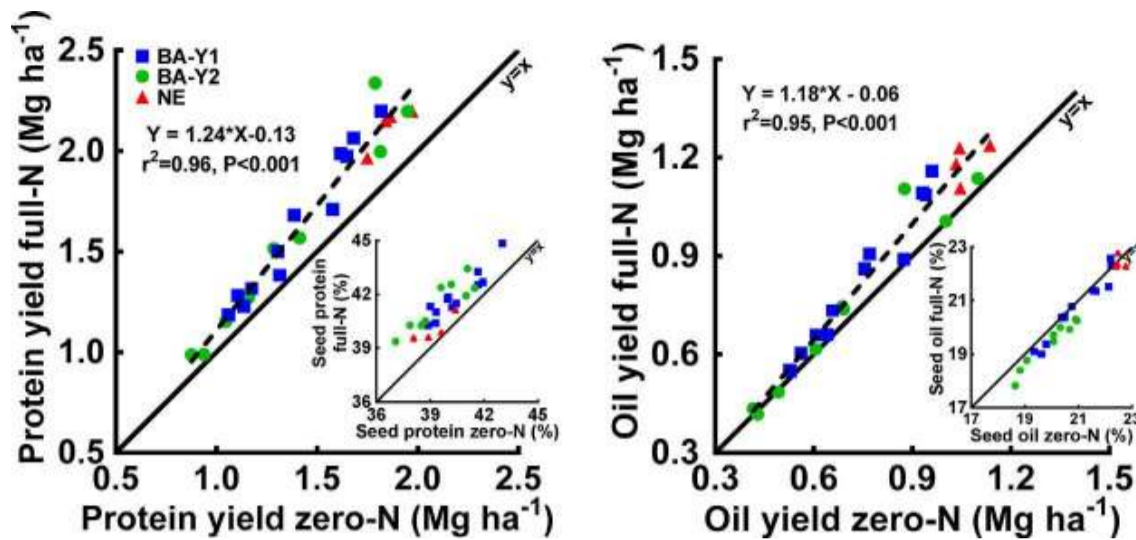


Fig. 6. Seed protein yield (left) and seed oil yield (right) in full-N versus zero-N treatments. Each data point represents the average yield for a given year x sowing date x variety (Balcarce; BA) or producer field (Nebraska; NE). Experiments were conducted in BA over two crop seasons (BA-Y1 and BA-Y2) and at four sites in NE during one crop season. Solid diagonal line indicates $y = x$. Parameters of the fitted linear regression (dashed line) and coefficient of determination (r^2) are also shown. Insets show full-N seed protein and seed oil concentration percentages relative to the zero-N treatments for those two measured variables.

especially in environments with high Y_p , without trade-offs on seed protein and oil concentration. Increases in primary yield components of seed number and individual seed mass (*i.e.*, weight) were consistent in terms of accounting for the higher seed yield in the full-N treatment. In that regard, greater seed yield was also highly associated with higher ADM, while no consistent trend was found in biomass partitioning to seed (*i.e.*, HI). We do not exclude the possibility that the yield difference found between zero and full-N treatments could also be partly related with smaller carbon cost due to reduced symbiotic N fixation in the full-N treatment (Ryle et al., 1979; Pate and Layzell, 1990; Connor et al., 2011). However, we note that (i) protein yields were higher in full-N versus zero-N treatments, suggesting greater N uptake in the full-N crops, and (ii) magnitude of yield increase was dependent upon the Y_p of the production environment. These two observations were consistent with our hypothesis that there is a gap between crop N requirements and N supply (which is comprised of N fixation and soil mineralization) and that gap becomes detectably larger with the increasing N demand needed to support a higher Y_p .

While we acknowledge that the N fertilizer amounts applied in the present study were far from being economically profitable (and environmentally sound), our N fertilizer protocol was specifically designed to allow us to experimentally identify a Y_p threshold above which *in situ* N fixation and soil mineralization were not capable of meeting the crop N demand. On average, seed yield in the full-N treatment increased, relative to the zero-N treatment, by *ca.* 200 kg per Mg increase in Y_p , but this N fertilizer induced yield response was experimentally evident only in crops with Y_p level above 2.5 Mg ha^{-1} , suggesting that yield response to N fertilizer is more likely in fields with a Y_p above this threshold. Our finding is not consistent with Ray et al. (2006) who found a persistent yield response to N fertilizer over a wide yield range, from 1.5 to 5.0 Mg ha^{-1} . We note, however, that most of the experiments in the lower yield range reported in the Ray et al. (2006) study were conducted in rainfed crop settings, for which N fertilizer may have helped to alleviate the effect of N limitation due to

seasonally sporadic, temporary water deficits (Purcell, 2014). Likewise, yield responses to small N additions have been reported for low-yield environments with very low indigenous N supply, where a relatively small fertilizer N addition early in the season helps establish N fixation (Van Kessel and Hartley, 2000; Giller and Cadisch, 1995). In contrast, our experiments were conducted in fertile soils with relatively high indigenous N supply and in which irrigation was efficiently used to ensure that only non-water limiting conditions prevailed relative to our experimental objectives. Another contribution from our study is to set an upper limit with respect to soybean yield increases that could be induced by N fertilization. The upper limit for seed yield response to N fertilizer increased by 250 kg per Mg increase in Y_p between the 2.5 Mg ha^{-1} Y_p threshold and Y_p levels approaching 6 Mg ha^{-1} . The maximum yield response we reported here ($\approx 0.9 \text{ Mg ha}^{-1}$ at $Y_p = 6 \text{ Mg ha}^{-1}$) is consistent with the upper range of soybean yield responses to N fertilizer reported by Salvagiotti et al. (2008) and Wilson et al. (2014).

Results presented in this paper challenge current estimates of Y_p in soybean, and more broadly, legume crops. Findings reported here indicate that thoughtful focus on N supply during the temporal phases of seasonal crop development can help ensure non-N limiting conditions in field-grown soybean. Hence, we argue that current estimates of Y_p derived from field observations have likely underestimated soybean Y_p (if N was limiting), given the lack of studies in which high-yield soybean has been grown following an explicit protocol to ensure ample N supply in time and space. The protocol developed in this study provided an experimental approach to minimize N limitation in high-yield conditions and thus ensured that measured yields can be taken as a reliable estimate of non-N-limited Y_p . In our experiment, we found that crop model simulated Y_p values closely matched full-N yields, indicating that crop simulation models are still robust at simulating Y_p for soybean when N is not limiting. A possible explanation is that the procedure used to calibrate soybean models considers optimal leaf N for the different phenological stages, without limitations by soil nutrient

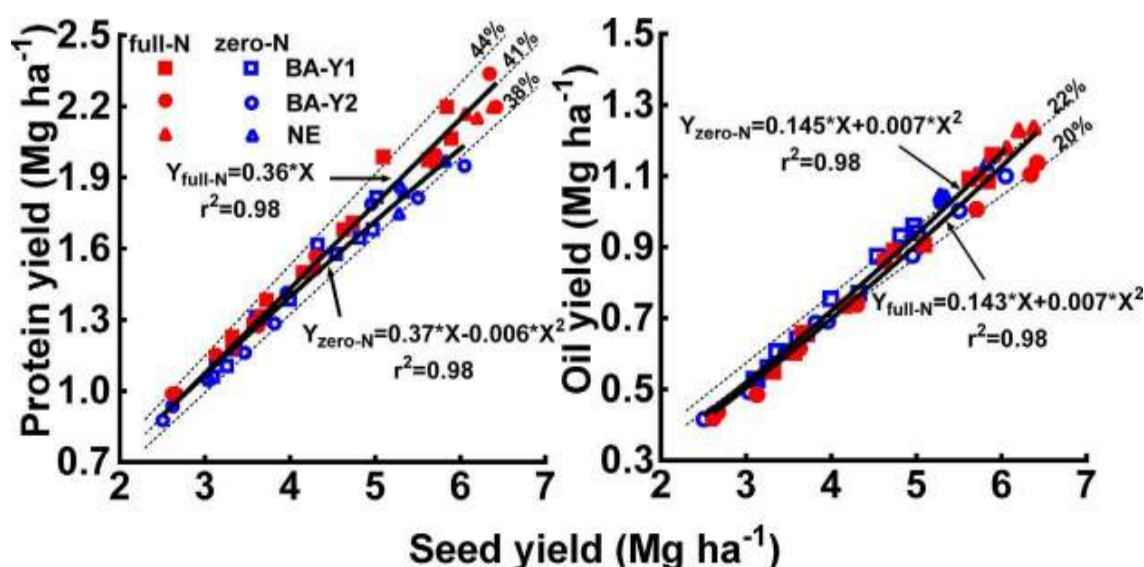


Fig. 7. Protein (left) and oil (right) yields versus seed yield in full-N (solid red symbols) and zero-N treatments (empty blue symbols). Each data point corresponds to a sowing date x variety x N treatment (Balcarce; BA) or producer field x N treatment (Nebraska; NE). Experiments were conducted in BA over two crop seasons (BA-Y1 and BA-Y2) and at four sites in NE during one crop season. Fitted linear or quadratic equations (solid lines) were forced through zero; their equations are shown. Shown for comparison are isolines for larger and smaller seed protein and oil concentration values (dotted lines). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

availability or symbiotic N fixation in root nodules (Boote et al., 2008; Setiyono et al., 2010 and references cited therein).

Finally, our results suggest that N fixation alone is not sufficient to fulfill soybean N demand in production environments with high Y_p , which include irrigated soybean fields located in the central US Great Plains (Grassini et al., 2015). Hence, insufficient N supply may explain part of the current yield gap between simulated Y_p and average producer yield reported for these cropping systems. For example, using Fig. 3 as basis to determine the yield loss due to insufficient N supply for irrigated soybean in the US central Great Plains, given respective Y_p and actual average producer irrigated yield of 5.7 and 4.5 Mg ha⁻¹ (Grassini et al., 2015; USDA-NASS, 2015–2016; https://www.nass.usda.gov/Quick_Stats/), we inferred that at least half of the current estimated yield gap may result from a seasonal N supply limitation. In a broader context, we speculate that N supply will likely become (if not already) a major yield-limiting factor in soybean production systems with high Y_p as producer yields in those systems continue to fine-tune their agronomic practices and adopt higher yielding cultivars (Grassini et al., 2014a, 2014b; Specht et al., 2014).

5. Conclusions

In the present study, we developed a novel N fertilization protocol to ensure non-N limiting conditions in field-grown soybean. The protocol was implemented across a wide range of environments of varying Y_p to evaluate the degree of N limitation on seed yield and quality. Measured yield in treatments receiving N fertilizer closely matched simulated Y_p . We found that soybean yields are limited by N supply in environments with Y_p above ≈ 2.5 Mg ha⁻¹. Above this threshold, yield response to N fertilizer had an upper limit of 250 kg per Mg increase in Y_p . Differences in seed yield between full-N and zero-N treatments were associated with higher seed number and weight and higher ADM. Protein and oil yields were also higher in the full-N treatments due to higher yields, with a slight increase and decrease in protein and oil concentration, respectively. Remarkably, full-N crops maintained the same seed protein concentration across the entire yield range and exhibited higher protein yields, especially at high yield levels. This study provides a framework to assess N limitations in field-grown soybean, and possibly other legume crops, and to explore opportunities to tune current N management in high-yield soybean cropping systems.

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References

- Aramburu Merlos, F., Monzon, J.P., Mercu, J.L., Taboada, M., Andrade, F.H., Hall, A.J., Jobbagy, E., Cassman, K.G., Grassini, P., Grassini, P., 2015. Potential for crop production increase in Argentina through closure of existing yield gaps. *Field Crops Res.* 184, 145–154.
- Bender, R.R., Haegle, J.W., Below, F.E., 2015. Nutrient uptake, partitioning, and remobilization in modern soybean varieties. *Agron. J.* 107, 563–573.
- Boote, K.J., Jones, J.W., Hoogenboom, G., 1998. Simulation of crop growth: CROPGRO model. In: Peart, R.M., Curry, R.B. (Eds.), *Agricultural Systems Modeling and Simulation*. Marcel Dekker, Inc, New York, USA, pp. pp. 651–692.
- 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. *Quantifying and Understanding Plant Nitrogen Uptake for Systems Modeling*. CRC Press, Florence, USA, pp. 13–46.
- Brevedan, R.E., Egli, D.B., Leggett, J.E., 1978. Influence of N nutrition on flower and pod abortion and yield of soybeans. *Agron. J.* 70, 81–84.
- Cassman, K.G., Dobermann, A., Walters, D.T., 2002. Agroecosystems, nitrogen-use efficiency: and nitrogen management. *AMBIO* 31, 132–140.
- Cassman, K.G., Dobermann, A., Walters, D.T., Yang, H., 2003. Meeting cereal demand while protecting natural resources and improving environmental quality. *Annu. Rev. Environ. Resour.* 28, 315–358.
- Connor, D.J., Loomis, R.S., Cassman, K.G., 2011. *Crop Ecology: Productivity and Management in Agricultural Systems*. Cambridge University Press.
- Evans, L.T., 1993. *Crop Evolution, Adaptation, and Yield*. Cambridge University Press, Cambridge, UK.
- FAOSTAT, F. Available online: <http://faostat3.fao.org> (Accessed on January 15, 2017).
- Fehr, W.R., Caviness, C.E., 1977. Stages of Soybean Development. Special Report 80. Iowa Agriculture and Home Economics Experiment Station, Iowa State University, Ames.
- Gaspar, A., Laboski, C., Nave, S., Conley, S., 2017. Dry matter and nitrogen uptake, partitioning, and removal across a wide range of soybean seed yield levels. *Crop Sci.* <http://dx.doi.org/10.2135/cropsci2016.05.0322>. (in press).
- Giller, K.E., Cadisch, G., 1995. Future benefits from biological nitrogen fixation: an ecological approach to agriculture. In *Management of Biological Nitrogen Fixation for the Development of More Productive and Sustainable Agricultural Systems*. Springer, Netherlands, pp. 255–277.
- Grassini, P., Specht, J., Tollenaar, T., Ciampitti, I., Cassman, K.G., 2014a. High-yield maize-soybean cropping systems in the U.S. Corn Belt. In: Sadras, V.O., Calderini, D.F. (Eds.), *Crop Physiology—Applications for Genetic Improvement and Agronomy*, 2nd edition). Elsevier, The Netherlands.
- Grassini, P., Torrión, J.A., Cassman, K.G., Yang, H.S., Specht, J.E., 2014b. Drivers of spatial and temporal variation in soybean yield and irrigation requirements in the western US Corn Belt. *Field Crops Res.* 163, 32–46.
- Grassini, P., Torrión, J.A., Yang, H.S., Rees, J., Andersen, D., Cassman, K.G., Specht, J.E., 2015. Soybean yield gaps and water productivity in the western US Corn Belt. *Field Crops Res.* 179, 150–163.
- de Bruin, J.L., Pedersen, P., 2009. New and old soybean cultivar responses to plant density and intercepted light. *Crop Sci.* 49, 2225–2232.
- de Bruin, J.L., Pedersen, P., Conley, S.P., Gaska, J.M., Naeve, S.L., Kurle, J.E., Elmore, R.W., Giesler, L.J., Abendroth, L.J., 2015. Probability of yield response to inoculants in fields with a history of soybean. *Crop Sci.* 50, 265–272.
- Herridge, D.F., Brockwell, J., 1988. Contributions of fixed nitrogen and soil nitrate to the nitrogen economy of irrigated soybean. *Soil Biol. Biochem.* 20, 711–717.
- Hoogenboom, G., Jones, J.W., Wilkens, P.W., Porter, C.H., Boote, K.J., Hunt, L.A., et al., 2010. Decision Support System for Agrotechnology Transfer (DSSAT) Version 4.5. Univ. of Hawaii, Honolulu.
- Jones, J.W., Hoogenboom, G., Porter, C.H., Boote, K.J., Batchelor, W.D., Hunt, L.A., Wilkens, P.W., Singh, U., Gijssman, A.J., Ritchie, J.T., 2003. The DSSAT cropping system model. *Eur. J. Agron.* 18, 235–265.
- Leggett, M., Diaz-Zorita, M., Koivunen, M., Bowman, R., Pesek, R., Stevenson, C., Leister, T., 2017. Soybean response to inoculation with in the United States and Argentina. *Agron. J.* 109, 1031–1038.
- Milliken, G.A., Johnson, D.E., 2009. second edition. *Analysis of Messy Data Volume 1: Designed Experiments*, vol. 1 CRC Press.
- Moore, K.J., Dixon, P.M., 2015. Analysis of combined experiments revised. *Agron. J.* 107, 763–771.
- Pate, J.S., Layzell, D.B., 1990. Energetics and biological costs of nitrogen assimilation. In: Mifflin, B.J., Lear, P.J. (Eds.), *The Biochemistry of Plants*, vol. 16 Academic Press, San Diego.
- Purcell, L.C., Serraj, R., Sinclair, T.R., De, A., 2004. Soybean N fixation estimates, ureide concentration: and yield responses to drought. *Crop Sci.* 44, 484–492.
- R Development Core Team, 2016. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
- Ray, J.D., Heatherly, L.G., Fritsch, F.B., 2006. Influence of large amounts of nitrogen on nonirrigated and irrigated soybean. *Crop Sci.* 46, 52–60.
- Ryle, G.J.A., Powell, C.E., Gordon, A.J., 1979. The respiratory costs of nitrogen fixation in soybean, cowpea: and white clover II. Comparisons of the cost of nitrogen fixation and the utilization of combined nitrogen. *J. Exp. Bot.* 30, 145–153.
- Salvagiotti, F., Cassman, K.G., Specht, J.E., Walters, D.T., Weiss, A., Dobermann, A., 2008. Nitrogen uptake: fixation and response to fertilizer N in soybeans: a review. *Field Crops Res.* 108, 1–13.
- Setiyono, T.D., Cassman, K.G., Specht, J.E., Dobermann, A., Weiss, A., Yang, H., Conley, S.P., Robinson, A.P., Pedersen, P., De Bruin, J.L., 2010. Simulation of soybean growth and yield in near-optimal growth conditions. *Field Crops Res.* 119, 161–174.
- Sinclair, T.R., De Wit, C.T., 1976. Analysis of the carbon and nitrogen limitations to soybean yield. *Agron. J.* 68, 319–324.
- Sinclair, T.R., Rufty, T.W., 2012. Nitrogen and water resources commonly limit crop yield increases: not necessarily plant genetics. *Global Food Secur.* 1, 94–98.
- Specht, J.E., Hume, D.J., Kumudini, S.V., 1999. Soybean yield potential—a genetic and physiological perspective. *Crop Sci.* 39, 1560–1570.
- Specht, J.E., Diers, B.W., Nelson, R.L., Toledo, J.F., Torrión, J.A., Grassini, P., 2014. Soybean (*Glycine max* (L.) merr.). In: Smith, J.S.C., Carver, B., Diers, B.W., Specht, J.E. (Eds.), *Yield Gains in Major US Field Crops: Contributing Factors and Future Prospects*. CSSA Special Publication #33. ASA-CSSA-SSSA, Madison, WI.
- Streeter, J., Wong, P.P., 1988. Inhibition of legume nodule formation and N₂ fixation by nitrate. *Crit. Rev. Plant Sci.* 71–123.
- Tamagno, S., Balboa, G.R., Assefa, Y., Kovács, P., Casteel, S.N., Salvagiotti, F., García, F.O., Stewarte, W.M., Ciampitti, I.A., 2017. Nutrient partitioning and stoichiometry in soybean: a synthesis-analysis. *Field Crops Res.* 200, 18–27.

- Tenorio, F.M., Specht, J.E., Arkebauer, T.J., Eskridge, K.M., Graef, G.L., Grassini, P., 2017. Co-ordination between primordium formation and leaf appearance in soybean (Glycine Max) as influenced by temperature. *Field Crops Res.* <http://dx.doi.org/10.1016/j.fcr.2017.03.015>. (in press).
- Thies, J.E., Singleton, P.W., Bohlool, B.B., 1995. Phenology, growth: and yield of field-grown soybean and bush bean as a function of varying modes of N nutrition. *Soil Biol. Biochem.* 27, 575–583.
- USDA-NASS, Quick stats 2.0. USDA-NASS 2005–2014; Available online: https://www.nass.usda.gov/Quick_Stats/ (Accessed on January 15, 2017).
- van Ittersum, M.K., Cassman, K.G., Grassini, P., Wolf, J., Tittonell, P., Hochman, Z., 2013. Yield gap analysis with local to global relevance—a review. *Field Crops Res.* 143, 4–17.
- Van Kessel, C., Hartley, C., 2000. Agricultural management of grain legumes: has it led to an increase in nitrogen fixation? *Field Crops Res.* 65, 165–181.
- Van Roekel, R.J., Purcell, L.C., 2014. Soybean biomass and nitrogen accumulation rates and radiation use efficiency in a maximum yield environment. *Crop Sci.* 54, 1189–1196.
- Wilson, R.F., 2008. Soybean: market driven research needs. *Genetics and Genomics of Soybean*. Springer, New York, pp. pp. 3–15.
- Wilson, E.W., Rowntree, S.C., Suhre, J.J., Weidenbenner, N.H., Conley, S.P., Davis, V.M., Diers, B.W., Esker, P.D., Naeve, S.L., Specht, J.E., Casteel, S.N., 2014. Genetic gain × management interactions in soybean: II. Nitrogen utilization. *Crop Sci.* 54 (1), 340–348.