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Does biological nitrogen fixation modify soybean nitrogen dilution curves?

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ABSTRACT

Biological nitrogen fixation (BNF) in soybean [*Glycine max* (L.) Merr.] represents, on average, 60% of total nitrogen (N) uptake. Nitrogen dilution curves link aboveground crop N concentration (%N) to biomass accumulation (W). It has been reported that BNF is an energy-intensive process that might reduce biomass production per unit of captured N (physiological N use efficiency or NUE). This increased energy cost could lead to a more attenuated N (i.e. less efficient) dilution curve. However, there are no reports comparing N dilution curves for soybean crops differing in N source. Our objectives were to: (i) evaluate the impact of BNF on soybean N dilution curves and how it influences NUE, and (ii) establish independent N dilution curves for soil and atmospheric N. Our working hypothesis is that relying on BNF attenuates the N dilution curve and reduces NUE. The experiment consisted of a control and a fertilized treatment, 0 and 600 kg N ha^{-1} respectively, applied to four soybean genotypes in order to establish two differential BNF situations. While the control and fertilized treatments had differential N accumulation from BNF, ~70% and ~16%, respectively, there were no differences observed in seed yield (~5000 kg ha^{-1}), NUE (~36 kg kg^{-1}) and only slight differences in total N uptake (~365 kg N ha^{-1} in fertilized treatment compared to ~389 kg h^{-1} in the control treatment). Results suggest that reliance on BNF for N does not influence substantially the attenuation of the N dilution curve and has no impact on NUE. The N dilution parameter ("b") ranged from −0.128 to −0.218 among cultivars and fertilization treatments. The less negative values (more attenuated curve) corresponded to the fertilized plots likely associated with luxury N consumption. Interestingly, dilution curves from soil mineral N showed the typical dilution pattern, while N derived from the atmosphere followed a concentration pattern as the crop developed. This most likely reflects the continuous N flux from BNF to the plant as opposed to the decreasing soil mineral N supply. Recognizing these concentration/dilution curves for atmospheric and soil N has three immediate implications. First, the atmospheric N concentration curve might indicate an upper benchmark for evaluating symbiosis performance during crop development. Second, the concentration pattern observed for BNF could potentially help to reverse the observed decline in seed protein concentration in modern soybean cultivars. Third, the N concentration/dilution curves for the individual N sources could be incorporated into crop models for estimating BNF at different crop biomass levels during soybean development.

1. Introduction

Nitrogen accumulation is frequently the most limiting process to attain maximum yield in several crops (Plénet and Lemaire, 1999; Ziadi et al., 2010; Rotundo et al., 2014). Nitrogen plays a key role as a constituent of carbon assimilation enzymes like ribulose-1,5-bisphosphate carboxylase/oxygenase (RUBISCO; Rotundo and Cipriotti, 2017). It is also involved in leaf area generation and radiation use efficiency at the crop level (Sinclair and Horie, 1989). Soybean shows a strong positive correlation between seed yield and N uptake (Salvagiotti et al., 2008; Rotundo et al., 2014), and has the highest N requirement compared to all other legumes and cereal crops (Sinclair and de Wit, 1975). To fulfill this high N requirement, soybean, like all legumes, utilizes two complementary sources for N uptake: soil mineral N absorption and atmospheric N via biological N fixation in association with rhizobium bacteria (Layzell, 1990).

On average, atmospheric N accounts for 60% of total N uptake (Salvagiotti et al., 2008). A regional survey in Argentina showed that the range of N derived from BNF varied from 46 to 71% in farmers' fields (Collino et al., 2015). However, recent evidence supports that

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Abbreviations: BNF, biological nitrogen fixation; N, nitrogen; %N, nitrogen concentration; NUE, nitrogen use efficiency; W, above ground biomass accumulation

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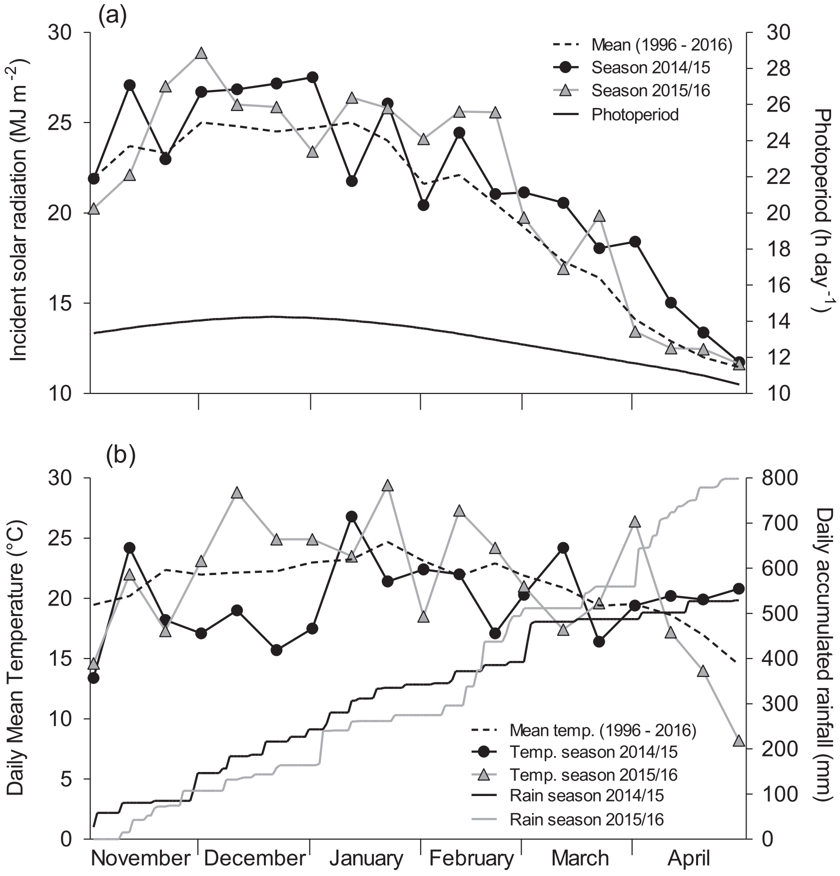

Fig. 1. Solar radiation 10-day average and daily photoperiod (a) and daily mean temperature 10-day average and daily accumulated rainfall (b) for the November-April period. Dashed lines show historic (1996–2016) solar radiation (a) and daily mean temperature (b).

high BNF is not required for maximizing yields when soil N supply is adequate for sustaining this potential yield, indicating a trade-off between both N sources (Santachiara et al., 2017). This view is also supported by the marginal soybean yield response to soil N fertilization with conventional fertilization practices (i.e. applying low N rates broadcast on the surface; Kaschuk et al., 2016). There may be, however, particular situations of high-yielding systems and/or unfavorable environmental conditions for nodulation where N fertilization can overcome N limitations (e.g. Salvagiotti et al., 2009; Cafaro La Menza et al., 2017). Regardless of the relative importance of each source, some evidence suggests that the BNF process entails an extra energy cost for the host as compared to soil nitrate absorption (Silsbury, 1977; Andrews et al., 2009). If this differential energy cost at the cellular level is expressed at a higher level of organization, then a reduction in physiological NUE may happen at the plant or crop scale. However, the impact of this extra energy cost to whole plant performance remains controversial (Salsac et al., 1984). For example, Kaschuk et al. (2009) suggested that rhizobial symbiosis stimulates photosynthesis due to increased carbon sink demand compensating any extra energy cost.

Physiological NUE may be defined as the production of biomass at physiological maturity per unit of accumulated N (Xu et al., 2012). High yielding cultivars from Argentina and the USA ranged from 28 to 35 kg of biomass produced per kg of N uptake (Rotundo et al., 2014). However, biomass production is dynamic, interacting with N uptake during the crop cycle, thus affecting NUE. Therefore, an approach relating plant N status to crop development is needed for analyzing differential energy cost on biomass production and NUE (Sadras and Lemaire, 2014). One such approximation is the concept of N dilution curve which relates aboveground N concentration to biomass accumulation, based on the equation proposed by Lemaire and Salette (1984):

$$\%N = aW^{b} \tag{1}$$

where W is total aboveground biomass (Mg ha^{-1}), parameter “a” is crop %N when W = 1 Mg ha^{-1} and parameter “b” is a dimensionless coefficient, than when takes negative values, represents the rate of decline in %N as biomass accumulation progresses.

The *critical* N dilution is further defined as the N dilution curve for the minimum N concentration that maximizes biomass production (Gastal and Lemaire, 2002). This framework was used for comparing N capture dynamics and fertilization needs in the C4 crop maize (Plénet and Lemaire, 1999), and C3 crops wheat (Ziadi et al., 2010) and rice (Sheehy et al., 1998). The *critical* N dilution curve for non-legume species is determined by increasing levels of N fertilization to find the minimum N concentration that maximizes crop biomass. However, the construction of this critical curve is not possible for N-fixing species under normal conditions (Ney et al., 1997; Divito et al., 2016). Studies using non-fixing pea mutants and N fertilization show that N-fixing crops usually dilute N close to the critical dilution curve (Ney et al., 1997). Previous research in soybean showed no changes in the total N uptake dilution curve among genotypes and planting dates (Divito et al., 2016). However, no attempts were made to analyze if reliance more on BNF or soil mineral N absorption would modify N dilution curve parameters.

This study had two main objectives. The first objective was to evaluate whether BNF modifies the pattern of soybean N dilution during crop development and how this impacts NUE at maturity. Our working hypothesis was that BNF entails an extra-energy cost, and therefore it will lead to a more attenuated N dilution curve when compared to a full N fertilized treatment, expressing a reduced physiological NUE at maturity. The second objective was to establish, for the first time, individual N dilution curves for both soil and atmospheric