



Post-Flowering Phosphorus Deficiency Negatively Impacts Biological Fixation but not Nitrogen Utilization Efficiency of Soybean

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Abstract

Understanding how phosphorus (P) deficiency during the reproductive phase of soybean [*Glycine max* (L.) Merrill] affects nitrogen (N) acquisition via biological N fixation (BNF), and seed yield per unit of the accumulated nutrient remains incomplete. Soybean plants were fertigated with a sufficient concentration of P in the nutrient solution (500 $\mu\text{mol L}^{-1}$ P) until flowering. Subsequently, plants were maintained under this condition or subjected to nutrient deficiencies (20 or 100 $\mu\text{mol L}^{-1}$ P), resulting in three regimes of P supply during the reproductive phase. At the onset of maximum grain-filling rate and physiological harvest, various parameters were assessed, including nodulation traits, plant nutritional status and biomass production, accumulation, partitioning, and utilization efficiency of P and N. P deficiency after flowering negatively impacted soybean yield and dry mass production, as well as the concentration of P and N in plant organs, their total shoot content, and partitioning to grains. The poor BNF performance was associated with a reduction in the number and dry mass of nodules, triggered by a decrease in plant's N demand. Nevertheless, low-P stress did not affect seed yield per unit of acquired nutrient, which was related to the fact that the decline in N partitioning to grains was accompanied by a proportional decreasing in their N concentration. The down-regulation of BNF, rather than an impaired N utilization efficiency, contributes to explaining reduced yield of soybean plants facing post-flowering P deficiency. Therefore, the development of precise P fertilization management approaches to maximize BNF and crop yield should prioritize strategies that ensure adequate P supply across the reproductive phase of soybean.

Keywords Abiotic stress · Fertilizer management · *Glycine max* · Nodulation · Nutrient efficiency · Sustainable agriculture

1 Introduction

The sustainability of soybean [*Glycine max* (L.) Merrill] production relies heavily on efficient biological nitrogen fixation (BNF) to meet high plant's nitrogen (N) requirements. This process of N acquisition reduces environmental risks

associated with the overapplication of synthetic N fertilizers (Ciampitti and Salvagiotti 2018; Telles et al. 2023). Thus, addressing detrimental consequences of nutritional stresses on BNF is a crucial step toward precise nutrient management of soybean fields, aiming for long-term sustainable gains in resource use efficiency (O'hara et al. 1988; Santachiara et al. 2019; Latifinia and Eisvand 2022). In this context, ameliorating the effects of phosphorus (P) deficiency deserves special attention, as it is a widespread nutritional disorder in weathered tropical soils (Vance et al. 2003) impairing growth, yield, and BNF performance of soybean (Qin et al. 2012; Medina et al. 2022; Mirriam et al. 2022). As a response to this adverse condition, the application of the entire recommended rate of P at sowing has been a commonly adopted fertilization strategy in fields cultivated with this grain legume (Li et al. 2011; Farmaha et al. 2012). While proper P supply at sowing is crucial for maximizing seedling vigor and crop harvests (Grant et al. 2001), it also

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contributes to N acquisition by stimulating formation and functioning of nodules at early plant developmental stages (Kouas et al. 2005; Bulgarelli et al. 2017; Qiao et al. 2017).

Nevertheless, it is noteworthy that the demand and uptake rates of P by soybean are relatively low during the vegetative phase, with the majority of this nutrient accumulating after flowering (Bender et al. 2015; Gaspar et al. 2017a). Such a phenomenon, coupled with a simultaneous decline in the availability of P and its uptake capacity by the roots (Howard et al. 1999; Vengavasi and Pandey 2018; Yang et al. 2023), leads to the risks of late P deficiency. This might have further implications for N acquisition via BNF (Li et al. 2022), given that N₂ fixation continues at high rates until the end of grain-filling phase, unless the plants experience events of abiotic stresses (Mastrodenico and Purcell 2012; Mastrodenico et al. 2013; Van Roekel et al. 2015). Indeed, the peak of N demand (Bender et al. 2015; Gaspar et al. 2017b) and the stabilization of nodules production in soybean occur during the reproductive phase (Gray et al. 2012). Despite this and the vulnerability of N₂ fixation to unfavorable conditions across the post-flowering period, the specific effect of P deficiency on BNF performance has not been widely explored in soybean.

Moreover, it has been proposed that a disturbed plant P nutritional status contributes to hampering overall crop performance by constraining the conversion of the acquired N into biomass production (N utilization efficiency, NUtE). The decline in NUtE is associated with impaired reduction and assimilation of the inorganic N acquired by the roots (Ruffy et al. 1990; De Groot et al. 2003; Gan et al. 2016). In addition to this, the conversion of the accumulated N into yield formation depends directly also on the amount of the nutrient that is partitioned to grains to sustain their proper development (The et al. 2021; Liang et al. 2023). Accordingly, NUtE at crop maturity can be negatively impacted by adverse conditions hindering remobilization and allocation of N to the harvestable organs during the post-flowering period (Marmagne et al. 2020, 2022). However, in the case of nodulated soybean plants, the incorporation of soluble-reduced N into organic compounds has been shown to be less sensitive to the negative effects of low-P stress compared to N₂ fixation (Israel and Ruffy 1988; Sa and Israel 1995). In this sense, whether a restricted P availability after flowering affects the conversion of the fixed N₂ into grain yield at physiological harvest in nodulated soybean plants remains to be clarified.

Herein, we tested the hypothesis that detrimental effects on soybean yield resulting from a constrained N₂ fixation capacity under post-flowering P deficiency stress would be exacerbated by a less efficient conversion of the acquired N into yield formation. Therefore, we conducted a study examining the effects of varying levels of post-flowering P

supply (sufficiency, mild and severe deficiencies) on nodulation parameters, plant growth and nutritional status, yield performance, as well as the accumulation and partitioning of P and N. The P nutrition regimes were specifically implemented during the reproductive phase to replicate field conditions where a lack of synchrony between availability and demand of P could affect soybean nutritional status during grain-filling (Duarte et al. 2022; Laira et al. 2023).

2 Materials and Methods

2.1 Plant Material, Nutritional Management until Flowering, and P Treatments during the Reproductive Phase

The experiment was conducted in a greenhouse at the Agronomic Institute's experimental area from October 2019 to February 2020. We utilized the soybean variety NA 5909RG, known for its indeterminate growth habit, relative maturation group of 6.2, and widespread cultivation in major producing regions (Matei et al. 2017). Throughout the experimental period, the average air temperature and relative humidity were 38 °C and 85%, respectively. Soybean seeds were inoculated with *Bradrhizobium japonicum* (Biovita Turfa Soja[®]) at a rate of 1.2 g kg⁻¹ of seeds. Subsequently, eight seeds were sown in pots containing 7.5 dm³ of fine-textured vermiculite (Global Minérios[®]).

Thinning was performed five days after emergence (DAE), retaining the three most vigorous plants. Concurrently, we initiated fertigation with a nutrient solution diluted at 50% of its final concentration (details provided below). At ten DAE, we transitioned to a solution with 100% nutrient concentration, which was used until the end of the experiment. The nutrient solution, devoid of N source, contained the following concentrations (μmol L⁻¹): 5,000 Ca; 3,000 K; 500 P; 1,300 Mg; 1,300 S; 41.6 B; 0.3 Co; 1.0 Cu; 46.7 Fe; 10.0 Mn; 1.3 Mo; 0.01 Ni and 3.5 Zn (Duarte et al. 2022). Fertigation was performed every other day throughout the experimental period, with the volume of applied solution tailored to ensure leaching, ranging from 450 to 900 mL per pot depending on the plant's phenological phase.

Soybean plants were fertigated with the aforementioned nutrient solution until they reached the R1 phenological phase, characterized by the emergence of the first flower on the main stem (Fehr and Caviness 1977). To simulate varying P availabilities in the rooting medium during the reproductive phase, different concentrations of P were applied from R1 until physiological harvest (R8) (Fehr and Caviness 1977). Treatments included severe deficiency (20 μmol L⁻¹ P, P₂₀), mild deficiency (100 μmol L⁻¹ P, P₁₀₀),

and sufficiency ($500 \mu\text{mol L}^{-1} \text{P}$, P_{500}), defined according to previous studies (Singh et al. 2018; Duarte et al. 2022). The experiment followed a randomized block design with four replicates for each regime of post-flowering P supply. Each replicate consisted of two pots with three plants, totaling 24 pots.

2.2 Assessment of Soybean Responses to Post-flowering P Treatments: Nodulation Traits, Biomass Production, and Nutrient Efficiency

During the reproductive phase, in addition to the evaluation at physiological harvest (R8), an earlier assessment was performed at the R5.5 phenological phase, which corresponds to the beginning of the maximum grain-filling rate (Fehr and Caviness 1977). This evaluation was chosen because R5.5 represents also the period of maximum P and N accumulation rates in soybean plants (Bender et al. 2015; Gaspar et al. 2017a, b) and potentially the point when nodule production by this legume species is at its highest (Gray et al. 2012).

At R5.5, half of the experimental units (12 pots) were destructively sampled. The plants were separated into leaves, stems with petioles, reproductive structures (pods with grains), roots, and nodules. These parts were washed in tap water, rinsed with deionized water and dried at 60°C for three days to measure dry mass (DM) production. Nodules were counted to obtain nodule number (nodules pot^{-1}) and to estimate specific nodulation (nodules g^{-1} root DM). Furthermore, average nodule DM (mg nodule^{-1}) was calculated as the ratio between nodule DM (mg pot^{-1}) and nodule number.

At R5.5, shoot DM production (g pot^{-1}) was determined by summing DM of leaves, stems with petioles, and reproductive structures. Harvest index (HI, %) was calculated as the ratio between DM of reproductive structures and shoot DM. At R8, the assessment of DM production followed the same procedures, except for nodules, which were senescent and could not be separated from roots. In addition, pods and grains were individually evaluated, providing pod-shell DM (g pot^{-1}) and grain DM (g pot^{-1}). Then, HI at R8 was the ratio between grain DM and shoot DM.

The nodules collected at R5.5 and all plant shoot organs sampled at R5.5 and R8 were ground and P concentration (g kg^{-1}) was determined using inductively coupled plasma optical emission spectrometry (ICP-OES) after wet digestion with nitric and perchloric acids. The concentration of N (g kg^{-1}) in the same organs was measured through distillation after sulfuric acid digestion. Shoot accumulation (g pot^{-1}) of P and N was estimated as the sum of the content of P and N in each organ, which was calculated as the product of their respective nutrient concentration and DM production. Given absence of N supply through nutrient solution,

the amount of N accumulated (g pot^{-1}) in soybean shoots at R5.5 and R8 was assumed to originate from BNF (Prudent et al. 2016). Based on this, nodule activity (mg N nodule^{-1}) at R5.5 and R8 was the ratio of shoot N content at each sampling time and nodule number at R5.5.

Furthermore, utilization efficiency (g g^{-1}) of P (PUtE) and N (NUtE) was estimated as the ratio between DM of reproductive structures (at R5.5) or grain (at R8) and shoot content of P or N at each phenological phase. P harvest index (PHI, %) and N harvest index (NHI, %) were the ratio between the content (g pot^{-1}) of P and N in the reproductive structures (at R5.5) or grains (at R8) relative to their respective shoot content in each evaluation period.

2.3 Statistical Analysis

The obtained data were subjected to one-way analysis of variance (ANOVA) using Sisvar software (Ferreira 2011). Tukey's multiple range test ($p < 0.05$) was then employed to compare the means of the treatments, the concentration of P in the nutrient solution throughout the reproductive phase.

3 Results

3.1 Nodulation Traits

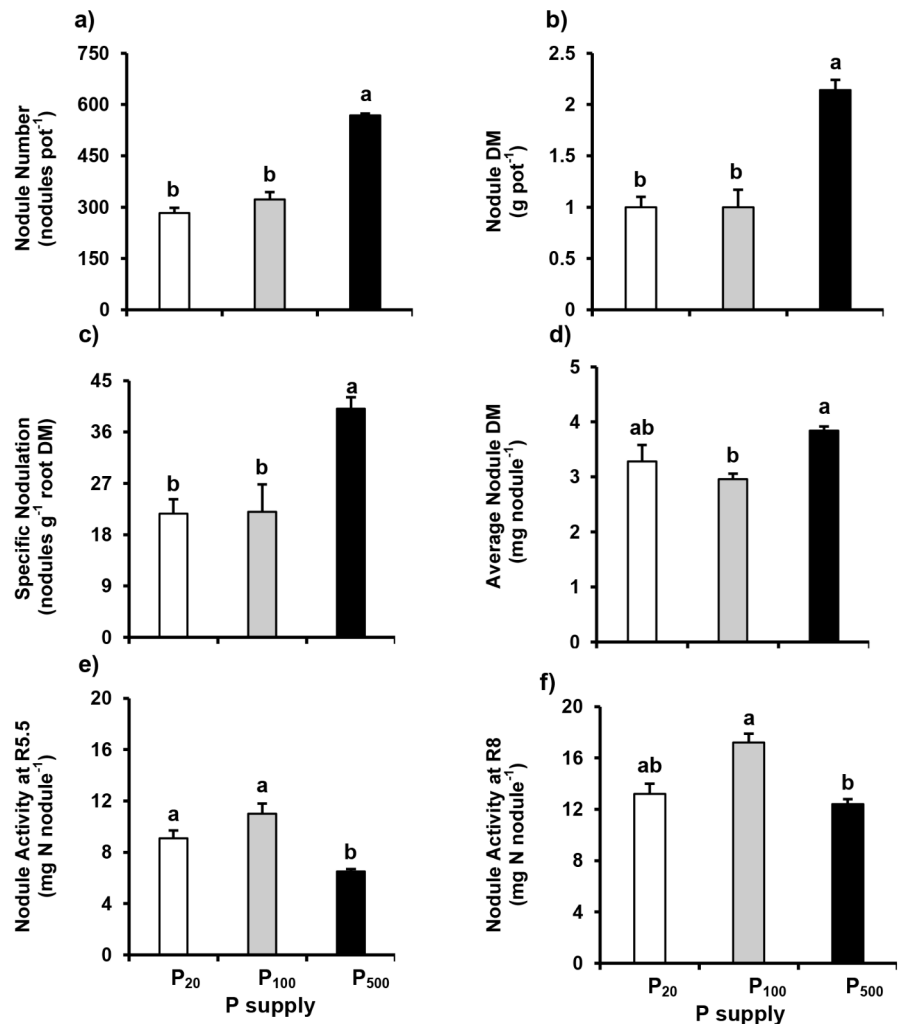
At R5.5, plants subjected to post-flowering P deficiency exhibited reduction in several nodulation traits compared to those under sufficiency. Specifically, for the average of P_{20} and P_{100} plants, nodule number decreased by 47%, nodule DM by 53%, and specific nodulation by 47% (Fig. 1a-c). Only P_{100} plants showed a lower average nodule DM compared to P_{500} plants and with a less pronounced effect size relative to the other nodulation traits (Fig. 1d). Nodule activity at R5.5 and R8 of P-limited plants was either higher or similar compared to those found in well-nourished plants (Fig. 1e, f).

3.2 Concentration of P and N in Shoot Organs and Nodules

At R5.5, the concentration of P in the shoot organs of plants grown under P_{500} condition was higher than those subjected to P_{20} and P_{100} conditions, with differences ranging from 21 to 90% (Fig. 2a, c, e). However, in the case of nodules, no response to varying P supply was found (Fig. 2g). P limitation decreased also N concentration in leaves and stems of soybean, but there was a lack of effect on reproductive structures and nodules (Fig. 2b, d, f, h).

By R8, post-flowering P supply did not affect the concentration of P and N in the leaves, while the concentration

Fig. 1 Nodule number (in **a**), nodule dry mass (DM) (in **b**), specific nodulation (in **c**), average nodule DM (in **d**) of soybean plants at the beginning of maximum grain-filling rate (R5.5) and nodule activity at R5.5 (in **e**) and physiological harvest (R8) (in **f**). Supply of phosphorus (P) during the reproductive phase corresponded to fertigation with $20 \mu\text{mol L}^{-1}$ P (P_{20} , severe deficiency), $100 \mu\text{mol L}^{-1}$ P (P_{100} , mild deficiency) and $500 \mu\text{mol L}^{-1}$ P (P_{500} , sufficiency). Columns followed by different letters indicate significant differences based on Tukey's multiple range test ($p < 0.05$). Bars indicate the standard error ($n = 4$)



of these nutrients in the stems decreased under P limitation (Fig. 3a-d). Low-P stress reduced the concentration of P in pod-shells, but the opposite occurred in the case of N (Fig. 3e, f). In the grains, concentration of both P and N were lower under P deficiency than P sufficiency; however, the magnitude of the decreasing was greater for P, a reduction of 32% in P_{20} and P_{100} plants compared to P_{500} plants (Fig. 3g, h).

3.3 Plant Biomass Production and Partitioning

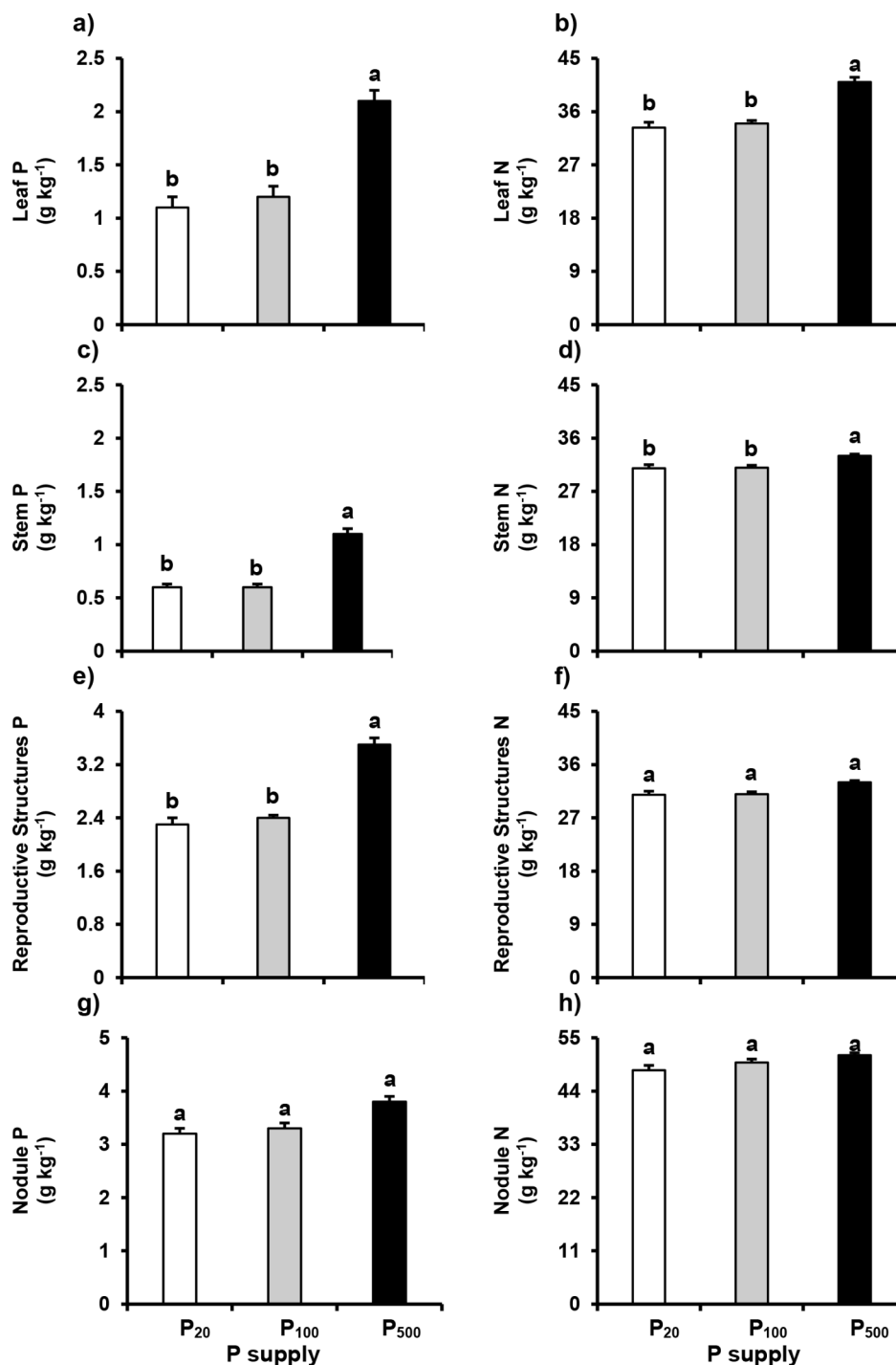
At R5.5, DM production of reproductive structures was 17% lower in P_{20} and 10% lower in P_{100} plants compared to P_{500} ones (Fig. 4a). By R8, the imposition of P limitation during the reproductive phase also resulted in compromised yield performance: the relative grain DM of P_{20} and P_{100} plants, compared to P_{500} plants, corresponded to 58% and 67%, respectively. Additionally, grain DM of plants under P_{100} was 15% higher than that in P_{20} plants (Fig. 4b). DM production of vegetative organs (leaves combined with

stems and petioles) at R5.5 and R8 as well as shoot DM at R5.5 did not vary as a function of P supply (Fig. 4c, d, e). However, shoot DM at R8 was 27% lower in P_{20} plants and 16% lower in P_{100} plants compared to those grown under sufficiency (Fig. 4f). Regarding partitioning of DM, HI of P-limited plants decreased by 9% at R5.5 and by 22% at R8 compared to P_{500} plants (Fig. 4g, h).

3.4 Accumulation, Partitioning and Utilization Efficiency of P and N

At the earlier sampling time (R5.5), the content of P in the reproductive structures and in the whole shoot of P-limited plants was on average 41% and 43% lower, respectively, than under P sufficiency; and, in the case of N, these differences corresponded to 20% and 22%, respectively (Fig. 5a-d). By R8, content of P and N in the grains was negatively impacted by P deficiency, with reductions of 57% and 45%, respectively (Fig. 5e, f). At R8, shoot P content was 2.3- and 2.0-fold higher in P_{500} plants than in P_{20} and P_{100} ones,

Fig. 2 Concentration of phosphorus (P) or nitrogen (N) in the leaves (in **a** and **b**), stems (in **c** and **d**), reproductive structures (pods with grains, in **e** and **f**) and nodules (in **g** and **h**) of soybean plants at the beginning of maximum grain-filling rate (R5.5). Supply of P during the reproductive phase corresponded to fertigation with $20 \mu\text{mol L}^{-1}$ P (P_{20} , severe deficiency), $100 \mu\text{mol L}^{-1}$ P (P_{100} , mild deficiency), and $500 \mu\text{mol L}^{-1}$ P (P_{500} , sufficiency). Different letters indicate significant differences based on Tukey's multiple range test ($p < 0.05$). Bars indicate the standard error ($n = 4$)

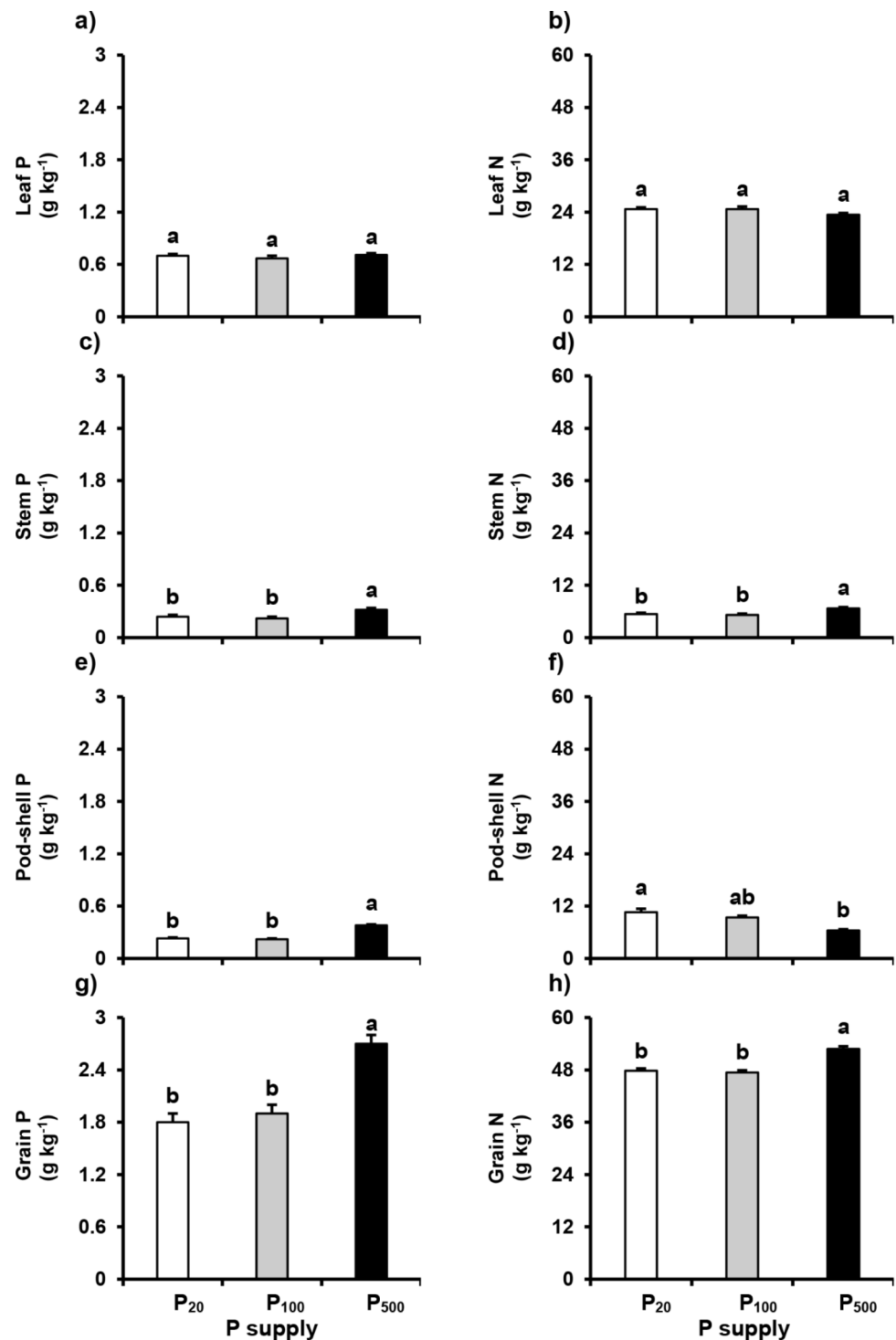


respectively; also, shoot P content under P_{100} condition was 14% higher than under P_{20} (Fig. 5g). Compared to P_{500} plants, shoot N content decreased by 41% and 33% in P_{20} and P_{100} plants, respectively (Fig. 5h).

At R5.5, post-flowering P supply did not influence the partitioning of P and N to the reproductive structures and NUtE, but PUtE was 37% lower under P_{500} than other P supplies (Fig. 6a-d). At R8, while P deficiency had a negative effect on PHI, PUtE was higher in P limited plants than

well-nourished ones (Fig. 6e, g). Restricted P availability posed a negative impact on NHI, with a decrease of 14% on this parameter compared to P_{500} plants (Fig. 6f). However, P deficiency did not influence NUtE at R8, and the average value across nutrition treatments corresponded to 14.9 g g^{-1} (Fig. 6h).

Fig. 3 Concentration of phosphorus (P) or nitrogen (N) in the leaves (in **a** and **b**), stems (in **c** and **d**), pod-shells (in **e** and **f**) and grains (in **g** and **h**) of soybean plants at the physiological harvest (R8). Supply of P during the reproductive phase corresponded to fertigation with 20 $\mu\text{mol L}^{-1}$ P (P_{20} , severe deficiency), 100 $\mu\text{mol L}^{-1}$ P (P_{100} , mild deficiency), and 500 $\mu\text{mol L}^{-1}$ P (P_{500} , sufficiency). Different letters indicate significant differences based on Tukey's multiple range test ($p < 0.05$). Bars indicate the standard error ($n = 4$)

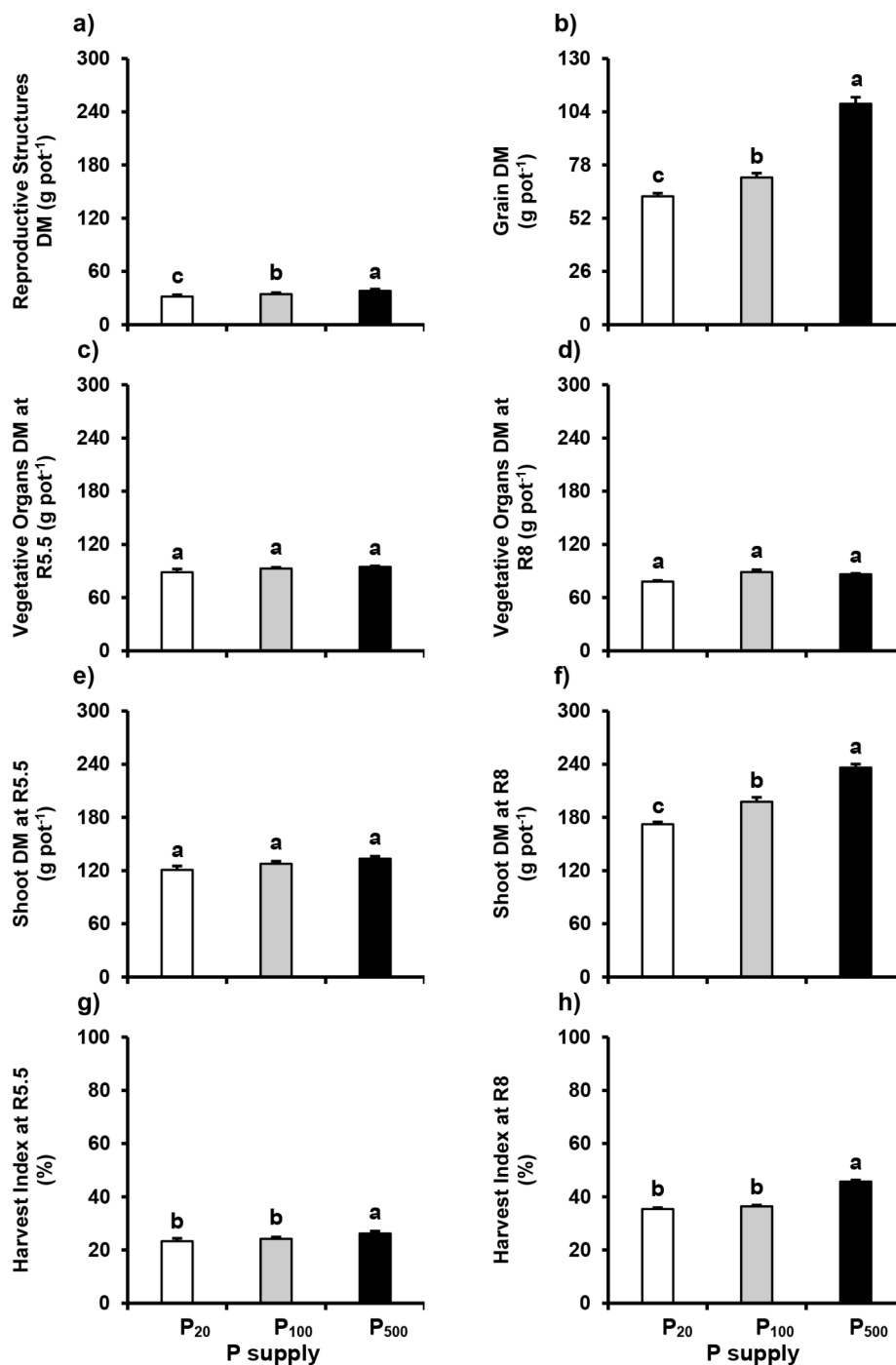


4 Discussion

The productivity of soybean can be described as the product of plant N content and grain DM produced per unit of the nutrient accumulated throughout the crop cycle (Rotundo et al. 2014; Ortez et al. 2019). Accordingly, examining the impact of nutritional disorders on these yield determinants

would be useful to understand how adverse growing conditions affect soybean performance and to propose avenues for crop improvement. In this respect, our results revealed that shoot N content of P_{20} and P_{100} plants was lower than that of P_{500} plants at both R5.5 and R8, with the difference becoming more pronounced at the latter phenological phase. Nevertheless, the efficiency of soybean in converting the

Fig. 4 Dry mass (DM) production of reproductive structures (pods with grains) at the beginning of maximum grain-filling rate (R5.5) (in **a**), grains at physiological harvest (R8) (in **b**), vegetative organs (leaves combined with stems and petioles) at R5.5 (in **c**) and R8 (in **d**), total shoot at R5.5 (in **e**) and R8 (in **f**), and harvest index at R5.5 (in **g**) and R8 (in **h**) of soybean plants. Supply of phosphorus (P) during the reproductive phase corresponded to fertigation with 20 $\mu\text{mol L}^{-1}$ P (P_{20} , severe deficiency), 100 $\mu\text{mol L}^{-1}$ P (P_{100} , mild deficiency), and 500 $\mu\text{mol L}^{-1}$ P (P_{500} , sufficiency). Different letters indicate significant differences based on Tukey's multiple range test ($p < 0.05$). Bars indicate the standard error ($n = 4$)

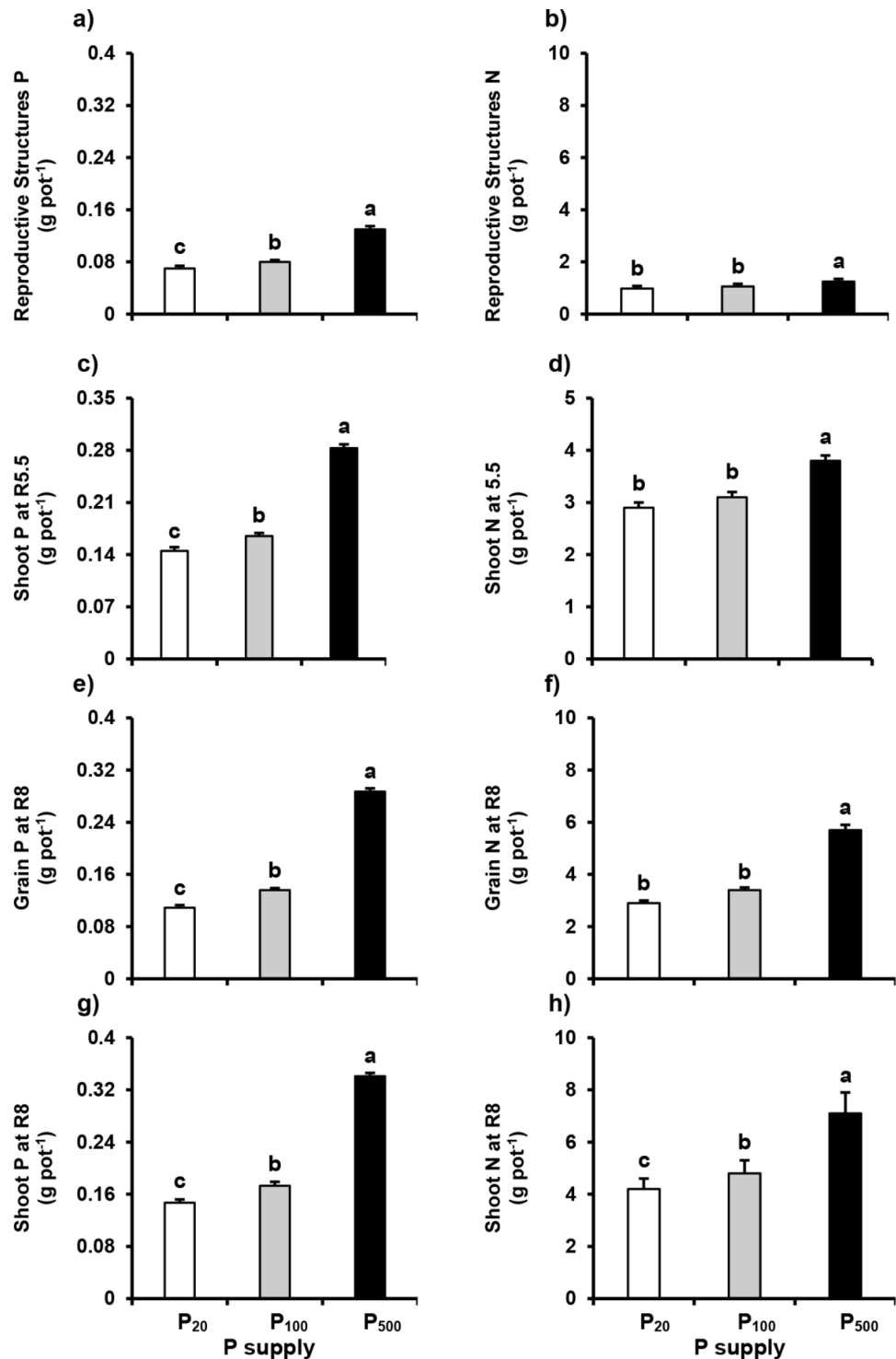


acquired N into DM production of reproductive structures at R5.5 and grains at R8 was not influenced by P supply regime. As a result, the poor crop performance under post-flowering P deficiency condition was a primary consequence of impairments in the plant's capability of accumulating N in their shoots over the reproductive phase. Hence, while a sufficient P availability since early phenological phases has proved to be essential for nodule formation and functioning (Qin et al. 2012; Bulgarelli et al. 2017; Qiao et al. 2017),

it is also evident that BNF is sensitive to post-flowering P deficiency, as already observed for other abiotic stresses (Mastrodenico and Purcell 2012; Mastrodenico et al. 2013; Van Roekel et al. 2015).

Considering that the proper functioning of shoot organs relies on a fine-tuned balance between the concentration of P and N, soybean plants appear to employ, in response to post-flowering P limitation, a primary strategy of down-regulating BNF to mitigate the risks of nutrient imbalance

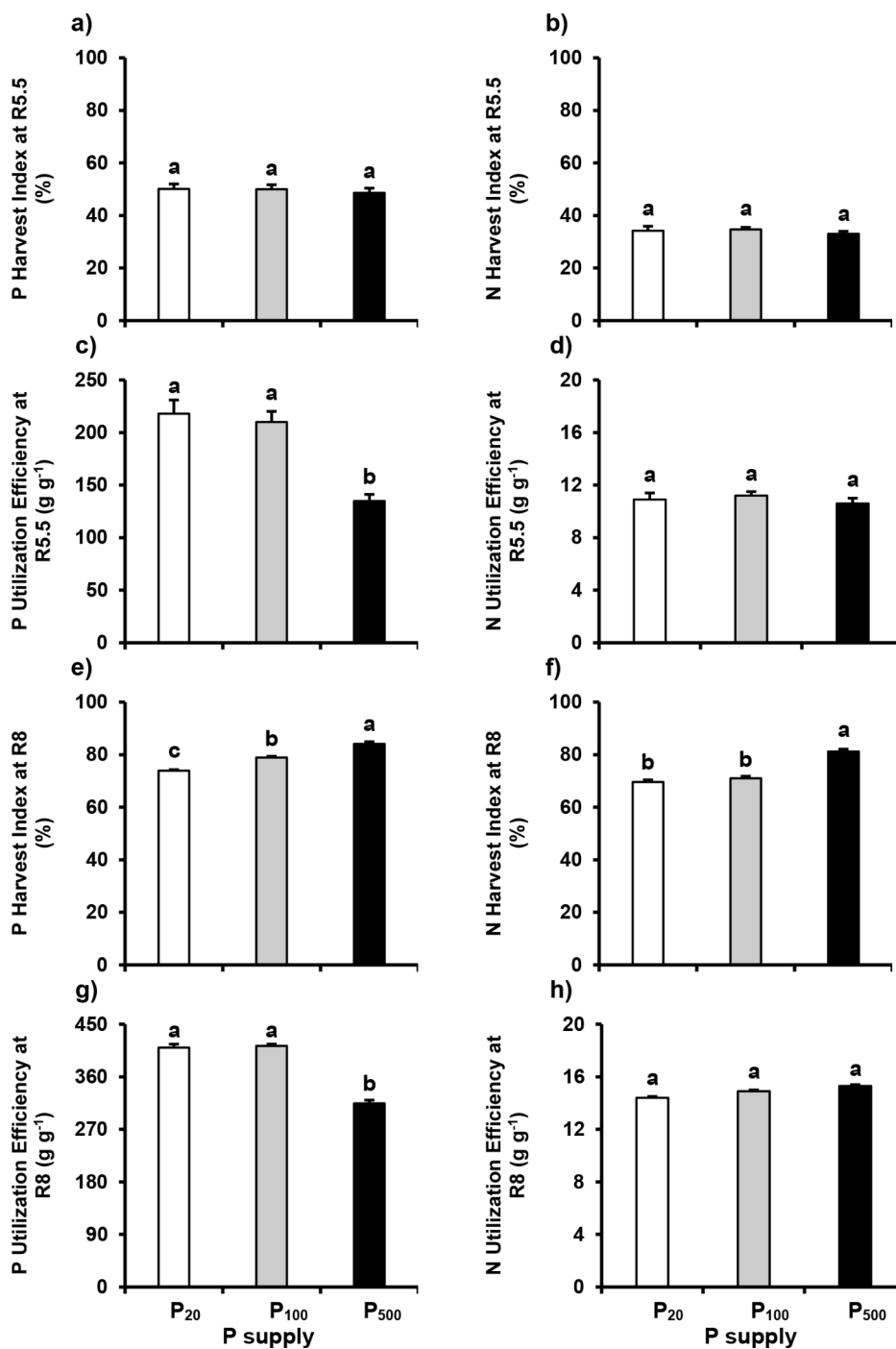
Fig. 5 Content of phosphorus (P) or nitrogen (N) in the reproductive structures (pods with grains) at the beginning of maximum grain-filling rate (R5.5) (in **a** and **b**), in the whole shoot at R5.5 (in **c** and **d**), in the grains at physiological harvest (R8) (in **e** and **f**) and in the whole shoot at R8 (in **g** and **h**) of soybean plants. Supply of P during the reproductive phase corresponded to fertigation with $20 \mu\text{mol L}^{-1}$ P (P_{20} , severe deficiency), $100 \mu\text{mol L}^{-1}$ P (P_{100} , mild deficiency), and $500 \mu\text{mol L}^{-1}$ P (P_{500} , sufficiency). Different letters indicate significant differences based on Tukey's multiple range test ($p < 0.05$). Bars indicate the standard error ($n = 4$)



(Sadras 2006; Vitousek et al. 2010; Li et al. 2023). Herein, this condition was found in P_{20} and P_{100} plants, that exhibited a concomitant reduction in the concentration of P and N in the leaves and stems at R5.5. In P-stressed tissues, an impaired synthesis of proteins leads to an increase in the content of free amino acids, which in turn triggers an N-feedback mechanism resulting in the down-regulation of BNF

(Schulze 2004). Moreover, given that shoot growth potential drives N requirement of the plants (Voisin et al. 2010), we argue that differences in yield performance posed also an important influence on the control of BNF: while there was no variation in the biomass of vegetative parts, DM of harvestable organs (pods with grains at R5.5 and grains at R8) and HI increased with a sufficient P supply. Indeed, actively

Fig. 6 Shoot harvest index of phosphorus (P) or nitrogen (N) at the beginning of maximum grain-filling rate (R5.5) (in **a** and **b**) and physiological harvest (R8) (in **e** and **f**) and utilization efficiency of P or N at R5.5 (in **c** and **d**) and R8 (in **g** and **h**) of soybean plants. Supply of P during the reproductive phase corresponded to fertigation with 20 $\mu\text{mol L}^{-1}$ P (P_{20} , severe deficiency), 100 $\mu\text{mol L}^{-1}$ P (P_{100} , mild deficiency), and 500 $\mu\text{mol L}^{-1}$ P (P_{500} , sufficiency). Different letters indicate significant differences based on Tukey's multiple range test ($p < 0.05$). Bars indicate the standard-error ($n = 4$)



filling grains act as strong sinks for N transport from vegetative tissues and plays a critical role on the stimulation of BNF (Rotundo et al. 2014). Such a phenomenon might, in turn, explain the exacerbation of the difference in shoot N content between P-deficient and -sufficient plants from R5.5 to R8. Interestingly, P-induced DM production increases the demand for N, which in turn enables BNF to persist and potentially contribute to higher crop yields and grain N content (Cabeza et al. 2024).

Additional insights into how nutrient deficiencies impact BNF potential of grain legume crops can be obtained with the assessment of nodulation parameters (Kouas et al. 2005; Zhong et al. 2024; Koutroubas et al. 2023). In our study, such an evaluation was performed at a critical stage coinciding with the peak of N demand (Bender et al. 2015; Gaspar et al. 2017b) and stabilization of nodules production in soybean (Gray et al. 2012). It was found that nodule number, nodule DM and average nodulation were severely affected

in P₂₀ and P₁₀₀ plants, most likely because a lower plant's N demand, as a response to P limitation in the rooting medium, induces a decreasing on nodule production (Divito and Sadras 2014). However, P-deficient plants exhibited shoot N content per nodule that was either higher or similar compared to the P-sufficient ones and there was no variation in the concentration of P and N in the nodules across treatments. These findings suggest that, in order to cope with the damaging effects of late P limitation on the process of N₂ fixation, soybean plants tend to optimize the allocation of P to the formed nodules for proper functioning (Suliman and Tran 2015; Valentine et al. 2017).

Finally, our results revealed also that the condition of post-flowering P deficiency impacted negatively the efficiency by which N is partitioned to the grains, as indicated by the decline in NHI at the physiological harvest. Nevertheless, this phenomenon was not translated into a reduced NUtE because a comparable decreasing occurred in grain N concentration (Manschadi et al. 2014; Rotundo et al. 2014). There is positive relationship between the partitioning of P and N toward reproductive tissues in grain crops (Ma et al. 2016), which explains the fact that the harvest indexes of these nutrients responded in parallel to P supply regimes. However, compared to N, the nutritional status of P of harvestable organs seems to be more sensitive to the effects of post-flowering P deficiency. For instance, in addition to the decline in P concentration of reproductive structures at R5.5, the reduction in grain P concentration was higher than that of N at R8 (32% versus 9%); moreover, P concentration in pod-shells decreased under P deficiency, but an opposite response occurred in the case of N. These contrasting outcomes contribute to elucidate the finding that while NUtE efficiency remained unaltered across nutrition treatments, PUtE was higher under P limitation despite the reduction in PHI. It is worth noting even though the decrease in grain concentration of P and N ameliorated yield penalty of soybean facing post-flowering P deficiency, such a phenomenon could further also affect seed quality and seedling vigor (Wilson et al. 2014; Rose et al. 2020).

5 Conclusions

Our study highlights the critical role of P availability during the reproductive phase on N nutrition relationships of soybean. While P deficiency during such a period of plant development does not affect the conversion of acquired N into yield formation, it severely harms the process of N₂ fixation. Accordingly, there is no evidence to support the hypothesis that impaired N utilization efficiency exacerbates the detrimental effects of hindered N₂ fixation on crop productivity under post-flowering P deficiency. Moreover,

the damages caused by low-P on N acquisition cannot be directly ascribed to poor nodule functioning, as evidenced by the lack of variation in the content of N₂ fixed per nodule and concentration of P and N in this organ. Then, the decreasing in nodule production along the reproductive phase of soybean due to diminished N requirement is a primary factor governing N₂ fixation potential. In this context, optimizing strategies to maximize N acquisition should involve approaches that ensure a proper supply of P during the reproductive phase of soybean. Also, our findings shed light on the need of addressing the complex interplay between P and N in soybean production systems for advances in crop nutrient management and agricultural sustainability.

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Author Contributions FA performed the experiments and analyzed the data. SA and FZ contributed to supervision, conceptualization, analyzing and interpretation of the data. All the authors contributed to writing, reviewing, editing and approved the final version of the manuscript.

Data Availability Data available on request from the authors.

Declarations

Competing Interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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