

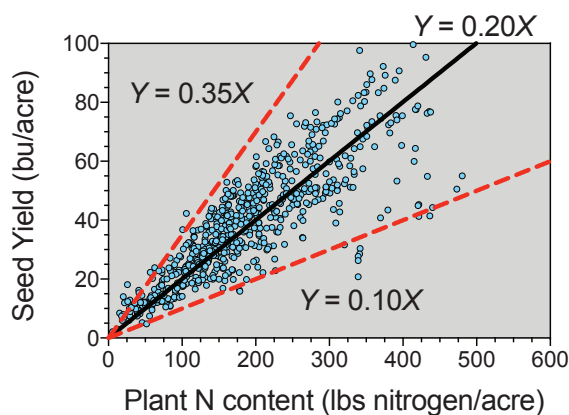
Legume crops offer many benefits to agricultural sustainability. Legumes capture atmospheric nitrogen through a symbiotic relationships with soil bacteria in a process called “biological nitrogen fixation.” This process reduces the reliance on synthetic nitrogen fertilizers.

Among legumes, soybeans are a major source of protein and oil. Soybeans are grown in a range of latitudes and environments. One of the challenges to improving soybean productivity is the high demand of nitrogen in comparison to cereals and oilseed crops.

Biological nitrogen fixation can be improved by breeding and selection that focuses on the plant, the nitrogen-fixing bacteria, and better matching plant and bacteria (Photo 1).

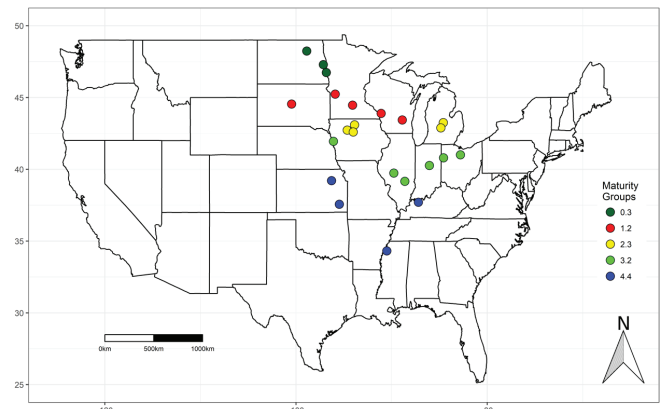
## Soybean Nitrogen Fixation and Yield

Soybean production depends heavily on the total nitrogen uptake supplied by the biological nitrogen fixation process. A strong connection has been documented between plant nitrogen demand and final yield for soybeans. For example, a soybean crop yielding 70 bushels per acre needs a total nitrogen uptake (above ground only) of approximately 320 pounds per acre (Figure 1). Plant nitrogen demand increases with yield at the rate of 100 pounds nitrogen per acre for each 20 bushels per acre. Approximately 50% of the total nitrogen uptake is coming after full-pod stage (R4 stage), increasing the nitrogen need of the crop during the later crop growth stages. From the end-season nitrogen uptake, on average 50-60% of this amount is met by biological nitrogen fixation.

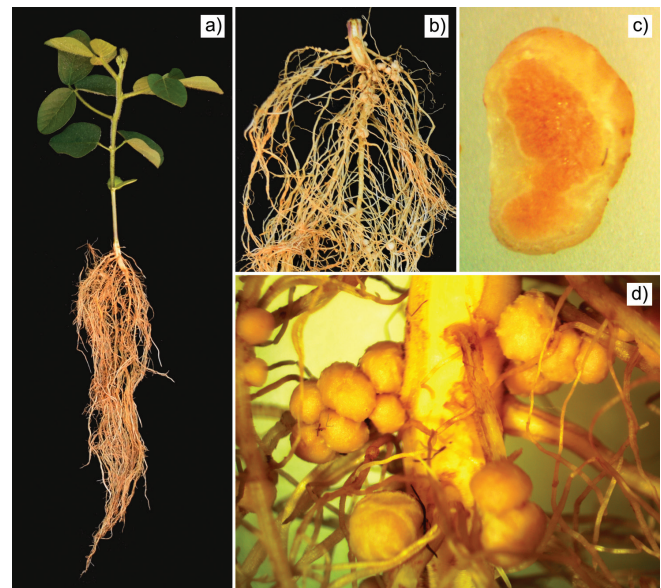


**Figure 1.** Relationship between seed yield and plant nitrogen content at maturity for soybean plant.

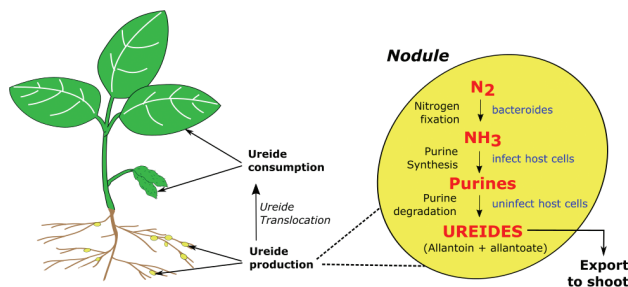
The primary nitrogen sources are: 1) biological nitrogen fixation and 2) inorganic nitrogen coming from the soil reservoir. The nitrogen fixed is assimilated and exported in the xylem as three nitrogen sources: 1) ureides (allantoin and allantoic acids), 2) amino-nitrogen, and 3) nitrate-nitrogen. As nodulation activity increases, the ureide content of the xylem



**Figure 2.** Map of the United States referencing all the experimental locations. Colors represent different soybean maturity groups (Tamagno et al., 2018, Scientific Reports Journal).



**Photo 1.** a) Soybean plant at V3 (three-leaf) stage with 43 centimeter total length, growing without inorganic nitrogen supply under greenhouse conditions. The seed was inoculated with an inoculant containing a minimum ( $3.0 \times 10^9$  colony forming unit  $mL^{-1}$ ) of *Bradyrhizobium japonicum* strain. b) and d) showing roots (and nodules). c) shows the nodule starting its activity, based on its internal coloration.



**Figure 3.** Ureide formation in the nodule and export to soybean shoots.



**Figure 4.** Theoretical description, based on data from this study, of the seasonal change of biological nitrogen fixation for soybeans, from planting until harvest (Tamagno et al., 2018, *Scientific Reports Journal*).

increases (Tajima and Yamamoto, 1975). Thus, the ureide concentration in the stem organ represents an indirect measurement of the nitrogen fixation activity for nodulated crops.

There are different methods to measure nitrogen fixation, each one presenting different complexity and limitations. Among the most popular and well-documented ones are: 1) nitrogen difference method, 2) natural abundance, 3) isotopic dilution, and 4) ureides. The natural abundance method represents a direct measure of biological nitrogen fixation, but it is expensive and requires previous calibration in a greenhouse and

the presence of a non-fixing crop (check plant). The ureide method is an indirect and point-measurement of biological nitrogen fixation, it is less labor intensive and expensive but needs calibration with isotopic labeled method ( $^{15}\text{N}$ , for example natural abundance).

### Study Description

In collaboration with WinField United this study established trials in 23 locations across the Midwest in the 2016 growing season (Figure 2). This study investigated the effect of nitrogen fertilizer application on biological nitrogen fixation and its implications for soybean productivity and protein concentration.

The study characterized the seasonal dynamics of biological nitrogen fixation (via ureide method, Figure 3) and its consequences for soybean productivity with an emphasis on growth and shoot biomass allocation mechanisms.

### Result

Application of nitrogen at different growth stages affected neither yield nor protein concentration. Overall, the biological nitrogen fixation process increased until the beginning of seed formation and then decreased until harvest (Figure 4).

Nitrogen fertilizer reduced the peak (up to 16%) of biological nitrogen fixation. Biomass allocation to seeds was reduced with increasing biological nitrogen fixation.

### Conclusions

Application of nitrogen fertilizer at different growth stages affect neither yield nor protein, but reduced the peak (up to 16%) of biological nitrogen fixation. This study highlights the importance of improving biological nitrogen fixation by breeding and selection targeting the plant, the nitrogen-fixing bacteria, and better matching plant and bacteria. This is particularly relevant in areas of the US Midwest where selection for maintenance of biological nitrogen fixation in dry soils is needed to improve soybean productivity under drought and heat stress conditions.

Based on: Tamagno, S., Sadras, V. O., Haegele, J. W., Armstrong, P. R., & Ciampitti, I. A. (2018). Interplay between nitrogen fertilizer and biological nitrogen fixation in soybean: implications on seed yield and biomass allocation. *Scientific Reports*, 8(1), 17502. <https://doi.org/10.1038/s41598-018-35672-1>

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