Nitrogen

Article

Effects of Nitrogen Fertilization on Weed Flora and Productivity of Soybean [*Glycine max* (L.) Merr.] Crop

Ioanna Kakabouki [1,*], Antonios Mavroeidis [1], Varvara Kouneli [1], Stella Karydogianni [1], Antigolena Folina [1], Vassilios Triantafyllidis [2], Aspasia Efthimiadou [3], Ioannis Roussis [1], Anastasios Zotos [4], Chariklia Kosma [2] and Nikolaos Katsenios [3]

[1] Laboratory of Agronomy, Department of Crop Science, Agricultural University of Athens, 11855 Athens, Greece; p1152016@aua.gr (A.M.); stud113072@aua.gr (V.K.); karidogianni@aua.gr (S.K.); folina@aua.gr (A.F.); roussis@aua.gr (I.R.)
[2] Department of Business Administration of Food and Agricultural Enterprises, University of Patras, 30100 Agrinio, Greece; vtrianta@upatras.gr (V.T.); xkosma@upatras.gr (C.K.)
[3] Department of Soil Science of Athens, Institute of Soil and Water Resources, Hellenic Agricultural Organization—Demeter, 14123 Athens, Greece; sissyefthimiadou@gmail.com (A.E.); katsenios@aua.gr (N.K.)
[4] Department of Biosystems and Agricultural Engineering, University of Patras, 30200 Mesolonghi, Greece; azotos@upatras.gr
* Correspondence: i.kakabouki@aua.gr

Abstract: The literature suggests that nitrogen (N) fertilization increases yield in soybean. This study aimed to investigate the effects of N fertilization on: (i) The performance of soybean, and (ii) the weed flora. A two-year field experiment was carried out in Agrinio, Western Greece. The experiment was set up in a randomized complete block design, with four organic fertilizer treatments and six replications. The four treatments included 0 kg N ha^{-1} (N0/unfertilized control) and the application of 80 kg N ha^{-1}, 100 kg N ha^{-1}, and 120 kg N ha^{-1}. The application of 120 N kg ha^{-1} resulted in the most notable increment of plant height (22.6–24%), biomass (10–13%), LAI values (14–17%), and yield (10–12%) compared to the N0. Compared to the N0, total weed biomass was increased by 26–32%, 34–49%, and 55–57% in N80, N100, and N120, respectively. The values of the H (Shannon), Dmg (Margalef), and J (Pielou) indices were unaffected by the fertilization, hence they did not affect weed biodiversity. CRI (crop resistance index), on the contrary, was negatively affected by N fertilization and was significantly reduced. Overall, our results indicate that the application of 80 kg N ha^{-1} is more efficient, can effectively improve the soybean performance, and enhance its yield.

Keywords: soybean; nitrogen fertilization; weed indices; weed biodiversity

check for updates

Citation: Kakabouki, I.; Mavroeidis, A.; Kouneli, V.; Karydogianni, S.; Folina, A.; Triantafyllidis, V.; Efthimiadou, A.; Roussis, I.; Zotos, A.; Kosma, C.; et al. Effects of Nitrogen Fertilization on Weed Flora and Productivity of Soybean [*Glycine max* (L.) Merr.] Crop. *Nitrogen* **2022**, *3*, 284–297. https://doi.org/10.3390/nitrogen3020019

Academic Editor: Marouane Baslam

Received: 9 March 2022
Accepted: 6 May 2022
Published: 10 May 2022

1. Introduction

Soybean [*Glycine max* (L.) Merr.] is one of the most important grain crops worldwide [1], with multiple uses ranging from biofuel production to human consumption [2,3]. Due to its high nutritional value (360 kg of protein per metric ton of soybean seed), soybean global consumption and demand is constantly growing [4]. Aside from its high nutritional value, the importance of soybean lies behind its ability to grow on low-N soils [5]. This is possible due to the process known as biological nitrogen fixation (BNF). Throughout this process, rhizobacteria of the *Rhizobium* genus colonize the surface of the roots and form nodules. Within these nodules, the bacteria convert the atmospheric N (N_2) to ammonia (NH_3) or related nitrogenous compounds, which can be assimilated by the plants [6–8]. N fixation is amplified during R3–R5 and is significantly reduced during the R5–R7 growth stages of soybean [9]. This reduction can result to N deficiency during seed filling and consequently to lower seed yield.

Inoculation with beneficial microorganisms could balance these fluctuations in N availability. These microorganisms are usually rhizobacteria [10]. Soybean inoculation with *Bradyrhizobium* spp. is considered as a novel biotechnological tool that improves the yield and the sustainability of the crop [11]. However, according to estimations by Leggett et al. [12], inoculation of soybean with *Bradyrhizobium japonicum* increases yield only by 1.67% in conventional cropping systems. In 2013, Ray et al. [13] predicted that a global food shortage was expected by 2050, thus the need to significantly increase the yield of crops such as soybean is evident. An agronomic practice that could ensure nitrogen availability for soybean and, thus, greater yields is the application of optimized N fertilization [14,15]. Even though it is widely accepted that N fertilization does not particularly benefit legumes [16], several studies have suggested that it can increase the yield in soybean [17,18]. Furthermore, according to Salvagiotti et al. [19], N fixation can meet only 50–60% of the soybeans' N demand. However, Khaledian et al. [20] reported that although N fertilization enhanced soybean performance, fertilization rates between 25 and 100 kg ha^{-1} might have a negative impact on the number of nodules, and therefore N fixation. Tamagno et al. [21] expressed the same concern in their study. As a result, an important research query is whether N fertilizers can moderate N limitations in favor of achieving greater seed yield values without compromising the N fixation capacity of the crop [22].

Fertilization is not the sole factor that affects soybean yield. Weeds pose a major threat to soybean. It has been estimated that weed infestation can reduce soybean global production up to 37% [23]. Weed competition is crucial during the vegetative growth stages V2 and V4 and can result in yield losses up to 10%, a quite unacceptable yield reduction from an economic perspective [24–27]. In addition, the presence of weeds during harvest reduces the seed quality and hinders the process [28]. Studies have demonstrated that N fertilization enhances the presence of weeds in the field. For instance, Sweeney et al. [29] reported that N fertilization increased weed biomass. Kakabouki et al. [30] observed a significant increase in total weed density and biomass under the application of either organic or inorganic fertilization.

Weed indices can facilitate the assessment of weed-induced yield losses. For this assessment, weed flora composition, weed density, and weed competition should be considered [31]. Indices such as the crop resistance index (CRI) [32] can be used for this evaluation. The CRI is a ratio of the crop and weed biomass and represents the crops' ability to withstand competition for nutrients [33]. Weed flora composition and the competition amongst weed species can be evaluated by the H-Shannon–Weiner diversity index [34], the (J) Pielou index [35], and the Margalef diversity index (Dmg) [36]. These indices are used as a measure of diversity in ecology and can describe the biodiversity status of the weed flora. Recent studies have highlighted the importance of introducing such indices into integrated weed management [37,38].

High demand and land limitations force us to emphasize the increasing soybean yield per unit area [5,39]. Although research is being conducted regarding the improvement in BNF [5], N fertilization seems to be a sufficient temporary solution. However, the interaction between fertilization, weed flora, and crop performance is complex. The objective of this study was to evaluate the effects of three different nitrogen fertilization rates on: (i) Weed presence on soybean fields and (ii) soybean yield and performance. In order to perform this evaluation, all of the aforementioned indices were utilized within the context of this evaluation.

2. Materials and Methods

2.1. Study Area

A field experiment was conducted in 2018 (first year), and then repeated in 2019 (second year), in Agrinio, Western Greece (38°35′18″ N, 21°25′40″ E). The soil properties of the experimental field are presented in Table 1. The mean temperature and total precipitation recorded during the experiment are presented in Figure 1. Even though maize (*Zea mays* L.) has been cultivated ever since 2015, soybean has also been regularly cultivated in this field, so the seeds were not inoculated [40].

Table 1. Soil properties of the experimental field.

Soil Type	Clay Loam
Clay	29.3%
Silt	34.9%
Sand	35.8%
pH (1:1 H_2O)	7.38
Organic matter	3.11%
$CaCO_3$	13.4%
Total Mineral Nitrogen	0.156%
Phosphorus–P Olsen	178 mg kg^{-1} soil
Potassium	625 mg kg^{-1} soil

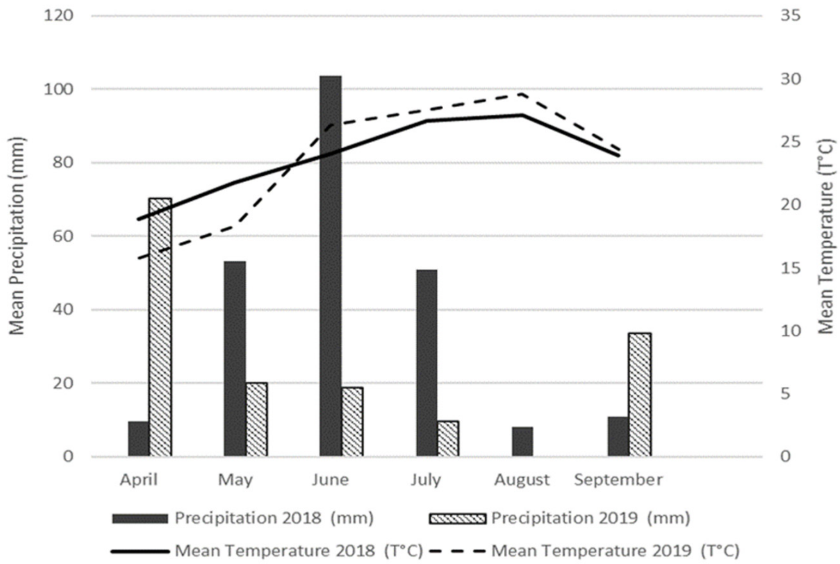

Figure 1. Meteorological data, mean air temperature (°C), and precipitation (mm) during the experimental periods (April–September) for the years 2018–2019.

The literature regarding the effects of herbicide application on the nodulation of soybean is not conclusive. Even though nodulation seems unaffected by the application of herbicides, several studies have reported that they reduced the number or size of soybean nodules [41–44]. One of the aims of the present study was to evaluate the interaction between fertilization, weed flora, and nodulation. Concurrently, the literature regarding the interplay between the nodulation of the cultivar used in our study (PR92B63) and post- or pre-emergence herbicide application is inadequate, thus chemical weed management was avoided in the present study. Instead, three weeks prior to the sowing (each year), the stale seed bed technique was applied to the field. Three light irrigations were performed (once per week) in order to stimulate weed emergence. The emerged weed flora was controlled by moldboard ploughing during the seedbed preparation. The ploughing was carried out at a 25 cm depth on 27 April and 28 April in 2018 and 2019, respectively. Seeds of the PR92B63 cultivar were sown by hand on 4 May and 6 May for the first and second experimental year, respectively. The sowing rate was 300,000 seeds ha^{-1} and the row spacing was 38 cm (8 rows per plot). The experiment was set in a randomized complete block design (RCBD) with four treatments and six replications. In each treatment, different rates of N fertilization were broadcasted (following the ploughing) in a single application, according to the recommendations of the Soybean Nutrient Management guidelines by the University of Minnesota [45]. The applied inorganic fertilizer's trademark is NUTRIPLUS (N–P–K: 21–0–0) by Phytothreptiki S.A. (Ano Liosia, Attika, Greece). The treatments consisted of N0 (0 kg N ha^{-1}/control), N80 (80 kg N ha^{-1} were applied), N100 (100 kg N ha^{-1} were applied), and N120 (120 kg N ha^{-1} were applied). Each plot was 3 m wide and 4 m long. Depending on the precipitation, irrigation was performed with a drip system every 8–10 days (when necessary), which provided 400 mm of water to the crop over the growing season [46,47]. The crop was harvested by hand on 17 September 2018 and 15 September 2019 when the seeds reached full maturity (seed moisture was approximately at 13%).

2.2. Measurements

For the estimation of leaf area index (LAI) values, a Sun-Scan Delta-T device (Delta-T Devices Ltd., Cambridge, UK) was used at 50 days after sowing (DAS) (R1 growth stage). On the day of the LAI measurement, one row was randomly selected in each plot and hand weeded. On these rows (weed-free rows), five LAI measurements per plot were taken at the height of the crop. Twenty days following the LAI measurement (70 DAS), 10 plant samples per plot were collected from the weed-free rows in order to record the number of nodules per plant. Plant sampling was performed with shovels in order to keep the root system of the plants intact [48]. The roots were then separated from the rest of the plant, washed with water, and the number of nodules was recorded. The height, biomass, and yield of soybean were measured on the day of harvest. The selection of plant-samples for the assessment of the plant height and biomass and the yield was conducted by randomly placing six 50 cm × 50 cm (0.25 m^2) quadrats in each plot. The quadrats were placed on the remaining rows (the weed-free rows were excluded). The soybean plants within the quadrats were clipped at ground level. Once the samples were gathered, the seeds were extracted and weighed, the height of the plants was recorded, and then the samples were dried for 72 h at 80 °C in order to measure the dry weight of the above-ground plant tissues.

Following the sowing, the experimental field was inspected weekly in order to keep track of the different weed species. Throughout the experiment, the major weed species that were recorded included goosefoot (*Chenopodium album* L.), red-root amaranth (*Amaranthus retroflexus* L.), cockspur (*Echinochloa crus-galli* (L.) Beauv.), and black nightshade (*Solanum nigrum* L.). On the day of the harvest, once the soybean plants were collected, the number of weeds within the quadrats was recorded and thus the weed density of each plot was estimated. These weeds were then collected and dried for 72 h at 80 °C. During this process, the root system of the weeds was disposed. Once the samples were completely dried, their biomass were recorded. These data (number of different weed species, weed density, weed biomass, and soybean biomass) were utilized for the assessment of the weed presence, biodiversity, and competitiveness according to the equations of Table 2.

Table 2. Weed indices.

Index Name	Equation	References
Crop Resistance Index	$\mathrm{CRI} = \left(\frac{\mathrm{Wn}}{\mathrm{Wcontrol}}\right) * \left(\frac{\mathrm{Kcontrol}}{\mathrm{Kn}}\right)$ [1]	[32]
H (Shannon) index	$\mathrm{H} = -\sum_{i=1}^{S} \mathrm{pi} * (\ln(\mathrm{pi}))$ [2]	[34]
J (Pielou) index	$\mathrm{J} = \frac{\mathrm{H}}{\mathrm{Hmax}}$ [3]	[35]
Margalef diversity index	$\mathrm{Dmg} = \frac{(\mathrm{S}-1)}{\ln(\mathrm{N})}$ [4]	[36]

[1] n: Treatment; W: Crop biomass; K: Weed biomass; [2] pi: The proportion of individuals belonging to the ith species; S: The total number of species; [3] H: The number derived from the Shannon diversity index; Hmax: The maximum possible value of H; [4] S: The number of species; N: The total number of individuals in the sample.

2.3. Statistical Analysis

All data were subjected to the Shapiro–Wilk normality test. The STATISTICA 11 (Stat Soft, Tulsa, OK, USA) logistic package was used for the analysis of variance (ANOVA). The ANOVA used a mixed model, with years and replications as random effects and N fertilization as the fixed effect. Data that did not follow normal distribution were subjected to the Kruskal–Wallis test for non-parametric one-way ANOVA. The effects of N fertilization on soybean growth traits and on the weed flora were tested at a $p = 0.05$ significance level. Significant differences amongst the treatments were determined using

Fisher's least significant difference test (LSD) at the 5% level of probability ($p < 0.05$). Simple regression analysis was carried out at the $p = 0.05$ significance level to estimate the correlation levels between the studied variables.

3. Results

3.1. Soybean Growth Traits and Yield

The results of the two-year field experiment indicate that N fertilization positively affected both the growth traits and the yield of soybean as it significantly increased the plant height, the leaf area index (LAI) values, the dry weight of the above-ground plant tissues, and the seed yield per ha. In contrast, the number of nodules was not affected by fertilization (Table 3).

Table 3. Agronomic characteristics of soybean as affected by the different N fertilization rates.

	LAI	Height (cm)	Plant Biomass (kg ha^{-1})	Yield (kg ha^{-1})	Number of Nodules per Plant
			Year A		
Control	4.81 ^{a}	42.83 ^{a}	3863 ^{a}	4095 ^{a}	6.2 ^{a}
N80	5.09 ^{b}	46.17 ^{ab}	4021 ^{ab}	4225 ^{ab}	5.8 ^{a}
N100	5.21 ^{bc}	49.33 ^{b}	4141 ^{bc}	4327 ^{bc}	5.5 ^{a}
N120	5.62 ^{c}	53.17 ^{c}	4272 ^{c}	4441 ^{c}	5.3 ^{a}
Fertilization (F)	**	***	***	**	ns
			Year B		
Control	4.84 ^{a}	44.33 ^{a}	3754 ^{a}	4066 ^{a}	7.5 ^{a}
N80	5.02 ^{ab}	48.50 ^{b}	3962 ^{b}	4208 ^{ab}	7.0 ^{a}
N100	5.33 ^{bc}	52.50 ^{c}	4135 ^{bc}	4343 ^{bc}	6.5 ^{a}
N120	5.54 ^{c}	54.33 ^{c}	4258 ^{c}	4462 ^{c}	6.3 ^{a}
Fertilization (F)	***	***	**	***	ns
			Overall effects		
Fertilization (F)	***	***	***	***	ns
Year (Y)	ns	**	ns	ns	***
F × Y	ns	ns	ns	ns	ns

F-test ratios from ANOVA. Different letters (a, b, and c) within a column and growing season indicate significant differences according to the LSD test. Significance levels: ** $p < 0.01$; *** $p < 0.001$; ns, not significant ($p > 0.05$).

The application of 120 kg N ha^{-1} (N120) resulted in the most noteworthy enhancement of the soybeans' performance and yield. Plant height in N120 was increased by 24% during 2018 and by 22.6% during 2019 compared to the control (Table 2). Even though N80 and N100 increased (on average) plant height by 8 and 16.5%, respectively, the differences between N80 and the control during 2018 and between N100 and N120 during 2019 were statistically insignificant. Notably, the average plant height in all of the treatments was greater during the second experimental year. The N120 treatment also resulted in the greatest LAI values during both years (Table 2). In particular, LAI was increased by 17 and 14% during 2018 and 2019, respectively. Although N80 and N100 increased the LAI values compared to the control, the differences between them were not statistically significant. Additionally, the differences in above-ground plant biomass between the N80 and N100 treatments were also insignificant for both years (Table 2). Nevertheless, when compared to the control, N80 and N100 increased the above-ground biomass by 4–10%. The greatest biomass increment was reported in N120 for both years (by 11% during 2018 and 13% during 2019). Regarding the yield, a positive correlation was observed between the fertilization rates and the seed yield. Even though the yield increased with higher N fertilization rates, the differences between the control and N80, between the N80 and the N100, and between the N100 and N120 were statistically insignificant (Table 2).

Overall, N120 led to the highest yield as it was increased by 8 and 10% during 2018 and 2019, respectively.

3.2. Weed Flora and Weed Indices

Weed flora observations revealed that the weed density was not significantly affected by fertilization. The total density of the weeds was measured at 11–14 weeds per m^2 during both 2018 and 2019. Similarly, the number of different weed species per m^2 was also unaffected by fertilization (Table 4). Regarding the total weed biomass in each treatment, measurements during both years revealed that the differences between the N0 and the N80 treatments were statistically insignificant (Table 4). Similarly to the biomass accumulation of soybeans, N120 reported the highest total weed biomass. In particular, N120 increased the weed biomass by 55% during 2018 and by 58% during 2019 (compared to the control). However, the differences between N80, N100, and N120 were not significant. Out of the four indices used in the present study to evaluate the effect of N fertilization on weeds, the H index, the Dmg index, and the J index did not note any statistically significant differences amongst treatments (Table 5). The values of the CRI decreased as the fertilization rate increased. The application of N80 decreased the CRI values by 16% during 2018 and by 18% during 2019. The differences between N0 and N80, and between N100 and N120, were statistically insignificant. The most notable decrease in the CRI values was reported in N120 where they were almost halved (Table 5). In addition, the average density (plants per m^2) and biomass (g m^{-2}) per weed species is presented in Table 6. Amongst the weed species observed in the field, *A. retroflexus* reported the most notable and significant fertilization induced biomass accumulation.

Table 4. Weed density, biomass, and number of different weed species as affected by the different N fertilization rates.

	Weed Density (m^{-2})	**Total Weed Biomass (kg ha^{-1})**	**Number of Weed Species**
	Year A		
Control	7.67^{a}	411.7^{a}	4.17^{a}
N80	9.50^{b}	470.1^{ab}	3.33^{a}
N100	11.01^{bc}	528.3^{b}	4.01^{a}
N120	14.03^{c}	605.2^{c}	4.50^{a}
Fertilization (F)	**	***	ns
	Year B		
Control	9.67^{a}	416.7^{a}	4.33^{a}
N80	10.83^{a}	478.3^{a}	4.17^{a}
N100	13.04^{ab}	531.7^{b}	4.13^{a}
N120	14.33^{b}	615.0^{b}	4.50^{a}
Fertilization (F)	***	***	ns
	Overall effects		
Fertilization (F)	***	***	ns
Year (Y)	ns	ns	ns
(F) × (Y)	ns	ns	ns

F-test ratios from ANOVA. Different letters (a, b, and c) within a column and growing season indicate significant differences according to the LSD test. Significance levels: ** $p < 0.01$; *** $p < 0.001$; ns, not significant ($p > 0.05$).

Table 5. Weed indices as affected by the different N fertilization rates.

	Crop Resistance Index (CRI)	H (Shannon) Index	Dmg (Margalef) Index	J (Pielou) Index
		Year A		
Control	1.00 [a]	1.21 [a]	3.78 [a]	2.05 [a]
N80	0.91 [a]	1.14 [a]	4.24 [a]	2.21 [a]
N100	0.84 [ab]	1.16 [a]	3.75 [a]	1.98 [a]
N120	0.76 [b]	1.30 [a]	3.36 [a]	2.01 [a]
Fertilization (F)	**	ns	ns	ns
		Year B		
Control	1.00 [a]	1.16 [a]	3.72 [a]	1.91 [a]
N80	0.91 [a]	1.08 [a]	3.70 [a]	1.84 [a]
N100	0.86 [ab]	1.13 [a]	3.70 [a]	1.89 [a]
N120	0.77 [b]	1.15 [a]	3.36 [a]	1.78 [a]
Fertilization (F)	***	ns	ns	ns
		Overall effects		
Fertilization (F)	***	ns	ns	ns
Year (Y)	ns	ns	ns	ns
(F) × (Y)	ns	ns	ns	ns

F-test ratios from ANOVA. Different letters (a and b) within a column and growing season indicate significant differences according to the LSD test. Significance levels: ** $p < 0.01$; *** $p < 0.001$; ns, not significant ($p > 0.05$).

Table 6. The average density (plants m^{-2}) and biomass (g m^{-2}) of each major weed species per treatment.

	A. retroflexus		*C. album*		*S. nigrum*		*E. crus-galli*	
				Year A				
	Density	Biomass	Density	Biomass	Density	Biomass	Density	Biomass
N0	5.33 ± 1.09 [a]	70.50 ± 15.71 [a]	3.50 ± 0.66 [a]	56.73 ± 9.21 [a]	1.33 ± 0.61 [a]	26.57 ± 13.18 [a]	1.67 ± 0.42 [a]	14.47 ± 3.88 [a]
N80	6.00 ± 1.17 [a]	103.84 ± 21.54 [ab]	3.50 ± 0.43 [a]	70.16 ± 15.52 [a]	1.67 ± 0.67 [a]	45.22 ± 17.18 [a]	0.67 ± 0.33 [a]	7.78 ± 0.55 [a]
N100	6.00 ± 1.22 [a]	106.45 ± 21.86 [ab]	3.33 ± 0.70 [a]	81.86 ± 7.81 [a]	1.50 ± 0.56 [a]	45.03 ± 17.81 [a]	0.83 ± 0.48 [a]	9.47 ± 0.49 [a]
N120	6.83 ± 1.66 [a]	138.47 ± 29.62 [b]	2.83 ± 0.95 [a]	83.69 ± 8.21 [a]	2.83 ± 1.05 [a]	60.42 ± 13.16 [a]	1.67 ± 0.67 [a]	17.25 ± 6.25 [a]
				Year B				
	Density	Biomass	Density	Biomass	Density	Biomass	Density	Biomass
N0	5.83 ± 1.38 [a]	83.14 ± 21.44 [a]	2.67 ± 0.21 [a]	38.41 ± 3.35 [a]	1.67 ± 0.56 [a]	36.92 ± 13.28 [a]	1.50 ± 0.67 [a]	15.75 ± 7.33 [a]
N80	5.83 ± 1.58 [a]	106.20 ± 25.28 [a]	2.83 ± 0.31 [a]	48.48 ± 3.67 [a]	2.33 ± 1.12 [a]	53.13 ± 2.65 [a]	1.17 ± 0.65 [a]	12.67 ± 7.18 [a]
N100	8.17 ± 0.95 [a]	157.23 ± 18.52 [a]	2.00 ± 0.45 [a]	51.90 ± 6.56 [a]	1.33 ± 0.61 [a]	36.92 ± 16.81 [a]	2.00 ± 0.52 [a]	22.58 ± 6.39 [a]
N120	10.33 ± 1.43 [a]	175.04 ± 21.79 [b]	2.17 ± 0.17 [a]	54.89 ± 4.57 [a]	1.17 ± 0.65 [a]	47.32 ± 7.14 [a]	1.67 ± 0.76 [a]	17.38 ± 6.03 [a]

Different letters (a and b) within a column and growing season indicate significant differences according to Fisher's or Kruskal–Wallis test at a 0.05 significance level.

4. Discussion

The results of the present study revealed that the agronomic traits of soybean (height, LAI, biomass) were positively affected by the application of N fertilization (Table 3). A fertilization-induced increase in plant height has also been reported by Zhang et al. [48] in a pot experiment regarding soybean. In their experiment, they provided 0.1–0.7 additional g of N per kg of pot soil and observed an increment of 18–35% in plant height. Nevertheless, as pot experiments provide controlled conditions and exclude the potential competition for resources among plants (e.g., between the crop and the weeds), any further comparison between our results and the results of Zhang et al. [48] could be misleading. In another study by Popović et al. [49], the application of 150 kg N ha^{-1} in the form of calcium ammonium nitrate increased soybean plant height by 11%. In contrast to these findings, our results suggest that 120 kg N ha^{-1} increases plant height by 22–24%. It should be noted though that the experiment of Popović et al. [49] was performed under arid conditions. As drought stress is known to inhibit soybean plant growth [50], perhaps the arid conditions were responsible for the deviation in our results. Overall, the positive correlation between N fertilization and plant height was anticipated as the literature suggests that N may regulate stem elongation either via a N-signaling pathway or due to its accumulation on the shoot apical meristem [51].

Aside from plant height, augmented LAI values were also anticipated, as several studies have suggested that N fertilization increases the values of this index. According to Prahraj [52], application of 30 kg N ha^{-1} during the early flowering stage significantly increased the LAI values in soybean. Caliskan et al. [53] stated in 2008 that the application of 120 kg N ha^{-1} increased the LAI values by 10–20%, similar to our findings. The findings of Kakabouki et al. [54] are also in agreement with our results, as they reported that application of 120 kg N ha^{-1} in soybean cultivated under conventional tillage system increased LAI values by 11–16%. After all, N is fundamental for cell elongation and cell multiplication [55]. Perhaps the increased N accumulation due to fertilization in the leaves of the plants catalyzed these biological processes and resulted in wider leaves and thus better canopy and increased LAI values. Notably, according to Tagliapietra et al. [56], optimum LAI values at the R1 growth stage in soybean should range between 3.6 and 4.5 in order to achieve the full yield potential. Even though LAI values were significantly higher in our study, this contrast between our findings and the findings of Tagliapietra et al. [56] could be attributed to increased N availability, different soybean cultivars, or to environmental factors (the study of Tagliapietra et al. was conducted under subtropical conditions).

In the same study by Kakabouki et al. [54], N fertilization also increased the plant biomass. In particular, the application of 120 kg N ha^{-1} increased plant biomass by 12%, which was similar to our results. This augmented plant biomass can be attributed to a surplus of photosynthates. According to Salvagiotti et al. [19], there is a negative exponential relationship between N fertilization and N fixation in soybean. Concurrently, it has been estimated that BNF requires 6–7 g C g^{-1} N while the assimilation of mineral N requires 4 g C g^{-1} N [21]. Therefore, as the fertilization rate rises, the plants' C requirements (in the form of photosynthates) are reduced, and the resulting photosynthate surplus could potentially be directed to biomass accumulation. This hypothesis would justify the positive correlation between fertilization and plant biomass noted in the present study (Table 7). Overall, enhanced leaf N concentration is believed to stimulate photosynthesis as N is essential for the synthesis of Rubisco and chlorophylls [57,58]. The correlation between the high N accumulation in the leaves and the increased photosynthetic rate of soybean can also be interpretated as a response to changes in the source/sink ratio of the plant. Photosynthetic rates are believed to be affected by the source/sink ratio of the plant [59,60]. The source, though, in the source/sink ratio is determined by the LAI [61]. Therefore, the aforementioned proposed interaction between N and the LAI could result in increased photosynthetic rates and thus in increased plant biomass.

The correlation between the biomass and the seed yield of soybean has been intensively discussed due to the conflicting reports in the literature [62]. Even though several studies have indicated that augmented plant biomass does not improve the yield [63–65], others have suggested a positive correlation between these two factors [66–68]. Nevertheless, N fertilization-induced increased yields have been reported by numerous researchers [69–72]. Jadhav et al. [73] and Khaledian et al. [20] stated that N fertilization increased the pods and the seeds per pod. According to Lorenc-Kozik and Pisulewska [74], fertilization rates of 30–60 kg N ha^{-1} could increase the seed yield by approximately 22–25%. In contrast, Prusiński et al. [75] claimed that the same fertilization rates did not contribute to a significant increase in the yield. The positive correlation between fertilization and yield was also observed in our study (Table 7). As above-mentioned, our findings suggest that the application of N fertilization in soybean results in robust and vigorous plants with higher photosynthetic rates. According to Buttery et al. [76], these increased photosynthetic rates could also justify the improved yield. In the same study, Buttery et al. also proposed that perhaps the leaves of certain soybean cultivars also have a greater photosynthate storage capacity that grants improved seed-filling and thus increased yields. This would explain why the conflicting findings regarding the fertilization of soybean as the response of different cultivars, with different photosynthetic potential and capacities, to N fertilization may vary vastly. Similar to our findings, the literature indicates that N fertilization increases yields of the PR92B63 variety by 7–9% at a rate of 120 kg N ha^{-1} [54]. Besides the increased photosynthetic rates, fertilization also regulates nutrient competition. According to Saitoh et al. [77], nutrient competition between flowers of the same plant, or between the vegetative organs and the flowers of the plant, is one of the major factors that cause flowers to fall, hence limiting the yield.

Table 7. Correlation matrix between the agronomic characteristics of soybean, the yield, the weed density and biomass, and the weed indices.

	Yield	No. of Nodules	H Index	Dmg Index	J Index	CRI	Weed Density	Total Weed Biomass
Soybean biomass	0.1724 ^{ns}	−0.2962 ^{ns}	0.3182 *	−0.1560 ^{ns}	0.2661 ^{ns}	−0.5028 ***	0.1847 ^{ns}	−0.2029 *
Yield		0.3431 *	0.1949 ^{ns}	0.0120 ^{ns}	0.1474 ^{ns}	−0.2624 ^{ns}	0.1735 ^{ns}	0.1548 ^{ns}
No. of nodules			0.1302 ^{ns}	0.0394 ^{ns}	−0.1276 ^{ns}	0.5030 ***	−0.0882 ^{ns}	−0.0556 ^{ns}
H index				−0.6225 ***	0.9644 ***	−0.2911 *	0.2926 *	0.3315 *
Dmg index					−0.6780 ***	0.0739 ^{ns}	0.4854 ***	0.3588 *
J index						−0.2368 ^{ns}	0.1420 ^{ns}	0.2005 ^{ns}
CRI							−0.2654 ^{ns}	−0.2546 *
Weed density								0.7946 ***

Significance levels: * $p < 0.05$; *** $p < 0.001$; ns, not significant ($p > 0.05$).

The results of the present study also suggest a positive correlation between the yield and the number of nodules per plant (Table 7). In contrast to the literature, N fertilization did not reduce nodulation. Several studies have indicated that mineral N inhibits nodulation and N fixation in soybean [78–80]. However, in a study by Abdel-Wahab and Abd-Alla [81], the application of N fertilization significantly increased the number of nodules per plant, but only when much lower fertilization rates (compared to ours) were applied (16–32 kg N ha^{-1}). Moreover, the average number of nodules per plant in their study was significantly higher than in our study (more than 10 times-fold) of 56 DAS. It should be mentioned though that in their study, Abdel-Wahab and Abd-Alla [81] used different soybean cultivars that were inoculated, and applied fertilization to 27 DAS. According to Lofton and Arnall [82], a soybean plant should contain 25–100 nodules. In a study by Căpățână et al. [83], soybean non-inoculated plants of the PR92B63 cultivar indeed contained approximately 22–29 nodules. In contrast, Vlachostergios et al. [46], in a study they conducted in 2021, reported that non-inoculated PR92B63 soybean plants did not form nodules. Nevertheless, it is safe to assume that in our study, soybean plants were not properly nodulated as the control (0N) plants also reported a low number of nodules.

As weeds pose a major threat to the yield [23,27], and fertilization reportedly promotes weed flora [84,85], the fertilization–weed interaction should always be considered when evaluating the effects of different fertilization rates on the productivity of a crop. According to our findings, and in conformity with the literature, weed density and biomass are positively correlated to N fertilization (Table 7). After all, the major weed species reported in our study (*A. retroflexus*, *C. album*, *S. nigrum*, and *E. crus-galli*) are all considered nitrophilous [86–89]. Mekdad et al. [90], while investigating the interaction between fertilization and weeds in another legume crop [Peanut (*Arachis hypogaea* L.)], noticed that providing 100 of additional kg N ha^{-1} increased the total weed biomass by approximately 40%. Even though in our study the respective weed biomass increment was estimated at approximately 30%, this inconsistency between our findings and the findings of Mekdad et al. [90] could be attributed to the different weed flora. Nevertheless, the results of the present study further validate that N fertilization promotes weed growth. Unsurprisingly, both the weed density and the total weed biomass were dependent on the number of different weed species (Table 7); however, throughout the duration of our study, the effects of fertilization on the number of weed species was insignificant (Table 4).

From an agroecological point of view, weed biodiversity is important for the functions of an ecosystem and thus should not be neglected [85]. Even though weeds compromise the yield, they are an important resource for insects and birds as they are an integral part of an ecosystem's food web [85,91,92]. Interfering with weed biodiversity may affect these (and many more) taxa as weeds provide food, shelter, and mating sites [93,94]. Moreover, the literature indicates that species diversity could increase yield stability and agricultural sustainability [95,96]. Agricultural practices should focus on reducing the adverse effects of weed competition instead of disturbing the agroecological equilibrium. The aim of our study was to perform an integrated evaluation of N fertilization in soybean and therefore, contemplate the agroecological consequences of fertilization. In order to thoroughly examine this aspect, a simple record of the number of species was not enough. The diversity of weed species can be preferably described by the H (Shannon), Dmg (Margalef), and J (Pielou) indices [37]. These indices describe the richness and evenness of weed species within a community [37]. In contrast to the findings of Pyšek and Lepš [97] and Inouye and Tilman [98] that stated that high rates of N fertilization decreased weed biodiversity, our results suggest that fertilization did not affect the weed species richness. Although the Dmg was negatively affected by fertilization, the values of the index recorded statistically insignificant differences between the treatments, much like the J and H indices (Table 5). This could also be interpretated as low competition amongst these specific weed species.

Regarding the competition between the weeds and the crop, a simple yet efficient method to evaluate the effect of the weed flora on the yield is the use of the CRI. The CRI, as above-mentioned, describes the ability of the crop to withstand weed competition [32], and is therefore negatively correlated to weed density (Table 7). In our study, CRI values reduced as the fertilization rates increased, implying a negative correlation between these two factors (Table 5). Despite this negative correlation, the CRI values were significantly reduced only in the N120 treatment and not in the N100 (Table 5). Notably, the yield differences between the N120 and the N100 treatments were insignificant (Table 3). It is safe to assume that in the N120 application, a significant portion of the additional 20 kg N ha^{-1} (compared to N100) was assimilated by the weeds as the resistance of the crop to nutrient competition was reduced, and the yield of soybean as well as its agronomic traits were insignificantly improved. This hypothesis can be further validated by the significantly increased weed biomass recorded in the N120 treatment during 2018 (Table 4). Use of N indices (e.g., nitrogen use efficiency, nitrogen harvest index, etc.) and estimation of the N content of the weeds could provide conclusive results in the future. Based on the results of this study, the application of 120 kg N ha^{-1} is probably less efficient than the application of 100 kg N ha^{-1}.

The necessity of N fertilization in soybean is a rather controversial topic [98]. According to the literature, the high N demand of soybean cannot be fully met solely by N fixation [19]. Even though research regarding the optimization of N fixation has been conducted over the last decades [5,99], the rising global demand [100] calls for an immediate answer. Our results suggest that N fertilization could be viable solution to this problem. Nevertheless, further research should be conducted in order to maximize the efficiency of N fertilization in soybean (e.g., the use of slow release fertilizers).

5. Conclusions

The application of N fertilization in soybean improved both the performance of the crop and the yield. The height, biomass, LAI values, and the yield of soybean were positively affected by N fertilization. As N fertilization also increased the weed biomass, the CRI values suggest that increased fertilization rates partially promote weed flora. Concurrently, weed flora biodiversity remained unaffected even at the higher fertilization rate (N120). Out of the three fertilization rates that were evaluated in this study, the application of 100 kg N ha^{-1} seemed to be the most efficient. Further research should be conducted in order to conclusively validate our results, especially regarding the N uptake efficiency of the crop.

Author Contributions: Conceptualization, I.K.; Methodology, I.K., A.M., V.K., S.K., A.F., V.T., A.E., I.R., A.Z., C.K. and N.K.; Validation, I.K., V.T., A.E., A.Z., C.K. and N.K.; Formal Analysis, I.K., A.M., V.K., S.K., I.R. and A.E.; Investigation, I.K., V.K., S.K., V.T., C.K., A.Z., N.K. and I.R.; Resources, I.K.; Writing—Original Draft Preparation, I.K., A.M., V.K., S.K., A.F., V.T., A.E., I.R., A.Z., C.K. and N.K.; Writing—Review and Editing, I.K. and V.K.; Supervision, I.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: We thank Ilias Travlos (Agricultural University of Athens) for his comments on the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

N: Nitrogen; LAI, leaf area index; CRI, crop resistance index; BNF, biological nitrogen fixation; DAS, days after the sowing; C, carbon; H, H-Shannon–Weiner diversity index; J, J Pielou index; Dmg, Margalef diversity index.

References

1. Grassini, P.; Cafaro La Menza, N.; Rattalino Edreira, I.G.; Monzón, J.P.; Tenorio, A.F.; Specht, E.J. Soybean. In *Crop Physiology Case Histories for Major Crops*, 1st ed.; Sadras, O.V., Calderini, F.D., Eds.; Academic Press: Cambridge, MA, USA, 2021; pp. 282–319.
2. Hartman, G.L.; West, E.D.; Herman, T.K. Crops that feed the World 2. Soybean–worldwide production, use, and constraints caused by pathogens and pests. *Food Secur.* **2011**, *3*, 5–17. [CrossRef]
3. Hellal, F.A.; Abdelhamid, M.T. Nutrient management practices for enhancing soybean (*Glycine max* L.) production. *Acta Biol. Colomb.* **2013**, *18*, 239–250.
4. Pagano, M.C.; Miransari, M. The importance of soybean production worldwide. In *Abiotic and Biotic Stresses in Soy-Bean Production*, 1st ed.; Miransari, M., Ed.; Academic Press: Cambridge, MA, USA, 2016; Volume 1, pp. 1–26.
5. Keyser, H.H.; Li, F. Potential for increasing biological nitrogen fixation in soybean. In *Biological Nitrogen Fixation for Sustainable Agriculture*; Springer: Dordrecht, The Netherlands, 1992; pp. 119–135.
6. Zimmer, S.; Messmer, M.; Haase, T.; Piepho, H.P.; Mindermann, A.; Schulz, H.; Habekuß, A.; Ordon, F.; Wilbois, K.P.; Heß, J. Effects of soybean variety and *Bradyrhizobium* strains on yield, protein content and biological nitrogen fixation under cool growing conditions in Germany. *Eur. J. Agron.* **2016**, *72*, 38–46. [CrossRef]
7. Albuquerque, T.M.; Ortez, O.A.; Carmona, G.I.; Ciampitti, I.A. Soybean: Evaluation of inoculation. *Kans. Agric. Exp. Stn. Res. Rep.* **2017**, *6*, 1–5. [CrossRef]
8. Kibido, T.; Kunert, K.; Makgopa, M.; Greve, M.; Vorster, J. Improvement of rhizobium-soybean symbiosis and nitrogen fixation under drought. *Food Energy Secur.* **2020**, *9*, e177. [CrossRef]
9. Zapata, F.; Danso, S.K.A.; Hardarson, G.; Fried, M. Time Course of Nitrogen Fixation in Field-Grown Soybean Using Nitrogen-15 Methodology. *Agron. J.* **1987**, *79*, 172–176. [CrossRef]
10. Santos, M.S.; Nogueira, M.A.; Hungria, M. Microbial inoculants: Reviewing the past, discussing the present and previewing an outstanding future for the use of beneficial bacteria in agriculture. *AMB Express* **2019**, *9*, 205. [CrossRef]
11. Hungria, M.; Nogueira, M.A.; Araujo, R.S. Soybean seed co-inoculation with *Bradyrhizobium* spp. and *Azospirillum brasilense*: A new biotechnological tool to improve yield and sustainability. *Am. J. Plant Sci.* **2015**, *6*, 811–817. [CrossRef]
12. Leggett, M.; Diaz-Zorita, M.; Koivunen, M.; Bowman, R.; Pesek, R.; Stevenson, C.; Leister, T. Soybean response to inoculation with *Bradyrhizobium japonicum* in the United States and Argentina. *Agron. J.* **2017**, *109*, 1031–1038. [CrossRef]
13. Ray, D.K.; Mueller, N.D.; West, P.C.; Foley, J.A. Yield trends are insufficient to double global crop production by 2050. *PLoS ONE* **2013**, *8*, e66428. [CrossRef]
14. Kumawat, S.M.; Dhakar, L.L.; Maliwal, P.L. Effect of irrigation regimes and nitrogen on yield, oil content and nutrient uptake of soybean (*Glycine max* (L.) Merrill). *Indian J. Agron.* **2000**, *45*, 361–366.

15. Chang, W.S.; Lee, H.I.; Hungria, M. Soybean production in the Americas. In *Principles of Plant-Microbe Interactions*; Springer: Cham, Switzerland, 2015; pp. 393–400.
16. Griffith, W.K. Satisfying the nutritional requirements of established legumes. *Forage Fertil.* **1974**, 147–169. [CrossRef]
17. Liu, Q.; Xu, H.; Mu, X.; Zhao, G.; Gao, P.; Sun, W. Effects of different fertilization regimes on crop yield and soil water use efficiency of millet and soybean. *Sustainability* **2020**, *12*, 4125. [CrossRef]
18. Wood, C.W.; Torbert, H.A.; Weaver, D.B. Nitrogen fertilizer effects on soybean growth, yield, and seed composition. *J. Prod. Agric.* **1993**, *6*, 354–360. [CrossRef]
19. Salvagiotti, F.; Cassman, K.; Specht, J.; Walters, D.; Weiss, A.; Dobermann, A. Nitrogen uptake, fixation and response to fertilizer N in soybeans: A review. *Field Crop. Res.* **2008**, *108*, 1–13. [CrossRef]
20. Khaledian, M.S.; Mohammadi, K.; Javaheri, M. Grain yield and yield components of soybean affected by integrated fertilization methods. *Int. J. Agric. For.* **2014**, *4*, 1–3.
21. Tamagno, S.; Sadras, V.O.; Haegele, J.W.; Armstrong, P.R.; Ciampitti, I.A. Interplay between nitrogen fertilizer and biological nitrogen fixation in soybean: Implications on seed yield and biomass allocation. *Sci. Rep.* **2018**, *8*, 17502. [CrossRef]
22. Folina, A.; Tataridas, A.; Mavroeidis, A.; Kousta, A.; Katsenios, N.; Efthimiadou, A.; Travlos, I.S.; Roussis, I.; Darawsheh, M.K.; Papastylianou, P.; et al. Evaluation of Various Nitrogen Indices in N-Fertilizers with Inhibitors in Field Crops: A Review. *Agronomy* **2021**, *11*, 418. [CrossRef]
23. Oerke, E.C. Crop losses to pests. *J. Agric. Sci.* **2006**, *144*, 31. [CrossRef]
24. Samseemoung, G.; Soni, P.; Jayasuriya, H.P.; Salokhe, V.M. Application of low altitude remote sensing (LARS) platform for monitoring crop growth and weed infestation in a soybean plantation. *Precis. Agric.* **2012**, *13*, 611–627. [CrossRef]
25. Fickett, N.D.; Boerboom, C.M.; Stoltenberg, D.E. Soybean yield loss potential associated with early-season weed competition across 64 site-years. *Weed Sci.* **2013**, *61*, 500–507. [CrossRef]
26. Knezevic, S.Z.; Datta, A. The critical period for weed control: Revisiting data analysis. *Weed Sci.* **2015**, *63*, 188–202. [CrossRef]
27. Travlos, I.; Tataridas, A.; Kanatas, P.; Kakabouki, I.; Papastylianou, P. Weed management in soybean with a special focus on the control of purple nutsedge (*Cyperus rotundus*). *Agron. Res.* **2020**, *18*, 1–8.
28. Werner, E.L.; Curran, W.S.; Lingenfelter, D.D. Management of eastern black nightshade in agronomic crops: An integrated approach. *Agron. Facts* **2014**, *58*, 1–6.
29. Sweeney, A.E.; Renner, K.A.; Laboski, C.; Davis, A. Effect of fertilizer nitrogen on weed emergence and growth. *Weed Sci.* **2008**, *56*, 714–721. [CrossRef]
30. Kakabouki, I.; Karkanis, A.; Travlos, I.S.; Hela, D.; Papastylianou, P.; Wu, H.; Chachalis, D.; Sestras, R.; Bilalis, D. Weed flora and seed yield in quinoa crop (*Chenopodium quinoa* Willd.) as affected by tillage systems and fertilization practices. *Int. J. Pest Manag.* **2015**, *61*, 228–234. [CrossRef]
31. Dew, D.A. An index of competition for estimating crop loss due to weeds. *Can. J. Plant Sci.* **1972**, *52*, 921–927. [CrossRef]
32. Rana, S.S.; Kumar, S. *Research Techniques in Agronomy*; Department of Agronomy, College of Agriculture, CSK Himachal Pradesh Krishi Vishvavidyalaya: Palampur, India, 2014; p. 64.
33. Garko, M.S.; Yawale, M.A.; Gaya, U.H.; Mohammed, I.B.; Bello, T.T. Weed persistence, crop resistance and phytotonic effects of herbicides in maize (*Zea mays*) production under different weed control method and poultry manure in Kano State Nigeria. *J. Biol. Agric. Healthc.* **2020**, *10*, 11–17.
34. Shannon, C.E.; Weaver, W. *The Mathematical Theory of Communication*; University of Illinois Press: Urbana, IL, USA, 1963.
35. Pielou, E. *An Introduction to Mathematical Ecology*; Wiley Interscience: New York, NY, USA, 1969.
36. Margalef, R. Information theory in ecology. *Gen. Syst. Yearb.* **1958**, *3*, 36–71.
37. Travlos, I.S.; Cheimona, N.; Roussis, I.; Bilalis, D.J. Weed-species abundance and diversity indices in relation to tillage systems and fertilization. *Front. Environ. Sci.* **2018**, *6*, 11. [CrossRef]
38. Kumar, A.; Dhaka, A.K.; Kumar, S.; Singh, S.; Punia, S.S. Weed management indices as affected by different weed control treatments in pigeon pea [*Cajanus cajan* (L.) Millsp.]. *J. Pharmacogn. Phytochem.* **2019**, *8*, 3490–3494.
39. Martey, E.; Goldsmith, P. Heterogeneous demand for soybean quality. *Afr. J. Agric. Resour. Econ.* **2020**, *15*, 27–50. [CrossRef]
40. 2012–2013 Lime and Nutrient Recommendations, University of Kentucky, College of Agriculture. Available online: https://uknowledge.uky.edu/cgi/viewcontent.cgi?article=1024&context=anr_reports (accessed on 14 April 2022).
41. Ribeiro, V.H.V.; Maia, L.S.G.; Arneson, N.J.; Oliveira, M.C.; Read, H.W.; Ané, J.M.; Santos, B.J.; Werle, R. Influence of PRE-emergence herbicides on soybean development, root nodulation and symbiotic nitrogen fixation. *Crop Prot.* **2021**, *144*, 105576. [CrossRef]
42. Reddy, K.N.; Zablotowicz, R.M. Glyphosate-resistant soybean response to various salts of glyphosate and glyphosate accumulation in soybean nodules. *Weed Sci.* **2003**, *51*, 496–502. [CrossRef]
43. Bohm, G.M.; Alves, B.J.; Urquiaga, S.; Boddey, R.M.; Xavier, G.R.; Hax, F.; Rombaldi, C.V. Glyphosate and imazethapyr-induced effects on yield, nodule mass and biological nitrogen fixation in field-grown glyphosate-resistant soy-bean. *Soil Biol. Biochem.* **2009**, *41*, 420–422. [CrossRef]
44. Bollich, P.K.; Dunigan, E.P.; Jadi, A.W.M. Effects of Seven Herbicides on N_2 (C_2 H_2) Fixation by Soybeans. *Weed Sci.* **1985**, *33*, 427–430. [CrossRef]
45. Soybean Nutrient Management Guidelines, University of Minnesota. Available online: https://drive.google.com/file/d/1VMhWf7uxmBu8WeIMTZsCEq1Yu7NMMZBY/view (accessed on 14 April 2022).

46. Vlachostergios, D.; Noulas, C.; Baxevanos, D.; Raptopoulou, C.; Aggelopoulos, V.; Karanika, C.; Kantartzi, S.K.; Mavromatis, A. Response of early maturity soybean cultivars to row spacing in full-season crop and double-crop systems. *Plant Soil Environ.* **2021**, *67*, 18–25. [CrossRef]
47. Specht, J.E.; Chase, K.; Macrander, M.; Graef, G.L.; Chung, J.; Markwell, J.P.; Germann, M.; Orf, J.H.; Lark, K.G. Soybean response to water: A QTL analysis of drought tolerance. *Crop Sci.* **2001**, *41*, 493–509. [CrossRef]
48. Zhang, X.; Huang, G.; Bian, X.; Zhao, Q. Effects of root interaction and nitrogen fertilization on the chlorophyll content, root activity, photosynthetic characteristics of intercropped soybean and microbial quantity in the rhizosphere. *Plant Soil Environ.* **2013**, *59*, 80–88. [CrossRef]
49. Popović, V.; Tatić, M.; Spalević, V.; Rajičić, V.; Filipović, V.; Šarčević-Todosijević, L.J.; Stevanović, P. Effect of nitrogen fertilization on soybean plant height in arid year. In Proceedings of the 2nd International and 14th National Congress of Soil Science Society of Serbia "Solutions and Projections for Sustainable Soil Management", Novi Sad, Srbia, 25–28 September 2017; pp. 65–73.
50. Dong, S.; Jiang, Y.; Dong, Y.; Wang, L.; Wang, W.; Ma, Z.; Yan, C.; Ma, C.; Liu, L. A study on soybean responses to drought stress and rehydration. *Saudi J. Biol. Sci.* **2019**, *26*, 2006–2017. [CrossRef]
51. Souza, L.A.; Tavares, R. Nitrogen and Stem Development: A Puzzle Still to Be Solved. *Front. Plant Sci.* **2021**, *12*, 181. [CrossRef]
52. Prahraj, C.S. Growth and Productivity of Soybean (*Glycine max* L. Merrill) as Affected by Interacting Influence of *Rhizobium*, Nitrogen and Potassium and Herbicide Use and Their Residual Effect on Wheat. Doctoral Thesis, Punjab Agricultural University, Ludhiana, India, 1994.
53. Caliskan, S.; Ozkaya, I.; Caliskan, M.E.; Arslan, M. The effects of nitrogen and iron fertilization on growth, yield and fertilizer use efficiency of soybean in a Mediterranean-type soil. *Field Crop. Res.* **2008**, *108*, 126–132. [CrossRef]
54. Kakabouki, I.; Folina, A.; Zisi, C.; Karydogianni, S. Fertilization expression via nitrogen indices in soybean crop under two system tillage. *Not. Bot. Horti Agrobot. Cluj Napoca* **2020**, *48*, 799–813. [CrossRef]
55. Virk, H.K.; Singh, G.; Manes, G.S. Growth, symbiosis, productivity, and profitability of soybean at varying planting methods and nitrogen levels. *J. Plant Nutr.* **2018**, *41*, 1184–1196. [CrossRef]
56. Tagliapietra, E.L.; Streck, N.A.; da Rocha, T.S.M.; Richter, G.L.; da Silva, M.R.; Cera, J.C.; Jerson Guedes, C.V.J.; Zanon, A.J. Optimum leaf area index to reach soybean yield potential in subtropical environment. *Agron. J.* **2018**, *110*, 932–938. [CrossRef]
57. Evans, J.R. Photosynthesis and nitrogen relationships in leaves of C_3 plants. *Oecologia* **1989**, *78*, 9–19. [CrossRef] [PubMed]
58. Hikosaka, K.; Terashima, I. A model of the acclimation of photosynthesis in the leaves of C_3 plants to sun and shade with respect to nitrogen use. *Plant Cell Environ.* **1995**, *18*, 605–618. [CrossRef]
59. Lawn, R.J.; Brun, W.A. Symbiotic Nitrogen Fixation in Soybeans. I. Effect of Photosynthetic Source-Sink Manipulations. *Crop Sci.* **1974**, *14*, 11–16. [CrossRef]
60. Ainsworth, E.A.; Rogers, A.; Nelson, R.; Long, S.P. Testing the "source–sink" hypothesis of down-regulation of photosynthesis in elevated [CO_2] in the field with single gene substitutions in *Glycine max*. *Agric. For. Meteorol.* **2004**, *122*, 85–94. [CrossRef]
61. Basuchaudhuri, P. Source-sink relationships in soybean. *Indian J. Plant Sci.* **2016**, *5*, 19–25.
62. Nagasuga, K. Soybean Seed Production and Canopy Photosynthesis in Cultivation. In *Soybean-Biomass, Yield and Productivity*; Kasai, M., Ed.; IntechOpen: London, UK, 2018. [CrossRef]
63. Voldeng, H.D.; Cober, E.R.; Hume, D.J.; Gillard, C.; Morrison, M.J. Fifty-eight years of genetic improvement of short-season soybean cultivars in Canada. *Crop Sci.* **1997**, *37*, 428–431. [CrossRef]
64. Shibles, R.M.; Weber, C.R. Leaf area, solar radiation interception and dry matter production by soybeans. *Crop Sci.* **1965**, *5*, 575–577. [CrossRef]
65. Weber, C.; Shibles, R.M.; Byth, D.E. Effect of Plant Population and Row Spacing on Soybean Development and Production. *Agron. J.* **1966**, *58*, 99–102. [CrossRef]
66. Hardman, L.L.; Brun, W.A. Effect of atmospheric carbon dioxide enrichment at different developmental stages on growth and yield components of soybeans. *Crop Sci.* **1971**, *11*, 886–888. [CrossRef]
67. Board, J.E.; Harville, B.G. Soybean yield component responses to a light interception gradient during the reproductive period. *Crop Sci.* **1993**, *33*, 772–777. [CrossRef]
68. Board, J.E.; Zhang, W.; Harville, B.G. Yield rankings for soybean cultivars grown in narrow and wide rows with late planting dates. *Agron. J.* **1996**, *88*, 240–245. [CrossRef]
69. Gan, Y.; Stulen, I.; van Keulen, H.; Kuiper, P.J. Effect of N fertilizer top-dressing at various reproductive stages on growth, N_2 fixation and yield of three soybean (*Glycine max* (L.) Merr.) genotypes. *Field Crop. Res.* **2003**, *80*, 147–155. [CrossRef]
70. Dong, S.K.; Gong, Z.P.; Zu, W. Effects of nitrogen nutrition levels on N-accumulation and yields of soybean. *Plant Nutr. Fertil. Sci.* **2010**, *16*, 65–70.
71. Mourtzinis, S.; Kaur, G.; Orlowski, J.M.; Shapiro, C.A.; Lee, C.D.; Wortmann, C.; Holshouser, D.; Nafziger, E.D.; Kandel, H.; Niekamp, J.; et al. Soybean response to nitrogen application across the United States: A synthesis-analysis. *Field Crop. Res.* **2018**, *215*, 74–82. [CrossRef]
72. Capatana, N.; Bolohan, C.; Marin, D.I. Research regarding the influence of mineral fertilization along with *Bradyrhizobium japonicum* on soybean grain yield (*Glycine max* (L.) Merrill) under the conditions of south-east Romania. *Sci. Pap. Ser. A Agron.* **2017**, *60*, 207–214.
73. Jadhav, A.S.; Andhale, R.P.; Patil, P.A. Effect of integrated nutrient management on yield attributes and yield of soybean. *J. Maharashtra Agric. Univ.* **2009**, *34*, 86–88.

74. Lorenc-Kozik, A.M.; Pisulewska, E. Effect of increasing levels of nitrogen fertilizer and microelements on seed yield of selected soybean cultivars. *Rośliny Oleiste-Oilseed Crop.* **2003**, *24*, 131–142.
75. Prusiński, J.; Baturo-Cieśniewska, A.; Borowska, M. Response of soybean (*Glycine max* (L.) Merrill) to mineral nitrogen fertilization and *Bradyrhizobium japonicum* seed inoculation. *Agronomy* **2020**, *10*, 1300. [CrossRef]
76. Buttery, B.R.; Buzzell, R.I.; Findlay, W.I. Relationships among photosynthetic rate, bean yield and other characters in field-grown cultivars of soybean. *Can. J. Plant Sci.* **1981**, *61*, 190–197. [CrossRef]
77. Saitoh, K.; Nishimura, K.; Kuroda, T. Characteristics of flowering and pod set in wild and cultivated types of soybean. *Plant Prod. Sci.* **2004**, *7*, 172–177. [CrossRef]
78. Streeter, J.; Wong, P.P. Inhibition of legume nodule formation and N2 fixation by nitrate. *Crit. Rev. Plant Sci.* **1988**, *7*, 1–23. [CrossRef]
79. Gulden, R.H.; Vessey, J.K. Low concentrations of ammonium inhibit specific nodulation (nodule number g^{-1} root DW) in soybean (*Glycine max* [L.] Merr.). *Plant Soil* **1998**, *198*, 127–136. [CrossRef]
80. Cigelske, B.D.; Kandel, H.; DeSutter, T.M. Soybean Nodulation and Plant Response to Nitrogen and Sulfur Fertilization in the Northern US. *Agric. Sci.* **2020**, *11*, 592. [CrossRef]
81. Abdel-Wahab, A.M.; Abd-Alla, M.H. Effect of different rates of N-fertilizers on nodulation, nodule activities and growth of two field grown cvs. of soybean. *Nutr. Cycl. Agroecosyst.* **1995**, *43*, 37–41.
82. Oklahoma Cooperative Extension Service. Available online: https://extension.okstate.edu/fact-sheets/print-publications/pss/understanding-soybean-nodulation-and-inoculation-pss-2169.pdf (accessed on 14 April 2022).
83. Căpățână, N.; Bolohan, C.; Oprea, C.A.; Marin, D.I. Influence of Soil Tillage Systems and Inoculation on Soybean Nodulation and Yield. *Sci. Pap.-Ser. A Agron.* **2018**, *61*, 46–52.
84. Bilalis, D.; Karkanis, A.; Pantelia, A.; Patsiali, S.; Konstantas, A.; Efthimiadou, A. Weed populations are affected by tillage systems and fertilization practices in organic flax *('Linum usitatissimum'* L.) crop. *Aust. J. Crop Sci.* **2012**, *6*, 157–163.
85. Tang, L.; Cheng, C.; Wan, K.; Li, R.; Wang, D.; Tao, Y.; Pan, J.; Xie, J.; Chen, F. Impact of fertilizing pattern on the biodiversity of a weed community and wheat growth. *PLoS ONE* **2014**, *9*, e84370. [CrossRef] [PubMed]
86. Holzner, W.; Numata, M. *Biology and Ecology of Weeds*; Springer: Dordrecht, The Netherlands, 2013; p. 400.
87. Costea, M.; Weaver, S.E.; Tardif, F.J. The biology of Canadian weeds. 130. *Amaranthus retroflexus* L., *A. powellii* S. Watson and *A. hybridus* L. *Can. J. Plant Sci.* **2004**, *84*, 631–668.
88. Edesi, L.; Jaervan, M.; Adamson, A.; Lauringson, E.; Kuht, J. Weed species diversity and community composition in conventional and organic farming: A five-year experiment. *Zemdirb.-Agric.* **2012**, *99*, 339–346.
89. Chauhan, B.S.; Abugho, S.B. Effects of water regime, nitrogen fertilization, and rice plant density on growth and re-production of lowland weed *Echinochloa crus-galli*. *Crop Prot.* **2013**, *54*, 142–147. [CrossRef]
90. Mekdad, A.A.; El-Enin, M.M.A.; Rady, M.M.; Hassan, F.A.; Ali, E.F.; Shaaban, A. Impact of Level of Nitrogen Fertilization and Critical Period for Weed Control in Peanut (*Arachis hypogaea* L.). *Agronomy* **2021**, *11*, 909. [CrossRef]
91. Marshall, E.J.P.; Brown, V.K.; Boatman, N.D.; Lutman, P.J.W.; Squire, G.R.; Ward, L.K. The role of weeds in supporting biological diversity within crop fields. *Weed Res.* **2003**, *43*, 77–89. [CrossRef]
92. Fried, G.; Petit, S.; Dessaint, F.; Reboud, X. Arable weed decline in Northern France: Crop edges as refugia for weed conservation? *Biol. Conserv.* **2009**, *142*, 238–243. [CrossRef]
93. Schumacher, M.; Dieterich, M.; Gerhards, R. Effects of weed biodiversity on the ecosystem service of weed seed predation along a farming intensity gradient. *Glob. Ecol. Conserv.* **2020**, *24*, e01316. [CrossRef]
94. Finger, R.; Buchmann, N. An ecological economic assessment of risk-reducing effects of species diversity in managed grasslands. *Ecol. Econ.* **2015**, *110*, 89–97. [CrossRef]
95. Schütte, G.; Eckerstorfer, M.; Rastelli, V.; Reichenbecher, W.; Restrepo-Vassalli, S.; Ruohonen-Lehto, M.; Saucy, W.A.G.; Mertens, M. Herbicide resistance and biodiversity: Agronomic and environmental aspects of genetically modified herbicide-resistant plants. *Environ. Sci. Eur.* **2017**, *29*, 1–12. [CrossRef] [PubMed]
96. Pyšek, P.; Lepš, J. Response of a weed community to nitrogen fertilization: A multivariate analysis. *J. Veg. Sci.* **1991**, *2*, 237–244. [CrossRef]
97. Inouye, R.S.; Tilman, D. Convergence and divergence of old field vegetation after 11 years of nitrogen addition. *Ecology* **1995**, *76*, 1872–1887. [CrossRef]
98. Chețan, F.; Chețan, C.; Bogdan, I.; Pop, A.I.; Moraru, P.I.; Rusu, T. The Effects of Management (Tillage, Fertilization, Plant Density) on Soybean Yield and Quality in a Three-Year Experiment under Transylvanian Plain Climate Conditions. *Land* **2021**, *10*, 200. [CrossRef]
99. Lavres, J.; Castro Franco, G.; de Sousa Câmara, G.M. Soybean seed treatment with nickel improves biological nitrogen fixation and urease activity. *Front. Environ. Sci.* **2016**, *4*, 37. [CrossRef]
100. Feng, L.; Wang, H.; Ma, X.; Peng, H.; Shan, J. Modeling the current land suitability and future dynamics of global soybean cultivation under climate change scenarios. *Field Crop. Res.* **2021**, *263*, 108069. [CrossRef]