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Edamame Yield and Quality Response to Nitrogen and Sulfur Fertilizers

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Abstract: As United States farmers adapt soybean (*Glycine max*) production methods from oilseed to vegetable (edamame), key management practices will need to be considered. The key objective of this study was to determine the optimal nitrogen (N) rate and N application timing for edamame in the mid-Atlantic coastal plain system. The study was conducted for three years in Painter, VA, USA on sandy loam soils. A factorial arrangement of four N rates was applied with two application timing strategies: at-planting, and split application. Leaf tissue samples were collected and analyzed at R1. At harvest, the Normalized Difference Vegetation Index (NDVI) was measured, whole pods were mechanically collected, and yield was recorded. Additionally, pod and bean physical and chemical quality were assessed. Nitrogen fertilization significantly increased pod yield in two out of three years. R1 leaf N and sulfur (S) concentrations correlated to the yield, and R1 leaf and R6 whole-plant N concentrations correlated to the total N uptake. None of the tested parameters indicated that N fertilizer decreased yield or quality. In conclusion, we found that N fertilizer applied at planting may aid edamame yield and profit for sandy loam soils in the mid-Atlantic, USA.

Keywords: fertilizer rate; fertilizer timing; maturity; nutrient management; soybean; vegetable quality

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1. Introduction

Edamame, the Japanese name for immature soybean in the pod, also called vegetable soybean, is the same species as commercial soybean (*Glycine max* (L.) Merr.) grown for oilseed production throughout the world. Edamame consumption in the U.S. has increased as consumers have desired healthful, plant-based protein sources [1]. Soyatech, LLC, recorded that U.S. frozen edamame sales increased 40% between 2003 and 2007 from USD 18 million to USD 30 million [2]. However, U.S. edamame production has not matched this increased demand, with approximately 70% of U.S. edamame consumed being imported from China [3,4]. Edamame is imported as a frozen product, but domestic edamame demonstrates a great opportunity for U.S. producers to provide consumers with a local fresh product. In 2001, the University of Kentucky estimated the return for hand-harvested, wholesale, fresh market edamame at 638 USD ha^{-1} [5]. To produce high-quality, high-yielding edamame for U.S. domestic markets that can be mechanically harvested, proper agronomic management techniques must be developed for sustainable and economically viable production within the expanding edamame industry.

Proper edamame nutrient management is essential to ensure optimal growth and development, and also maintain a healthy plant to defend against pests. Nitrogen (N) is arguably the most important mineral nutrient as a key component of amino acids, DNA, and chlorophyll, and therefore, essential for photosynthesis. Not only is N essential for plant growth, but it also impacts bean quality, specifically protein and oil content. In a study of 54 genotypes, shell-dried edamame protein and oil concentrations were 6 and 10 g kg^{-1} higher than mature soybean [6].

As a monocarpic plant, soybean transfers mobile nutrients from vegetative structures to the seed during senescence; thus, the total N uptake throughout the growing season impacts seed yield [7]. Between R4 and R5, daily N uptake can reach 4.3 kg N ha^{-1} [8]. In soybean, N accumulation contained within vegetative structures is highest during R5 around 60 days after sowing (DAS) and begins to decrease around 73 DAS during R6 when N is transferred from vegetative structures to the reproductive structures [9]. After R5.5 550–590 g kg^{-1} of vegetative N is translocated to the seed [10,11]. Edamame is harvested at R6, right after translocation begins, and consequentially many nutrients remain in the leaves and stems.

For every 1000 kg harvested, the total soybean uptake is 82 kg N which must be obtained from atmospheric deposition, biological N fixation, soil N, or fertilizer [12]. According to the Environmental Protection Agency (EPA), the annual N deposition for the study area was 8.61 kg ha^{-1} in 2016 [13]. Bezdicek et al. [14] reported N fixation over 300 kg ha^{-1} in inoculated soybeans grown in N deficient soils, with N fixation contributing 71–80% of total plant N. Leaching, runoff, volatilization, and denitrification all threaten soil N in Virginia, due to environmental conditions and management practices [15]. In the mid-Atlantic, N fertilizer programs for snap bean *Phaseolus vulgaris*—a legume with similar growth and development to edamame—recommend split N applications when grown in coarse-textured soils to ensure proper nutrition is available throughout the growing season [16,17]. Split applications can combat N losses by providing lower amounts of N throughout the season to ensure adequate nutrition during critical growth stages.

Soybean response to N fertility is highly dependent on the environment and management. A synthesis analysis combining results from sixteen states, covering 86% of U.S. soybean production, concluded that N management decisions should be made in conjunction with non-N management decisions as many interactions between the two were observed [18]. For example, N application timing was significant in irrigated environments, while in rainfed environments, N application timing was only significant at high seeding rates. Mourtzinis et al. [18] also stated that the N rate increased the yield in only 13 out of 207 environments, and in environments where the yield was increased the range was 0.9–3.0 kg ha^{-1} for every kg N applied. Salvagiotti et al. [7] also concluded that high-yielding environments (>4500 kg ha^{-1}) were more likely to respond to N fertilizers.

Although N additions may improve the yield, N fertilizer is not typically economical for soybean production [7,19]. Being a specialty crop with higher prices per unit, N additions in edamame may increase profit margins. In 2001, Ernst and Woods [5] approximated the breakeven price of edamame production at 0.45 US$ kg^{-1}. A separate high-management study using high tunnels required yields of 20,000 kg ha^{-1} and a fresh market cost of 5 USD kg^{-1} to sustain edamame production [20]. Varying production costs across management styles and the volatility of new commodity market prices make profitability analysis challenging.

Sometimes labeled the fourth-most-important nutrient, sulfur (S) is essential for the formation of proteins, oils, and chlorophyll; aids in nodule formation and enzyme activation; and is a structural component of two essential amino acids: cysteine and methionine. Sulfur-containing compounds are responsible for bitter acrid flavors, undesirable to consumers in vegetables [21]. Sulfur and N in the plant are closely related as one nutrient influences the assimilation of the other. Increasing amounts of S will increase N uptake and increased N levels cause S deficiencies to be more apparent; for legumes, typical N:S ratios should be 17:1 [22]. Additionally, amino acid concentrations are also connected to N and S levels with the typical cystine and methionine concentration in the soybean seed being approximately 6.5 g kg^{-1} correlating to approximately 87 g kg^{-1} total N content [23].

The proper harvest of edamame is R6, also known as "full seed", when pod weight is maximized [24–26]. At this stage, the pods and beans are still green, beans are touching in the pod, and lower leaves may yellow and drop from the plant [24]. The ideal window for edamame harvest lasts approximately 18 days or less [27,28]. R7 is classified as physiological maturity and is indicated by one pod that has a mature color: brown, tan, lacking all green color [24]. While the bean still contains 60% moisture at the R7 stage [24], it is no longer desirable to the edamame consumer. As the plant matures mono- and disaccharides decrease while oligosaccharides increase [25]. Konovsky [29] reported that when the pod color and seed size seem optimal for harvest, flavor has already begun to deteriorate. Additionally, pests can damage pods, which triggers premature yellowing and can halt normal pod development [30]. Proper harvest time is essential to ensure crop quality and marketability. The longer a crop is in the field, the greater the potential for decreased quality. As a vegetable crop, pod and bean quality can determine crop failure versus crop success.

While extensive research was carried out to measure the impacts of N and S fertilization on soybean yield and seed quality, little was completed to document N and S fertility impacts on edamame. Edamame is harvested green, prior to senescence; therefore not all nutrients have been translocated to the seed as occurs with conventional oilseed soybean. For this reason, the overall edamame seed yield and quality responses to fertilizers may be different than soybean. The objective of our project was to determine the optimal N rate and N application timing for edamame in the mid-Atlantic coastal plain system and ascertain whether S is limiting in this system. Growers need region-specific recommendations to ensure optimal yield and seed/pod quality [17].

2. Materials and Methods

Trials were conducted in an irrigated Bojac sandy loam (coarse-loamy, mixed, semi-active, thermic Typic Hapludults) [31] with a history of soybean production in Accomack County, VA, USA (37.586917°, −75.823861°) from 2019–2021. The soil was conventionally tilled before planting. Soil samples were collected from each replication at planting to a depth of 60 cm. Samples were air dried and available nutrient concentration was measured by Mehlich-III extraction [32]. Soil pH was determined using 1:1 water solution [33]. Soil properties are listed in Table 1.

Table 1. Soil physical and chemical properties for edamame grown on sandy loams soils in the mid-Atlantic, USA from 2019 to 2021.

Year	DEPTH	pH	Organic Matter	NO_3-N	P	K	Ca	Mg	SO_4-S	Zn	Mn	B
	cm		%				ppm					
2019	0–15	5.9	1.1	14.8	175	89	610	47	10.3	1.8	45.5	0.48
	15–30	6.1	1.1	7.4	144	88	626	59	7.8	1.7	45.5	0.45
	30–45	6.2	0.9	7.5	43	98	438	66	15.0	1.2	26.5	0.31
	45–60	6.2	0.9	4.7	8	98	597	91	8.3	0.9	6.5	0.26
2020	0–15	5.3	1.7	77.5	324	193	509	72	12.3	1.3	65.0	0.56
	15–30	5.4	1.6	49.5	272	159	523	79	10.0	1.2	57.8	0.51
	30–45	5.5	1.6	16.3	52	140	638	154	23.5	0.7	34.5	0.37
	45–60	5.4	1.4	6.0	7	117	636	197	55.0	0.7	17.8	0.33
2021	0–15	6.1	1.3	4.7	77	123	501	67	6.5	1.9	30.8	0.40
	15–30	6.1	1.2	5.6	65	118	533	77	6.3	1.2	28.0	0.37
	30–45	6.4	1.2	3.9	10	115	695	116	11.8	0.6	16.3	0.30
	45–60	6.3	1.1	3.8	4	95	754	132	14.5	0.5	6.3	0.28

A total of 10 treatments were replicated four times for a total of 40 plots per year. Maturity group four edamame was planted at a population of 215,000 plants ha^{-1} with an experimental variety in 2019, and the commercial variety MFL2P59 in 2020 and 2021 (Montague Farms, Center Cross, VA, USA). Plots were 4, 12 m rows, spaced 0.91 m apart, a 3 m alley separated the rows of plots. In 2019, the row length was 9 m. Differences between the years occurred due to seed and land availability. A factorial arrangement of 4 N rates (22.4, 44.8, 67.2 and 89.6 kg N ha^{-1}) and two N timings (at-planting and split (50% applied at-planting and 50% at R1)) were broadcast applied by hand utilizing urea (460 g N kg) as a

N source. To determine if S was limiting, a S treatment of 22.4 kg S ha^{-1} and 44.8 kg N ha^{-1} was split applied each year. Sulfur was applied using ammonium sulfate (210 g N kg^{-1} and 240 g S kg^{-1}) with the remaining N necessary being supplied by urea. An untreated control was also included.

The outer rows for each plot were treated as border rows to avoid any edge effect. The uppermost fully developed trifoliate leaves (petiole removed) were collected randomly at flowering (R1) from 15 edamame plants per plot. Whole-plant samples of all above-ground biomass from 1 m of row were collected at full pod (R6) to investigate total N and S uptake. Leaf tissue and whole-plant samples were dried at 55 °C until a constant weight was obtained and then ground to pass through a 0.841-mm sieve. Leaf tissue and whole plants were analyzed for C, N, and S concentrations using the dry combustion method [34,35]. The C:N ratios and N:S ratios were calculated to further understand tissue sufficiency ratios at R1 and R6. The dry, whole-plant sample weight was multiplied by C, N, and S concentrations to determine total plant nutrient uptake at R6. One of the two center rows from each plot was harvested for pod yield at R6 with an Oxbo pixall BH100 one-row fresh bean harvester (Roosendall, North Brabant, The Netherlands), and any leaves or stems were removed.

Maturity was analyzed by examining the treatment effect on two different measurements of "greenness". The Normalized Difference Vegetation Index (NDVI) at harvest (R6) was measured using a Trimble GreenSeeker handheld crop sensor (Sunnyvale, CA, USA). Additionally, a 100-pod subsample of harvested pods was used to determine the percentage by weight of yellow pods. Both were utilized to account for discrepancies between pod maturity and leaf senescence [36].

Quality, relating to pod size, was measured at harvest by sampling 100 pods per plot. Samples were weighed to determine the average pod weight. The number of beans per pod was counted. Th average pod dimensions were determined by measuring the length, width, and thickness of 10 pods per sample. Average bean weight was determined by weighing 20 beans per sample. After physical quality measurements were taken, beans were dried and ground to determine total C, N, and S concentrations using the dry combustion method. Seed protein was calculated from total bean N using the conversion factor of 5.52 [37]. Similar to plant tissue samples, C:N ratios and N:S ratios were calculated.

Fertilizer prices were estimated using a three-year average of weekly prices from the USDA Ag Marketing Service. Prices were estimated as 0.52 USD kg^{-1} urea, and 0.47 USD kg^{-1} AMS. At these fertilizer prices, individual nutrient costs are 1.13 USD kg^{-1} N and USD 0.97 kg^{-1} S. Sulfur cost was calculated by using the N cost from urea to subtract N fertilizer value, with the remaining value being attributed to S.

Yield, in-season plant samples, and all post-harvest measurements were analyzed as response variables. The yield was analyzed with a regression model to determine the effects of N rate and timing. All three years were analyzed together in a mixed model with year, N rate, N rate × N rate, timing, timing × year, N Rate × year, N rate × timing, and N rate × timing × year interaction as fixed effects and replication nested within year was a random effect. If the quadratic effect (N rate × N rate) was not significant, it was removed and only the linear N rate was used. The S treatment was excluded from regression analysis. All plant samples and post-harvest measurements were analyzed with a mixed model where fertilizer treatment was a fixed effect and year and replication nested within a year were random effects. Total C, N, and S uptake, R1 leaf, R6 whole plant, and R6 bean C, N, and S concentrations were compared to yield. Additionally, R1 leaf, R6 whole plant, and R6 bean N concentrations were compared to total N uptake. A two-tailed *t*-test was conducted to test the S effect. The S treatment was compared to the treatment receiving equivalent rates of N, with no S applied. Significance is measured by $\alpha < 0.05$.

3. Results and Discussion

3.1. Yield Response

When yield was analyzed with the regression model, the N Rate × Year and the N Rate × Timing interactions were significant (p-value = 0.0099 and 0.0252 respectively) for yield. To better understand these interactions, individual analyses for each year were completed (Table 2). In 2019, the N Rate × Timing interaction was significant (p-value = 0.0204). In 2020, the N Rate was significant (p-value = 0.0426), and in 2021 no significance was found. In 2020, the N Rate was significant with the model, yield = 9.0(N Rate) + 6696, indicating a yield increase of 8.98 kg ha^{-1} was seen for every kg N ha^{-1} applied.

Table 2. Mixed model analyzing yield as impacted by N rate, N application timing, and year for edamame grown on sandy loams soils in the mid-Atlantic, USA from 2019 to 2021.

	2019		2020		2021		At-Planting		Split	
Effect	**F Ratio**	***p*-Value**	**F Ratio**	***p*-Value**	**F Ratio**	***p*-Value**	**F Ratio**	***p*-Value**	**F Ratio**	***p*-Value**
N Rate (R)	10.04	0.0033	4.45	0.0426	0.36	0.5499	10.84	0.0019	0.84	0.3632
Timing (T)	5.49	0.0253	0.15	0.6989	0.05	0.8179				
R × T	5.94	0.0204	0.08	0.7769	0.84	0.3663				
Year (Y)							55.85	<0.0001	67.13	<0.0001
T × Y										
R × Y							4.29	0.0197	1.46	0.2423

To further understand interactions, individual analyses were conducted for each timing (Figure 1). When all N was applied at planting, the N Rate × Year was significant (p-value = 0.0197), and when the N was split applied, only year was significant (p-value < 0.0001). In 2019, when all N was applied at planting, the N Rate was significant (p-value = 0.0108) with the model, yield = 29.9(N Rate) + 3387, indicating a yield increase of 29.9 kg ha^{-1} was seen for every kg N ha^{-1} applied at planting. Split applications did not impact yield. In 2019, because of seed availability, we used an experimental variety, while the in other two years, we used a commercial variety. These results may indicate that the N response may be dependent on variety. A similar soybean study conducted on sandy or silt loam soils in Mississippi—the same texture as soils observed in our study—when N application increased yield, an average of a 4% increase occurred when averaged across N rates (45–179 kg N ha^{-1}) and application timings (V4 or R1) [19].

Yield differences between years can be partially attributed to soil conditions (Table 1). In 2020, the highest-yielding year, soil organic matter, NO_3-N, P, K, and SO_4-S were all greater than the other two years. Similarly, in the lowest-yielding year, soil NO_3-N and P were the lowest of the three years. Temperatures for the growing season averaged 23.2 °C and differed by less than a degree between the three years.

In this study, N increased yields in two out of three years. Our results are similar to N fertility research on soybean, which produced mixed results. A study in Alabama in fine sandy loam, sandy loam, and silt loam soils observed positive and negative responses to N fertilizer concluding that positive responses were not reliable enough to recommend N applications for soybean [38]. Conversely, studies in Nebraska silt loam, silty clay loam and sandy loam soils and Argentina deep fine-loamy soils in high-yielding environments (>4500 kg ha^{-1}) demonstrated that soil N and N fixation do not supply sufficient N to achieve maximum yields [39,40]. Our results of N fertilizers increasing yields at high-yielding site-years and non-significance at low-yielding site-years agree with previous research which stated that N applications only benefit yield at high rates [18,40,41].

Mixed results are likely also related to biological nitrogen fixation. A hydroponic study documented delayed nodule formation at nutrient solution concentrations as low as 0.5 mM NO_3^- and higher NO_3^- concentrations further delayed nodulation [42]. In another hydroponic study, when high nitrate solution levels inhibited nodulation, the yield was less than control plants which utilized nitrate in solution and also N_2 fixed from nodules [43].

In a meta-analysis across 17 countries with diverse environments, Salvagoitti et al. [7] observed that increased N rates exponentially decreased N fixation when N was surface applied or incorporated in the topsoil layers. Furthermore, as yield increased, the ratio of biologically fixed N to total N uptake also decreased, indicating additional N is necessary at higher yields [7]. Although positive yield response to N fertilizer has been recorded, adding N may delay symbiotic biological N fixation, which is essentially a free N source for the farmer.

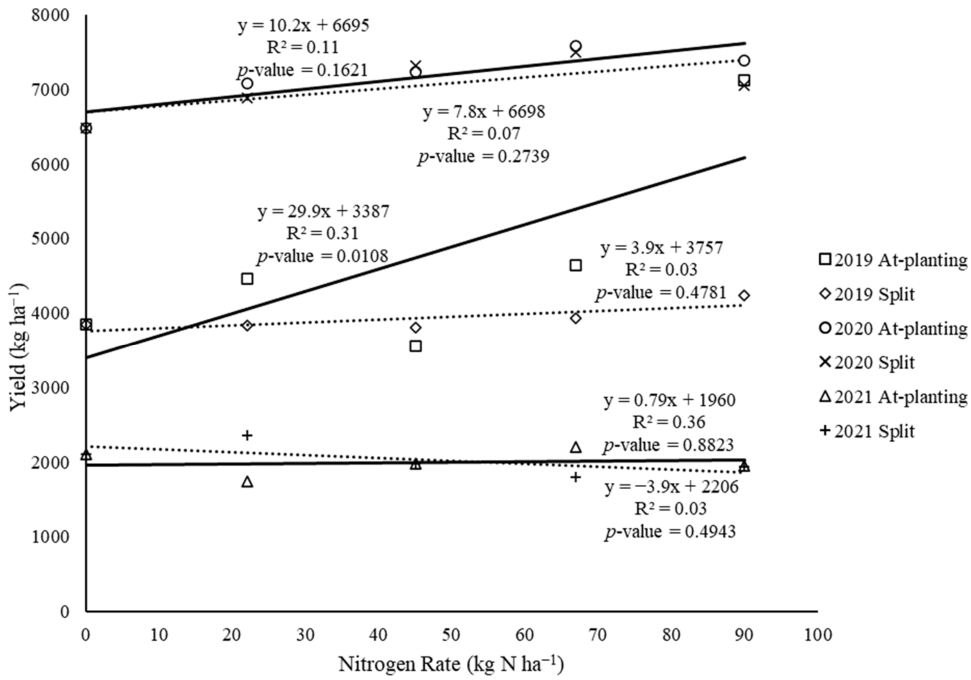

Figure 1. Pod yield as impacted by growing season, N rate, N application timing, and year for edamame grown on sandy loams soils in the mid-Atlantic, USA from 2019–2021. Solid lines indicate at-planting N applications and dotted lines indicate split N applications.

Split applications in this environment did not yield higher than at-planting applications in any of the years. These results contrast soybean research in which late-season N application increased soybean yield. In Kansas, a study demonstrated a 7.7 kg ha^{-1} yield increase when 22 kg N ha^{-1} was applied at R3 to the irrigated high-yielding soybean [41]. Chiluwal et al. [44] documented soybean yield increases when 202 kg N ha^{-1} was applied after R5 in Minnesota and Arkansas. Results from our study may differ from soybean literature due to edamame being harvested at R6 rather than R8, and late-applied N may still be in the soil or in vegetative structures at harvest. Our results indicate that leaching did not diminish the efficacy of at-planting applications. In this environment, producers can apply all N at planting, and should be aware that reactive N applications applied at R1 may not impact yield.

3.2. Tissue Analysis

No fertilizer treatment significantly impacted N, C, or S concentrations or the C:N, N:S ratios of R1 leaf tissue or whole-plant samples (Table 3). Tissue analysis indicated that in mid-Atlantic sandy loam soils when N is applied at rates at or below 90 kg ha^{-1}, leaf nutrient concentration at the R1 development stage and whole-plant nutrient concentration at the R6 development stage, are not significantly impacted.

In 2019 and 2021, N concentrations were within the sufficiency range of 32.5–50 g kg^{-1}, while in 2020 N concentrations exceeded the recommended sufficiency range by approximately 10 g kg^{-1} [22]. Sulfur concentrations for all three years were below sufficiency recommendations of 2.5–6.0 g kg^{-1}, indicating possible S deficiencies [22]. Available soil nitrate and sulfate were higher in 2020 when compared to the other two years (Table 1) which translated to higher nutrient uptake and higher R1 nutrient concentrations during 2020.

Table 3. Yearly means and mixed model results of R1 leaf tissue and whole-plant tissue N, C, S concentration and C:N, N:S ratios as impacted by N and S treatments for edamame grown on sandy loams soils in the mid-Atlantic, USA from 2019 to 2021.

	Nutrient	Treatment Effect		Means by Year		
		F Ratio	*p*-Value	2019	2020	2021
R1 Leaf Tissue	**N (g kg^{-1})**	0.79	0.6273	49.1	60.6	46.3
	C (g kg^{-1})	0.93	0.5070	467.3	447.1	468.1
	S (g kg^{-1})	0.77	0.6752	1.7	2.2	1.6
	C:N	0.78	0.6322	9.6	7.4	10.2
	N:S	0.78	0.6354	28.8	29.0	28.5
Whole-plant Tissue	**N (g kg^{-1})**	0.46	0.9006	20.7	26.5	30.2
	C (g kg^{-1})	1.20	0.3036	450.3	457.1	455.8
	S (g kg^{-1})	1.15	0.3379	1.0	1.5	1.2
	C:N	0.56	0.8233	22.2	17.4	15.3
	N:S	1.01	0.4401	19.7	17.7	26.2

Nitrogen R1 leaf concentrations were higher than R6 whole-plant N concentrations. In soybean production, the maximum total N accumulation occurred by R6.5, but approximately 40% of N was still in vegetative structures [10]. At R1 and R6, N was highly concentrated in the leaves and had not translocated to the seeds [9–11]. Thus, whole-plant samples at R6 indicate a lower overall N concentration than leaf samples at R1.

Figure 2 plots pod yield by C, N, and S leaf concentrations at R1. The quadratic form of S was significant (p-value < 0.0001), while the linear model of C and N concentration was significant (p-value < 0.0001 and <0.0001 respectively). Samples taken at R1 may be too late to adjust nutrient deficiencies as split applications in our study did not improve yields in any year, but leaf concentrations at R1 are good predictors of yield. Therefore, understanding leaf nutrient concentrations at R1 allows producers to estimate future returns weeks before harvest and also provides a better understanding of their fertility system so adjustments can be made in future years.

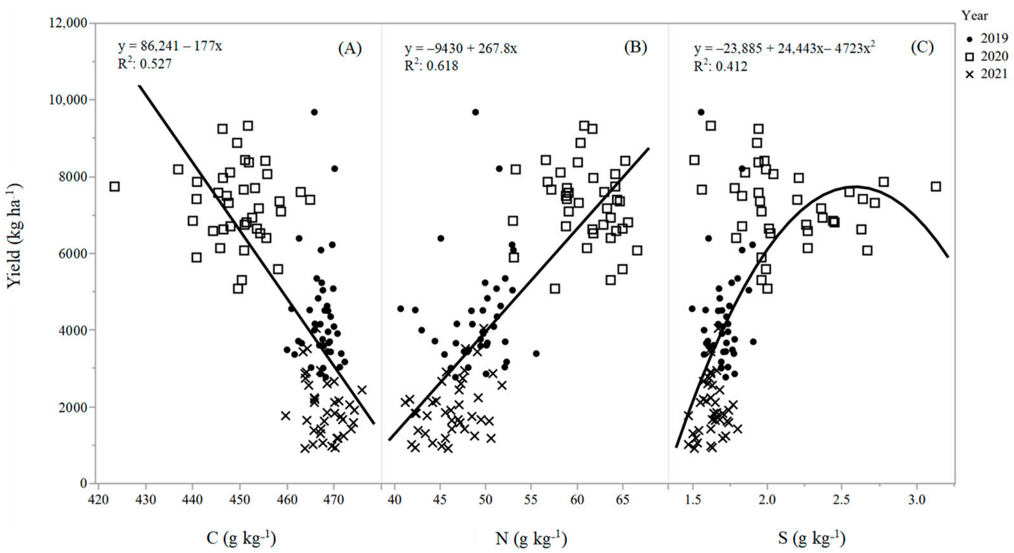

Figure 2. Pod yield correlated to C (**A**), N (**B**), and S (**C**) R1 concentration for edamame grown on sandy loam soils in the mid-Atlantic, USA from 2019 to 2021.

Leaf C concentration was inversely related to yield. Although carbon is a plant essential nutrient, it is abundant in growing environments. Therefore, plant C content is not typically reported in relation to yield. These results indicate further research on plant C content could provide insight into plant physiology. The N linear model suggested greater N concentrations may be necessary to reach peak yields in coastal plain systems leaf N never peaked with the experimental N rates tested. Additionally, yield did not level off between the suggested sufficiency range for soybean (32.5–50 g N kg^{-1}), indicating that this range may need to be adjusted for edamame N management. A similar positive correlation between N leaf tissue content and yield was found in soybeans [45]. Using the first derivative of the S model, the peak occurs at 2.59 g S kg^{-1}. This peak matches the reported sulfur sufficiency range of which the lower boundary is 2.5 g S kg^{-1} [22].

All three years, the N:S ratio was higher than the soybean recommended ratio of 17:1, even in the no-fertilizer control (Table 3). Excess N in the soil may have caused luxury consumption of N, but this is unlikely due to the linear equation seen when yield was plotted by N concentration (Figure 2). A similar study observed when grain soybeans were fertilized with AMS, S concentration increased and N:S ratio decreased in both leaf and whole-plant samples [46]. Additionally, the authors note that the N:S ratio was still higher than recommended critical values, despite no yield response to fertilization [46]. More research is necessary to determine if lowering the N:S ratio will increase yields or if the soybean recommendation for proper N:S ratios need to be adjusted for edamame.

Figure 3 plots yield by whole-plant C, N, and S concentration at harvest. Nitrogen and S concentration significantly correlated to yield (p-value = 0.0521 and <0.0001 respectively). With these models, the R2 is weaker, indicating that at this developmental stage, it is difficult to relate whole-plant nutrient concentration to the yield. At R6 leaves are still green, and not all nutrients have been transferred from the leaves to the beans. The beans have a high moisture content contributing to the yield weight. These factors make it difficult to estimate yield from whole-plant nutrient concentration at R6.

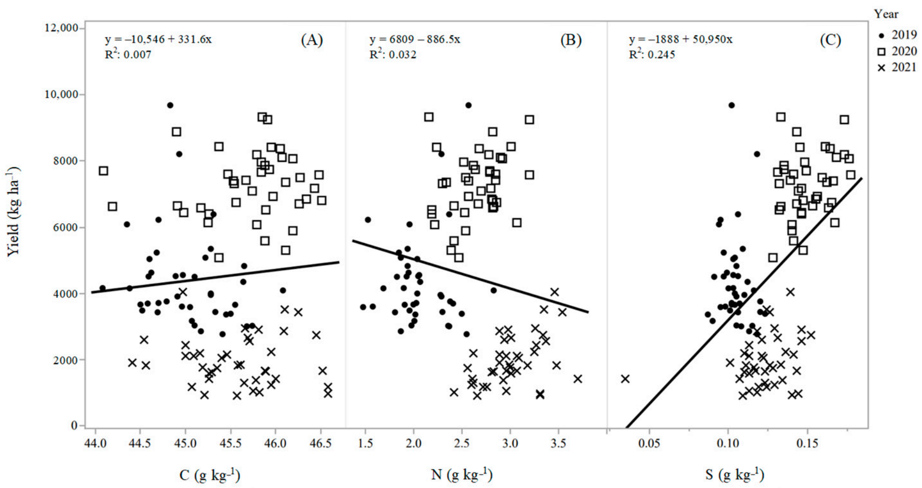

Figure 3. Pod yield correlated to C (**A**), N (**B**), and S (**C**) whole-plant concentration at harvest (R6) for edamame grown on sandy loams soils in the mid-Atlantic, USA from 2019 to 2021.

3.3. Plant Nutrient Uptake

Fertilizer additions did not impact the total uptake of N, C, or S (Table 4). Additionally, the average N total uptake is higher than the highest rate (90 kg N ha^{-1}) averaging 154.71 kg N ha^{-1}, indicating that soil N and nitrogen fixation are also providing substantial N for the edamame crop regardless of the N rate applied. Conversely, annual S total uptake averaged 7.6 kg ha^{-1}; therefore, treatments of 22 kg ha^{-1} supplied an excess of 14.4 kg S ha^{-1} left in the soil unused by the edamame plant. Fertilizer S not being taken up is either lost from the system by leaching or potentially banked for future use [47]. Based on these S uptake values (5.18, 10.52, 7.11 kg ha^{-1}), sulfur rates at 22 kg S ha^{-1} provided S greater than plant need; however, if no S fertilizer is applied to the system, soil mining will occur over time since annual mid-Atlantic S deposition is 2.72 kg ha^{-1} [48].

Table 4. Yearly means and mixed model results of total N, C, and S uptake concentration as impacted by N and S treatments for edamame grown on sandy loams soils in the mid-Atlantic, USA from 2019–2021.

Nutrient	Treatment Effect		Means by Year		
	F Ratio	***p*-Value**	**2019**	**2020**	**2021**
N (kg ha^{-1})	0.91	0.5230	102.7	185.6	175.8
C (kg ha^{-1})	1.12	0.3550	2237.9	3203.6	2640.0
S (kg ha^{-1})	1.27	0.2626	5.2	10.5	7.1

Carbon, N, and S uptake linearly correlated (p-value < 0.0001, 0.0001, and 0.0100 respectively) to yield (Figure 4). Plants with more nutrients were able to yield more.

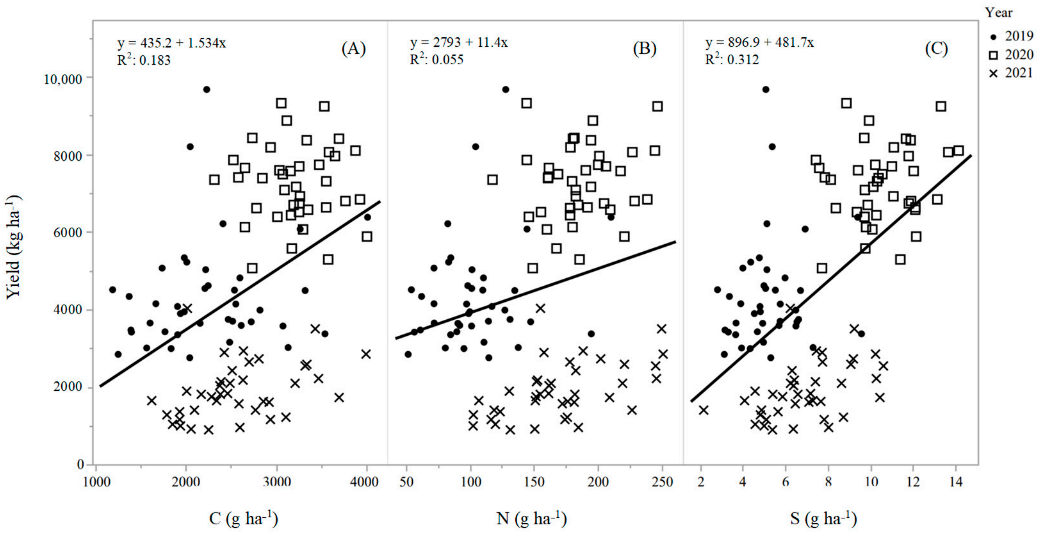

Figure 4. Pod yield correlated to total C (**A**), N (**B**), and S (**C**) uptake for edamame grown on sandy loams soils in the mid-Atlantic, USA from 2019–2021.

Although N, C, and S are not strongly correlated with edamame yield they are indicators. Again, it should be noted that total plant uptake measured nutrients within the whole plant, but edamame yield only measured the pod and seed weight. At the R6 developmental stage, when total nutrient uptake and yield are measured, much of the N, C, and S have not been translocated to the seed. Similar research in soybean also concluded that N uptake was an indicator of yield, but more strongly correlated (r^2 = 0.43) than these edamame results [49]. Differences in correlation may be due to the translocation of nutrients and lower moisture content of soybean compared to edamame.

R1 and whole-plant N concentrations were positively related to total N uptake (p-value < 0.0001 and <0.0001, respectively) (Table 4) (Figure 5). While both R1 and whole-plant concentrations significantly correlated to total N uptake, whole-plant concentration was more strongly correlated. Total N uptake estimations can aid in future edamame nutrient predictions and can also estimate N added or removed from the system for future crops on the same field.

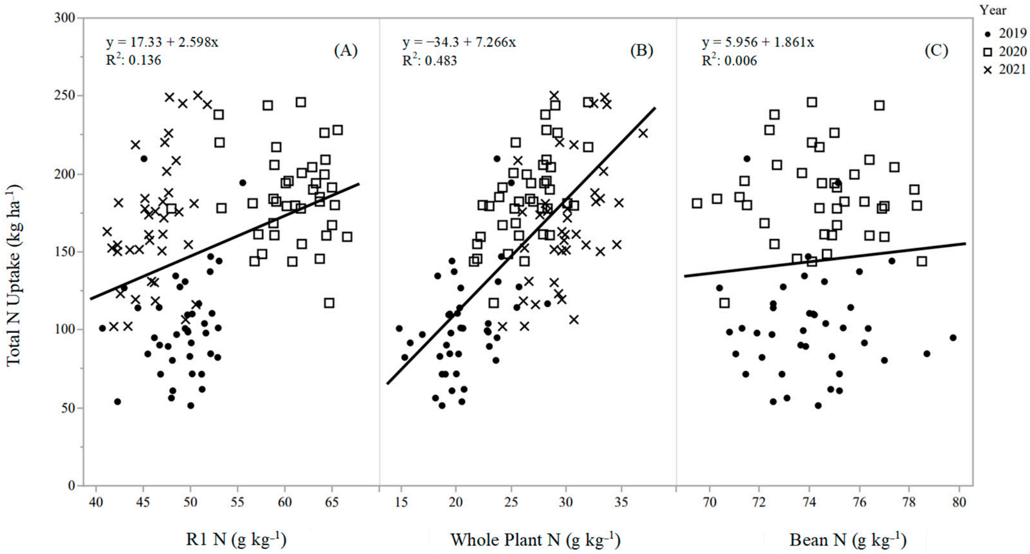

Figure 5. R1 (**A**), whole plant (**B**), and bean (**C**) N% correlated to total N uptake for edamame grown on sandy loams soils in the mid-Atlantic, USA from 2019 to 2021.

3.4. Maturity Analysis

None of the fertilizer rates or timings significantly impacted leaf senescence or maturity (Table 5). Soil solutions can impact plant growth and development rates. In a hydroponic study, nutrient solution N concentrations of 400 to 800 mg N L^{-1} delayed and even prevented leaf senescence; plants continued to mature, and beans began to yellow while the leaves and stems remained green [36]. Our results indicate soils in our study did not reach concentrations high enough to delay senescence. Fertilizing edamame with rates at or lower than 90 kg ha^{-1}, does not impact edamame maturity rates. The N rates applied in this study neither decreased pest susceptibility by accelerating maturity nor delayed maturity leaving the pest more susceptible to damage.

Table 5. Yearly means and mixed model results of NDVI and percent yellow pods as impacted by N and S treatments for edamame grown on sandy loams soils in the mid-Atlantic, USA from 2019 to 2021.

	Treatment Effect		Means by Year		
	F Ratio	***p*-Value**	**2019**	**2020**	**2021**
NDVI	0.74	0.6735	0.46	0.87	0.41
Yellow Pods (g kg^{-1})	1.08	0.3876	10.2	6.1	5.4

3.5. Bean Quality

3.5.1. Physical Quality

Beans per pod, pod weight, bean weight, and pod length, width, and height determined the N impact on quality parameters. In the mid-Atlantic on sandy loam soils, N and S additions did not significantly impact the physical quality of edamame (Table 6). These results correlated to previous research which states that pod and bean quality is largely related to cultivar [50,51]. At rates equal to or lower than 90 kg N ha^{-1} producers can apply N without concerns of impacting the physical quality of the pod or bean.

Table 6. Yearly means and mixed model results of physical pod components as impacted by growing season, and N and S treatments for edamame grown on sandy loams soils in the mid-Atlantic, USA from 2019 to 2021.

	Treatment Effect		Means by Year		
	F Ratio	*p*-Value	2019	2020	2021
beans pod^{-1}	0.74	0.6715	1.75	1.67	2.01
pod weight (g)	0.57	0.8166	1.44	1.84	1.30
bean weight (g)	0.87	0.5568	0.94	1.10	0.54
pod length (mm)	0.52	0.8591	11.53	12.36	13.08
pod width (mm)	1.15	0.3344	40.06	42.65	41.90
pod thickness (mm)	1.06	0.3984	8.13	9.38	8.33

3.5.2. Chemical Quality

Nitrogen is a main component of protein; therefore, a lot of research has been conducted measuring the impact of N fertilizer on protein concentration. In this study, bean nutrient concentration was reported for 2019 and 2020 (Table 7). None of the N or S rates significantly impacted the C, N, or S nutrient concentrations, ratios, or protein concentration (Table 7). At rates equal to or lower than 90 kg N ha^{-1} producers can apply N without concerns of impacting the elemental chemical quality of the bean. Similar to the yield discussion, previous studies have produced mixed results when measuring the impact of N fertility on soybean protein. While late-season N additions at R5 demonstrated increased protein concentration in the soybean seed in fine-silty soils in Arkansas, Kentucky, and Minnesota [44], other research in Kansas and Alabama concluded that N additions are not a reliable method for increasing protein content in soybean seeds [38,41]. Protein increases with N fertilizer mostly occurred with late-season applications. In edamame, late-season N additions may not be taken up by the plant or translocated to the seed before the pods are harvested. The rates applied in this study did not impact protein content, but more research is necessary to determine whether the N application can impact other edamame chemical qualities such as amino acids and oils.

Table 7. Yearly means and mixed model results of bean N, C, S concentration C:N, N:S ratios and protein concentration as impacted by N and S treatments for edamame grown on sandy loams soils in the mid-Atlantic, USA from 2019 to 2021.

Nutrient	Treatment Effect		Means by Year	
	F Ratio	*p*-Value	2019	2020
N (g kg^{-1})	0.50	0.8683	74.1	74.5
C (g kg^{-1})	0.70	0.7065	524.3	514.2
S (g kg^{-1})	0.14	0.9984	2.4	2.5
C:N	0.56	0.8205	7.1	6.9
N:S	0.33	0.9604	30.9	29.8
Protein (g kg^{-1})	0.50	0.8683	414.5	405.5

When edamame yield was correlated with bean nutrient concentration, C was negatively correlated (p-value < 0.0001) while S was positively correlated (p-value = 0.0053) (Figure 6). Nitrogen and S removal rates were estimated by multiplying yield by concentration. Nitrogen removal for this study was estimated as 74 kg N Mg^{-1}, while S removal was estimated as 2.5 kg S Mg^{-1}. These estimates are similar to soybean removal rates of 55 kg N Mg^{-1} and 3 kg S Mg^{-1} [12]. Notably, this study indicated that more N was removed from the field with edamame than with soybeans, meaning less N will remain in the system, and consequentially less N can be credited for the next crop. Further research is necessary to produce accurate removal rates. In this calculation, yield weight included pods while the nutrient concentration was calculated using only the edamame beans. Actual removal rates will be higher than those calculated here.

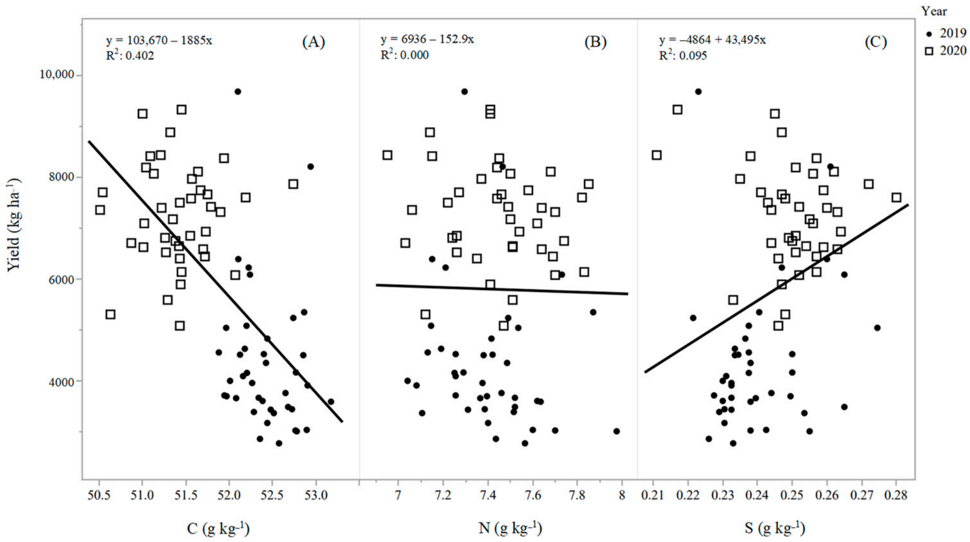

Figure 6. Pod yield correlated to C (**A**), N (**B**), and S (**C**) bean concentration for edamame grown on sandy loams soils in the mid-Atlantic, USA from 2019 to 2021.

3.6. Sulfur

When comparing the 22.4 kg S ha^{-1} ammonium sulfate treatment to the N urea treatment with a similar N rate, S did not significantly impact any variables measured (Table 8), indicating that the soil, air, and rainfall are providing all S necessary for proper edamame growth and development. Recent studies have produced mixed results of S additions on soybean seed yield. A meta-analysis of 44 site years in various environments and management programs across 8 states in the central U.S. concluded that S applied at planting increased seed yield and protein, but the high heterogeneity of results makes site-specific S recommendations difficult [52]. Another study, across 10 soybean-producing states reported that S additions increased yields at 3 out of 52 site-years and noted that responses to N and S fertilizers were not seen in environments with average yields below 3643 kg ha^{-1} [53]. Sulfur additions at rates as high as 33 kg S ha^{-1} did not increase yield in medium-textured soils with low OM and S deficiencies [54]. A study across the main U.S. soybean-producing region concluded that 10 kg S ha^{-1} did not impact seed yield, but did increase seed protein by 10 g kg^{-1} when S was applied at planting [55]. Fertilizing edamame with S may increase edamame yield or enhance nutrient quality, but, currently, in the mid-Atlantic coastal plain system, sulfur is not a yield-limiting factor for edamame production. Additional S is not recommended in this current system.

Table 8. Sulfur *t*-test comparing split application of 22.4 kg S ha^{-1} with ammonium sulfate to treatments of similar N rates without S for edamame grown on sandy loam soils in the mid-Atlantic, USA from 2019 to 2021.

	Response Variable	**Without S**	**With S**		
		Mean		**t Ratio**	***p*-Value**
Harvest Analysis	**pod yield (kg ha^{-1})**	4365	4566	0.19	0.8536
	NDVI	0.57	0.60	0.3	0.7699
	yellow pods (g kg^{-1})	212.1	175.3	−0.4	0.6954
	beans pod^{-1}	1.80	1.78	−0.3	0.7646
	pod weight (g)	1.55	1.54	−0.08	0.9396
	bean weight (g)	0.89	0.92	0.25	0.8085
	pod length (mm)	41.64	41.43	−0.25	0.8044
	pod width (mm)	12.36	12.19	−0.56	0.5829
	pod thickness (mm)	8.79	8.77	−0.06	0.9558

Table 8. *Cont.*

	Response Variable	Without S	With S		
		Mean		t Ratio	*p*-Value
R1 Leaf Tissue	**N (g kg^{-1})**	50.7	52.2	0.53	0.6033
	C (g kg^{-1})	461.9	461.8	−0.02	0.9809
	S (g kg^{-1})	1.8	1.8	0.24	0.8149
	C:N	9.3	9.0	−0.38	0.7071
	N:S	28.9	29.1	0.09	0.9305
Whole Plant	**N (g kg^{-1})**	25.9	25.9	0.03	0.9798
	C (g kg^{-1})	456.8	455.9	−0.36	0.7243
	S (g kg^{-1})	1.2	1.3	1.35	0.1903
	C:N	18.2	18.2	−0.02	0.9852
	N:S	21.4	19.4	−1.59	0.1292
Plant Uptake	**N (kg ha^{-1})**	143.7	157.7	0.74	0.4702
	C (kg ha^{-1})	2513.0	2785.4	0.93	0.3646
	S (kg ha^{-1})	6.9	8.3	1.16	0.2602
Bean	**N (g kg^{-1})**	74.1	74.4	0.42	0.6784
	C (g kg^{-1})	520.6	519.6	−0.44	0.6666
	S (g kg^{-1})	2.4	2.5	0.07	0.9423
	C:N	7.0	7.0	−0.58	0.5698
	N:S	30.3	30.4	0.15	0.8812
	protein (g kg^{-1})	408.8	410.4	0.42	0.6784

3.7. Economics

As a new crop, edamame prices are difficult to estimate, but production cost can be helpful when determining the profitability of management decisions. In 2019, yield increased 29.9 kg yield per kg N, at this rate, with an average N cost of \$1.13 kg^{-1} N every extra kg of edamame only cost \$0.04 in N inputs, or in 2020, where yield increased at a rate of 9.0 kg yield per kg N, every extra kg of edamame only cost \$0.13 in N inputs. Another estimate may be to average all three years since yield increases were not seen every year. When all three years are averaged, the yield increased 16 kg ha^{-1} for every 1 kg N applied, final edamame profit would be offset by \$0.07 kg^{-1} in N cost. Although the edamame market is still developing, and profit is difficult to determine, our results indicate that N fertility could increase the economic return for edamame producers.

4. Conclusions

In the mid-Atlantic coastal plain system, during a three-year study, N rates up to 90 kg ha^{-1} increased edamame yield in the two highest-yielding years. One year, at-planting applications yielded significantly higher than split applications. Split applications of N did not increase yield, but also, did not hamper yields. Split applications may prove to be more important in environments where N leaching is a concern. Treatments of N and S did not impact leaf tissue and total plant uptake. Leaf N and S concentration at R1 may be able to predict yield. Differences in sufficiency ranges and observed values in N and S R1 leaf concentrations suggest that current soybean recommendations may need to be adjusted to best manage edamame fertility regimes. R1 leaf and R6 whole-plant N concentrations are able to predict total N uptake. No fertilizer rates or timings impacted the maturity of the edamame plant or final pod or bean quality. In conclusion, edamame does have special recommendations regarding N fertility that should be followed for optimal yield and quality. Further research and farmer education is needed to better model nutrient interactions when a historically grown crop, such as soybean, is harvested at a different growth stage for fresh vegetable uses.

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References

1. Neill, C.L.; Morgan, K.L. Beyond Scale and Scope: Exploring Economic Drivers of US Specialty Crop Production with an Application to Edamame. *Front. Sustain. Food Syst.* **2021**, *4*, 582834. [CrossRef]
2. Bernick, K. Edamame Takes Root in US Corn & Soybean Digest. Available online: http://www.cornandsoybeandigest.com/edamame-takes-root-us (accessed on 18 May 2022).
3. Barlow, Z.; Virginia Tech Research Team Works across Disciplines to Boost U.S. Edamame Production. Available online: https://vtx.vt.edu/content/vtx_vt_edu/en/articles/2018/10/cals-edamame.html (accessed on 17 May 2022).
4. Yu, D.; Lin, T.; Sutton, K.; Lord, N.; Carneiro, R.; Jin, Q.; Zhang, B.; Kuhar, T.; Rideout, S.; Ross, J. Chemical Compositions of Edamame Genotypes Grown in Different Locations in the US. *Front. Sustain. Food Syst.* **2021**, *5*, 22. [CrossRef]
5. Ernst, M.; Woods, T. Marketing Challenges for Emerging Crops in Kentucky: Vegetable Soybeans. In Proceedings of the Second International Vegetable Soybean Conference, Tacoma, WA, USA, 10–12 August 2001.
6. Jiang, G.; Katuuramu, D.N.; Xu, Y.; Ren, S.; Rutto, L.K. Analysis and Comparison of Seed Protein, Oil, and Sugars in Edamame Dried Using Two Oven-drying Methods and Mature Soybeans. *J. Sci. Food Agric.* **2020**, *100*, 3987–3994. [CrossRef] [PubMed]
7. Salvagiotti, F.; Cassman, K.G.; Specht, J.E.; Walters, D.T.; Weiss, A.; Dobermann, A. Nitrogen Uptake, Fixation and Response to Fertilizer N in Soybeans: A Review. *Field Crops Res.* **2008**, *108*, 1–13. [CrossRef]
8. Gaspar, A.P.; Laboski, C.A.; Naeve, S.L.; Conley, S.P. Dry Matter and Nitrogen Uptake, Partitioning, and Removal across a Wide Range of Soybean Seed Yield Levels. *Crop Sci.* **2017**, *57*, 2170–2182. [CrossRef]
9. Islam, M.M.; Ishibashi, Y.; Nakagawa, A.C.; Tomita, Y.; Iwaya-Inoue, M.; Arima, S.; Zheng, S.-H. Nitrogen Redistribution and Its Relationship with the Expression of GmATG8c during Seed Filling in Soybean. *J. Plant Physiol.* **2016**, *192*, 71–74. [CrossRef]
10. Bender, R.R.; Haegele, J.W.; Below, F.E. Nutrient Uptake, Partitioning, and Remobilization in Modern Soybean Varieties. *Agron. J.* **2015**, *107*, 563–573. [CrossRef]
11. Hanway, J.; Weber, C. Accumulation of N, P, and K by Soybean (*Glycine max* (L.) Merrill) Plants 1. *Agron. J.* **1971**, *63*, 406–408. [CrossRef]
12. IPNI. IPNI Estimates of Nutrient Uptake and Removal. In *4R Plant Nutrition Manual*; IPNI: Norcross, GA, USA, 2012.
13. United States Environmental Protection Agency. Total Annual Nitrogen Deposition (Kg/Ha). [Map]. 2022. Available online: https://enviroatlas.epa.gov/enviroatlas/interactivemap/ (accessed on 16 February 2022).
14. Bezdicek, D.; Evans, D.; Abede, E.B.; Witters, R. Evaluation of Peat and Granular Inoculum for Soybean Yield and N Fixation Under Irrigation 1. *Agron. J.* **1978**, *70*, 865–868. [CrossRef]
15. Frame, W.H.; Reiter, M.S. *Enhanced Efficiency Fertilizer Materials: Nitrogen Stabilizers*; Virginia Polytechnic Institute and State University: Blacksburg, VA, USA, 2013.
16. Heinrich, A.; Sullivan, D.M.; Peachey, E. *Snap Bean—Western Oregon, Nutrient Management Guide*; Oregon State University Extension Service: Corvallis, OR, USA, 2016.
17. Reiter, M.S.; Samtani, J.B.; Torres Quezada, E.; Singh, V.; Doughty, H.B.; Kuhar, T.P.; Sutton, K.L.; Wilson, J.M.; Langston, D.B.; Rideout, S.L.; et al. *2022–2023 Mid-Atlantic Commercial Vegetable Recommendations*; Virginia Polytechnic Institute and State University: Blacksburg, VA, USA, 2022; Publ. 456-420 SPES-391P, 153.
18. 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 Crops Res.* **2018**, *215*, 74–82. [CrossRef]
19. McCoy, J.M.; Kaur, G.; Golden, B.R.; Orlowski, J.M.; Cook, D.; Bond, J.A.; Cox, M.S. Nitrogen Fertilization of Soybean in Mississippi Increases Seed Yield but Not Profitability. *Agron. J.* **2018**, *110*, 1505–1512. [CrossRef]

20. Bec, S.; Pfeiffer, T.; Slone, D. Production System for Extending the Harvest Time Frame of Fresh-Market Edamame in Kentucky. University of Kentucky. Available online: https://www.uky.edu/ccd/sites/www.uky.edu.ccd/files/edamame_extend_harvest.pdf (accessed on 18 May 2022).
21. Kopsell, D.E.; Kopsell, D.A.; Randle, W.M.; Coolong, T.W.; Sams, C.E.; Curran-Celentano, J. Kale Carotenoids Remain Stable While Flavor Compounds Respond to Changes in Sulfur Fertility. *J. Agric. Food Chem.* **2003**, *51*, 5319–5325. [CrossRef]
22. Bryson, G.M.; Mills, H.A.; Sasseville, D.N.; Jones, J.B.; Barker, A.V. *Plant Analysis Handbook III: A Guide to Sampling, Preparation, Analysis, Interpretation and Use of Results of Agronomic and Horticultural Crop Plant Tissue*; Micro-Macro Publishing, Incorporated: Athens, GA, USA, 2014; ISBN 1-878148-01-X.
23. Friedman, M.; Brandon, D.L. Nutritional and Health Benefits of Soy Proteins. *J. Agric. Food Chem.* **2001**, *49*, 1069–1086. [CrossRef]
24. McWilliams, D.; Berglund, D.R.; Endres, G. *Soybean Growth and Management Quick Guide*; North Dakota State University Extension: Fargo, ND, USA, 1999.
25. Saldivar, X.; Wang, Y.-J.; Chen, P.; Hou, A. Changes in Chemical Composition during Soybean Seed Development. *Food Chem.* **2011**, *124*, 1369–1375. [CrossRef]
26. TeKrony, D.; Egli, D.; Balles, J.; Pfeiffer, T.; Fellows, R. Physiological Maturity in Soybean. *Agron. J.* **1979**, *71*, 771–775. [CrossRef]
27. Nolen, S.; Zhang, B.; Kering, M. Increasing Fresh Edamame Bean Supply through Season Extension Techniques. *J. Hortic.* **2016**, *3*, 1000170.
28. Purcell, L.C.; Salmeron, M.; Ashlock, L. Soybean Growth and Development. In *Arkansas Soybean Production Handbook*; University of Arkansas System: Little Rock, AR, USA, 2014; pp. 1–8.
29. Konovsky, J.; Lumpkin, T.A.; McClary, D. Edamame: The Vegetable Soybean. In *Understanding the Japanese Food and Agrimarket*; CRC Press: Boca Raton, FL, USA, 1994; Volume 1988, pp. 173–181.
30. Martinelli, J.A.; Bocchese, C.A.; Xie, W.; O'Donnell, K.; Kistler, H.C. Soybean Pod Blight and Root Rot Caused by Lineages of the Fusarium Graminearum and the Production of Mycotoxins. *Fitopatol. Bras.* **2004**, *29*, 492–498. [CrossRef]
31. Soil Survey Staff Official Soil Series Descriptions. Available online: https://soilseries.sc.egov.usda.gov/OSD_Docs/B/BOJAC.html (accessed on 17 May 2022).
32. Maguire, R.; Heckendorn, S.E. *Laboratory Procedures: Virginia Tech Soil Testing Laboratory*; Virginia Polytechnic Institute and State University: Blacksburg, VA, USA, 2005.
33. Burt, R. *Soil Survey Laboratory Methods Manual*; United States Department of Agriculture, Natural Resources Conservation Service: Portland, OR, USA, 2004; Volume 42.
34. Dumas, J. Procédés de l'analyse Organique. *Ann Chim Phys* **1831**, *47*, 198–205.
35. Elementar. *Operating Instructions VarioMAX CNS*; Elementar: Ronkonkoma, NY, USA, 2000.
36. Islam, M.M.; Ishibashi, Y.; Nakagawa, A.C.S.; Tomita, Y.; Zhao, X.; Iwaya-Inoue, M.; Arima, S.; Zheng, S.-H. Nitrogen Manipulation Affects Leaf Senescence during Late Seed Filling in Soybean. *Acta Physiol. Plant.* **2017**, *39*, 42. [CrossRef]
37. Mosse, J. Nitrogen-to-Protein Conversion Factor for Ten Cereals and Six Legumes or Oilseeds. A Reappraisal of Its Definition and Determination. Variation According to Species and to Seed Protein Content. *J. Agric. Food Chem.* **1990**, *38*, 18–24. [CrossRef]
38. Wood, C.; Torbert, H.; Weaver, D. Nitrogen Fertilizer Effects on Soybean Growth, Yield, and Seed Composition. *J. Prod. Agric.* **1993**, *6*, 354–360. [CrossRef]
39. La Menza, N.C.; Monzon, J.P.; Specht, J.E.; Lindquist, J.L.; Arkebauer, T.J.; Graef, G.; Grassini, P. Nitrogen Limitation in High-Yield Soybean: Seed Yield, N Accumulation, and N-Use Efficiency. *Field Crops Res.* **2019**, *237*, 74–81. [CrossRef]
40. La Menza, N.C.; Monzon, J.P.; Specht, J.E.; Grassini, P. Is Soybean Yield Limited by Nitrogen Supply? *Field Crops Res.* **2017**, *213*, 204–212. [CrossRef]
41. Wesley, T.; Lamond, R.; Martin, V.; Duncan, S. Effects of Late-season Nitrogen Fertilizer on Irrigated Soybean Yield and Composition. *J. Prod. Agric.* **1998**, *11*, 331–336. [CrossRef]
42. Gibson, A.; Harper, J. Nitrate Effect on Nodulation of Soybean by Bradyrhizobium Japonicum. *Crop Sci.* **1985**, *25*, 497–501. [CrossRef]
43. Harper, J. Soil and Symbiotic Nitrogen Requirements for Optimum Soybean Production. *Crop Sci.* **1974**, *14*, 255–260. [CrossRef]
44. Chiluwal, A.; Haramoto, E.R.; Hildebrand, D.; Naeve, S.; Poffenbarger, H.; Purcell, L.C.; Salmeron, M. Late-Season Nitrogen Applications Increase Soybean Yield and Seed Protein Concentration. *Front. Plant Sci.* **2021**, *12*, 715940. [CrossRef]
45. Bhangoo, M.S.; Albritton, D. Effect of Fertilizer Nitrogen, Phosphorus, and Potassium on Yield and Nutrient Content of Lee Soybeans 1. *Agron. J.* **1972**, *64*, 743–746. [CrossRef]
46. Sweeney, D.; Granade, G. Yield, Nutrient, and Soil Sulfur Response to Ammonium Sulfate Fertilization of Soybean Cultivars. *J. Plant Nutr.* **1993**, *16*, 1083–1098. [CrossRef]
47. Eriksen, J.; Murphy, M.; Schnug, E. The Soil Sulphur Cycle. In *Sulphur in Agroecosystems*; Kluwer Academic Publishers: Dordrecht, The Netherlands, 1998; pp. 39–73.
48. United States Environmental Protection Agency. Annual Sulfur Deposition (Kg/Ha). [Map]. 2022. Available online: https://enviroatlas.epa.gov/enviroatlas/interactivemap/ (accessed on 16 February 2022).
49. Santachiara, G.; Borrás, L.; Salvagiotti, F.; Gerde, J.A.; Rotundo, J.L. Relative Importance of Biological Nitrogen Fixation and Mineral Uptake in High Yielding Soybean Cultivars. *Plant Soil* **2017**, *418*, 191–203. [CrossRef]
50. Ogles, C.; Guertal, E.; Weaver, D. Edamame Cultivar Evaluation in Central Alabama. *Agron. J.* **2016**, *108*, 2371–2378. [CrossRef]
51. Sánchez, E.; Kelley, K.; Butler, L. Edamame Production as Influenced by Seedling Emergence and Plant Population. *HortTechnology* **2005**, *15*, 672–676. [CrossRef]

52. De Borja Reis, A.F.; Rosso, L.H.M.; Davidson, D.; Kovács, P.; Purcell, L.C.; Below, F.E.; Casteel, S.N.; Knott, C.; Kandel, H.; Naeve, S.L.; et al. Sulfur Fertilization in Soybean: A Meta-Analysis on Yield and Seed Composition. *Eur. J. Agron.* **2021**, *127*, 126285. [CrossRef]
53. Brooks, K.; Mourtzinis, S.; Conley, S.P.; Reiter, M.S.; Gaska, J.; Holshouser, D.L.; Irby, T.; Kleinjan, J.; Knott, C.; Lee, C. Soybean Yield Response to Sulfur and Nitrogen Additions across Diverse US Environments. *Agron. J.* **2022**, *115*, 370–383. [CrossRef]
54. Cannon, K.; McClure, M.A.; Yin, X.; Sams, C. Corn and Soybean Response to Sulfur Fertilizer in West Tennessee. *Crop Forage Turfgrass Manag.* **2021**, *7*, e20092. [CrossRef]
55. Moro Rosso, L.; Carciochi, W.; Naeve, S.; Kovács, P.; Casteel, S.; Ciampitti, I. Nitrogen and Sulfur Fertilization in Soybean: Impact on Seed Yield and Quality. *Kans. Agric. Exp. Stn. Res. Rep.* **2020**, *6*, 18. [CrossRef]