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Growth and Yield Responses of Pot-Grown Long Bean and Luffa to Nitrogen Rates

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Abstract: Optimizing nitrogen (N) input rates for vegetable production is crucial in Florida to reducing production costs and enhancing environmental sustainability. Asian vegetables emerging and expanding in Florida not only increase profit for growers, but also enhance food diversity for consumers. The objective of this study was to gain a better understanding of the partitioning and usage of N and carbohydrates in two Asian vegetable crops: long bean (*Vigna unguiculata* ssp. *sesquipedalis* (L.) Verdc.) and angled luffa (*Luffa acutangular* (L.) Roxb.). Four N rates (0, 0.91, 1.36, and 1.81 g N pot^{-1}) were compared in a high tunnel trial to understand the influence of N fertilization on the two crops. For long bean, plant biomass was highest at the highest N input, and N-fertilized plants had significantly higher leaf greenness than the control at the flower initiation and mid-reproductive stages. However, N inputs had no apparent effect on yield, nitrogen use efficiency (NUE), blade total N concentration, roots (length, volume, dry biomass, and root-to-shoot ratio), or nodules (number plant^{-1} and biomass). For luffa, the highest N input had significantly greater total yield, fruit number, and leaf greenness at the flower initiation and mid-reproductive stages, although there was no significant difference in shoot biomass, blade total N content, or NUE among treatments. Within the range of these N rates, our results suggest that higher N inputs promoted vegetative growth of long bean, whereas reproductive growth was promoted in luffa. This study highlights differences in the sink–source relationship of N for long bean and luffa production in high tunnel, which can guide N input decisions for these two crops that are rapidly expanding in the USA.

Keywords: ammonium nitrate; high tunnel; *Luffa acutangular*; nodule; source partition; *Vigna unguiculata* ssp. *sesquipedalis*

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1. Introduction

Asian vegetable production is rapidly expanding in the USA. There were less than 40 hectares being cultivated in northeast Florida in 2013 compared to more than 2500 hectares in 2017 [1]. These "foreign" crops are much more profitable than traditional vegetable crops. One hectare of Asian vegetables can generate USD 99,000 gross income (personal communication with local grower John Sykes). More and more growers have switched from traditional vegetables to these new crops in recent years. Inputs of synthetic fertilizers are often excessive in vegetable production systems, which not only adds to production costs, but also increases the potential for environmental pollution [2]. Nitrogen (N) is the most common limiting nutrient in cropping systems; hence, N inputs are typically high in crop production. However, high N inputs often result in low N use efficiency due to leaching, runoff, soil erosion, denitrification, and volatilization. Nitrogen loss is typically more severe in vegetable systems due to their shallower roots relative to agronomic row crops and fruit crops [3], although this varies among vegetable crops [4]. As a result, N management must be optimized for individual crops to maximize yields while minimizing negative effects.

Long bean (*Vigna unguiculata* ssp. *sesquipedalis*) is an annual herbaceous vine in the legume family. Long bean pods are nutritious, with 5–60% starch and 24% protein (dry weight), including multiple amino acids; they also contain various vitamins, minerals, and cellulose [5,6]. Long bean is susceptible to root knot nematodes (*Meloidogyne* spp.), but it is relatively resistant to disease compared to pole beans [7]. Powdery mildew (*Erysipbe* spp.) and aphids (Aphididae) do not cause severe damage to plant growth or production [8]. Long bean is tolerant to hot, dry, and infertile soils [6]. It is an effective N-fixer, especially during blooming and with *Rhizobium* populations responsible for biological N fixation (BNF), peaking about 30 days after planting [9]. Several *Rhizobium* strains can significantly increase N content, dry matter, and yield in long bean [10]. However, root nodule growth and activity are negatively regulated by N concentration in the phloem [11]. Sinsiri and Homchan [9] reported that hydroponic long bean plants with a N input of 70 mg N L^{-1} had no *Rhizobium* nodules, while there was no significant difference in plant fresh weight between N input and *Rhizobium* inoculation.

Angled luffa (*Luffa acutangula*) is also an annual herbaceous vine in the cucumber family, the Cucurbitaceae (also called cucurbits or the gourd family), originating from tropical Asia [12]. Luffa is a good source of mineral nutrients like phosphorus, potassium, and vitamins A and C, as well as phytochemicals [13]. Fully mature and old fruits have medicinal uses and can also provide fibrous sponges for cleaning. Many crops in the cucumber family have antidiabetic activity due to cucurbitacin, and consuming immature luffa fruits can help manage diabetes [14,15]. Luffa has a wide distribution in various environments, including multiple soil types and nutrient and moisture conditions, and N is one of the major limiting factors of its growth, especially in sandy soils [16]. Increasing N inputs increases vegetative and reproductive growth in luffa, including vine length, dried biomass, leaf number, and fruit yield [17], with a strong correlation between leaf and fruit production [18]. Higher N concentration also increases the shoot–root ratio in luffa because shoots are more affected by N than roots [19].

Long bean and luffa are part of a group of rapidly expanding Asian vegetables in Florida, increasing growers' profit and consumers' food diversity. These two crops are the major ethnic vegetables from the cucumber and legume families grown in Florida. This was the first study of these crops in the state, which are all from Asia, and are treated as one group by growers in the state. They were therefore studied together in this manuscript.

Both crops are currently commercially grown in Ellzey fine sands; classified as sandy, siliceous, hyperthermic Areni Endoaqualfs [20]. However, cultivation recommendations are unavailable for both crops; thus, growers often over-fertilize with N, which can lead to leaching in the sandy soils of Florida, especially during rainy summers. This high tunnel study aims to gain a deeper understanding of the partitioning and usage of N and carbohydrates in long bean and luffa. The central hypothesis was that high N inputs would maximize luffa growth and yield, whereas moderate N inputs would favor long bean growth, root nodule formation, and yield.

2. Materials and Methods

2.1. Plant Materials and Cultivation

Angled luffa ('Chinese Okra') seeds were purchased from Kitazawa Seed Co. (Oakland, CA, USA). The seeds were put in petri dishes with moistened filter paper, sealed in a Ziploc bag, and put in an incubator (27–32 °C) on 6 September 2019 in the Horticultural Sciences Department at the University of Florida (Gainesville, FL, USA). Germinated seeds were transferred to a tray with vermiculite on 9 September 2019. Long bean ('Bai-lung') seeds were procured from Tainong Seeds (Vista, CA, USA) and directly sown in a tray with vermiculite. Both trays were put in the high tunnel until 21 September 2019, when 24 healthy and visually uniform seedlings of each crop were transplanted to two-gallon pots with potting medium (Premier PRO-MIX HP Mycorrhizae High Porosity Grower Mix). The potting medium contained sphagnum peat moss (65–75%), limestone, a wetting agent, and mycorrhizae. The potting mix label indicated a soil pH between 5.2 and 5.8, although this was not measured during the growing season. Transplanted seedlings were then moved to a high tunnel (west–east direction) for the experiment.

These 48 pots were organized in a randomized complete block design (RCBD) next to two irrigation pipelines on the north and south sides of the high tunnel. Although field production is typical for these crops, a high tunnel provides a controlled environment that allowed for a closer look at plant physiology, especially roots and nodules. The 24 plants of each crop were randomly assigned into six groups of four pots. Within each group, a pot was assigned to one of four N rates using ammonium nitrate: 0 (control), 0.91 (low), 1.36 (medium), and 1.81 (high) g N pot^{-1}. Given the soil volume and pot area used in this study, these N rates were equivalent to 0, 112, 168, and 224 kg N ha^{-1} for a plant density of 6250 plants ha^{-1} in field conditions. Other growing conditions (light, water, other nutrients) were identical among groups. Ammonium nitrate, potassium chloride (6.79 g pot^{-1}), and monosodium phosphate (4.53 g pot^{-1}) were applied as dry fertilizers four times during the experiment, from 13 October 2019 until 9 December 2019: at transplant (40%), blooming (20%), fruiting (20%), and after the first harvest (20%). We applied only one type of fertilizer per fertilization event, and there were approximately five-day gaps between two applications to avoid plant burning. Dry fertilizers were buried on the surface of each pot, opposite of the drip nozzle. The irrigation ran for 1–10 min (adjusted according to plant growth and weather) in the morning and in the late afternoon every day through a micro nozzle in each pot, to provide a moisture content just below the field capacity of about 400 mL water for each pot. The vines were trained onto the trellis and high-pressure water was used to protect plants from aphids. The high tunnel cover was rolled down to the ground when air temperature decreased below 10 °C. Luffa fruits are considered marketable if they are greater than 2.5 cm in diameter with tender dark green peels. Marketable long bean pods are about 0.8 cm in diameter. Non-marketable fruits (small, yellow, and dead fruits) were harvested and not considered as yield.

2.2. Leaf Sampling and SPAD

Ten newly matured leaves without defects were sampled from the top to the bottom of each plant during the afternoons of 24 October, 7 November, and 27 November in 2019, corresponding to flower initiation, first harvest, and mid harvest. Leaf greenness was measured using a SPAD 502 (Minolta Camera Co., Osaka, Japan) as a proxy for leaf chlorophyll content. Several long bean plants (one plant from each of the treatments of 0, 1.36, and 1.81 g N pot^{-1}, respectively) were determined with fewer than ten leaves per plant because there were not many leaves due to the early development stage or stress, such as water surplus, which resulted from the water demand of luffa plants in the same irrigation line, and aphids.

2.3. Aboveground Plant Dry Biomass

Three healthy and uniform plants were randomly harvested from each treatment for either crop. The plants were cut from the stem at the soil surface on 25 January 2020 for luffa, and on 1 February 2020 for long bean. Leaves from each plant were separated from the stem, dried (75 °C) for one week, and analyzed for total nitrogen concentration by combustion at Waters Agricultural Labs (Camilla, GA, USA). The stems for each plant were put in a paper bag and dried (75 °C) for eight (luffa) or three (long bean) days, and each plant, including the leaves, was weighed for dry biomass, based on a slightly modified method from Islam [21].

2.4. Root and Nodule Sampling for Long Bean

The roots of each sampled long bean plant (three plants per treatment) were first washed with high-pressure water using a Melnor 5-Pattern Watering Nozzle at harvest. Tweezers were used to pick out remaining wood and soil particles from each root. Main root length (the maximum length of roots) was measured with a ruler and root volume was measured by water displacement. Any nodules found on long bean roots were separated and counted. Nodules and bare roots were dried (75 °C) for three days and weighed. The dry weight of nodules of each plant was added to the bare root dry biomass:

$$\text{Root dry biomass (g)} = \text{Nodule dry biomass (g)} + \text{Bare root dry biomass (g)} \quad (1)$$

The root–shoot ratio (R/S) of long bean plants was calculated by dividing the root dry biomass by the aboveground dry biomass:

$$\text{R/S} = \text{Root dry biomass (g)/Aboveground dry biomass (g)} \quad (2)$$

2.5. Yield and NUE

Weekly or biweekly harvest of luffa fruits and long bean pods reaching marketable maturity started on 1 November 2019 (long bean) or 7 November 2019 (luffa) and continued until 20 January 2020. Long bean pods and luffa fruits were weighed and counted for total number per pot. We calculated nitrogen use efficiency (NUE) by dividing yield by the amount of nitrogen added (Figure 1):

$$\text{NUE (g fruit/g N)} = \text{(YieldTreatment} - \text{YieldControl) (g fruit)/Nitrogen input (g N)} \quad (3)$$

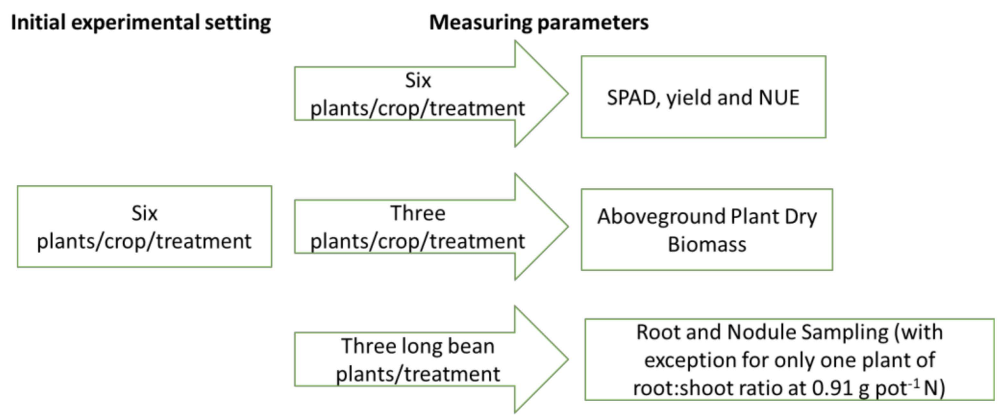

Figure 1. Initial experimental setting and measured parameters.

2.6. Statistical Analysis

All data were analyzed by using R (Version 4.0.0). Leaf SPAD readings, yield and fruit numbers for luffa, aboveground dry biomass, root volume, root length (log-transformed), R/S, nodule dry biomass, yield (square-root transformed) for long bean, blade total N content, and NUE for both crops were analyzed with a one-way ANOVA to find out if the means were significantly different from each other. Means with significant differences were separated via Tukey's HSD ($p \leq 0.05$). Homogeneity of variances was tested using Bartlett's test and normality of residuals was tested with the Shapiro–Wilk test for each variable, to ensure an ANOVA could be used.

When ANOVA conditions were not met, the Kruskal–Wallis test was used instead for SPAD readings, aboveground dry biomass for luffa, root dry biomass, average nodule mass, and nodule number per plant^{-1} for long bean. Means with significant differences were separated with a pairwise Wilcoxon test, with a Holm correction to correct for multiple testing ($p \leq 0.05$).

3. Results

3.1. Leaf SPAD Readings

SPAD readings for long bean leaves followed a decreasing trend over time (Figure 2A). Plants fertilized with N had significantly greater SPAD readings than the unfertilized control during flower initiation on 24 October 2019. At first harvest (7 November 2019), plants with the intermediate N inputs had statistically greater SPAD readings than the unfertilized control, whereas only plants with the highest N input treatment had significantly greater SPAD than the other treatments at mid-harvest, on 27 November 2019 (Table 1).

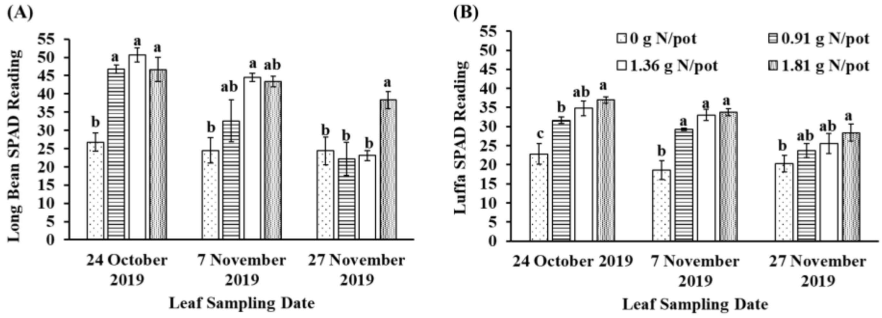

Figure 2. Long bean (**A**) and luffa (**B**) leaf SPAD reading throughout the reproductive stage: at flower initiation (24 October 2019), first harvest (7 December 2019), and mid-harvest (27 December 2019). Each bar represents the mean with a standard error of six pots per treatment. Different letters indicate statistically significant differences between the N rates for the crop on a specialized date.

Table 1. ANOVA *p* values of long bean and luffa. Luffa's root traits were not measured in this study.

Crop	Biomass	Blade Nitrogen	Yield	NUE	Number of Fruits	SPAD Reading			Root			Root/Shoot	Nodule	
						Treatment (A)	Date (B)	A × B	Biomass	Length	Volume		Number	Biomass
Long Bean	0.008	0.275	0.626	0.241	N/A	0.413	<0.001	<0.001	0.065	0.338	0.089	0.883	0.117	0.229
Luffa	0.312	0.347	0.007	0.830	0.079	<0.001	<0.001	0.281	N/A	N/A	N/A	N/A	N/A	N/A

For luffa (Figure 2B), there was a similar decreasing trend over time. At flower initiation, plants with the highest N input had significantly greater SPAD readings than the low N input and control treatments. At first harvest, plants with N input treatments had statistically higher SPAD reading than the unfertilized control, whereas plants with the highest N input were significantly higher than the control at mid-harvest.

3.2. Aboveground Plant Dry Biomass and Total Nitrogen Content

Long bean fertilized with the highest N rate had a significantly higher aboveground dry biomass than the plants with the low N rate and the control (Figure 3A), whereas there was no significant difference among treatments for luffa plants (Figure 3B). In addition, there was no significant difference in leaf blade N among treatments for both crops (Figure 4A,B).

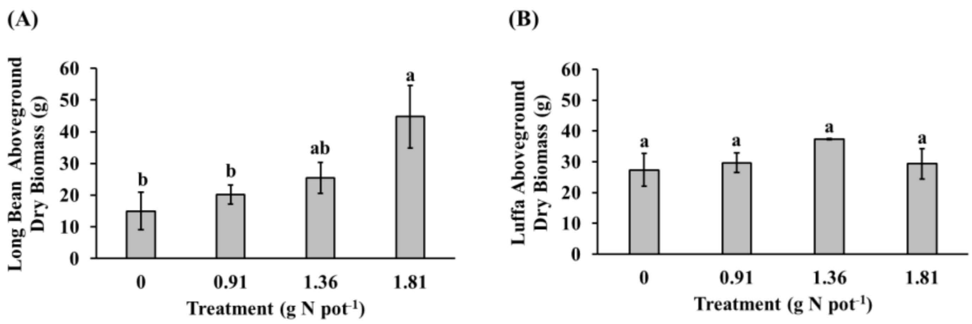

Figure 3. Long bean (**A**) and luffa (**B**) aboveground dry biomass. Letters indicating statistically significant differences should be interpreted within a specific date and crop only. Different letters indicate statistically significant differences between the N rates for the crop.

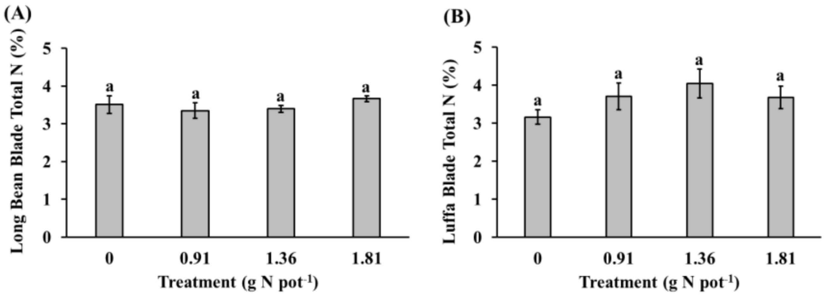

Figure 4. Long bean (**A**) and luffa (**B**) blade total N (%). Letters indicating statistically significant differences should be interpreted within a specific date and crop only. Different letters indicate statistically significant differences between the N rates for the crop.

3.3. Roots and Nodules of Long Bean

Nitrogen inputs had no significant effect on long bean main root length, root volume, root dry biomass, and R/S (Figure 5). Nodule number and nodule dry biomass were not significantly affected by N rates, but there were large variations among treatments (Figure 6).

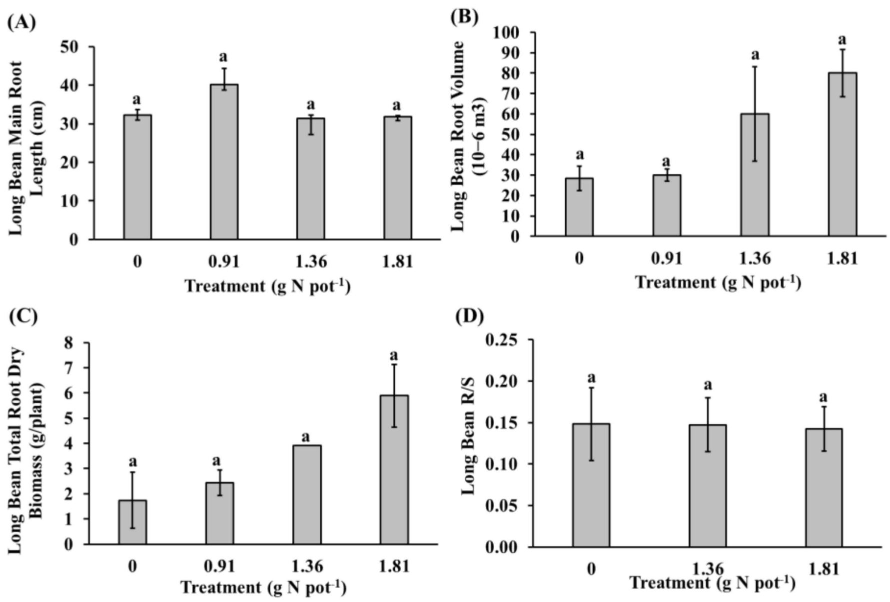

Figure 5. Long bean root traits: (**A**) main root length, (**B**) root volume, (**C**) root dry biomass, and (**D**) root–shoot ratio. Each bar represents the mean with a standard error of three pots per treatment, except for the root–shoot ratio at 0.91 g pot^{-1} N (**D**). where only one pot was measured, and no standard error could be computed (data not shown). Different letters indicate statistically significant differences between the N rates for the crop.

There was no significant effect of N inputs on long bean yield (Figure 7A). In contrast, luffa yields were significantly greater with the highest N input compared to the low N input and the control (Figure 7B). A similar trend was observed for luffa fruit number, although in this case the highest N input was only statistically greater than the control (Figure 7C).

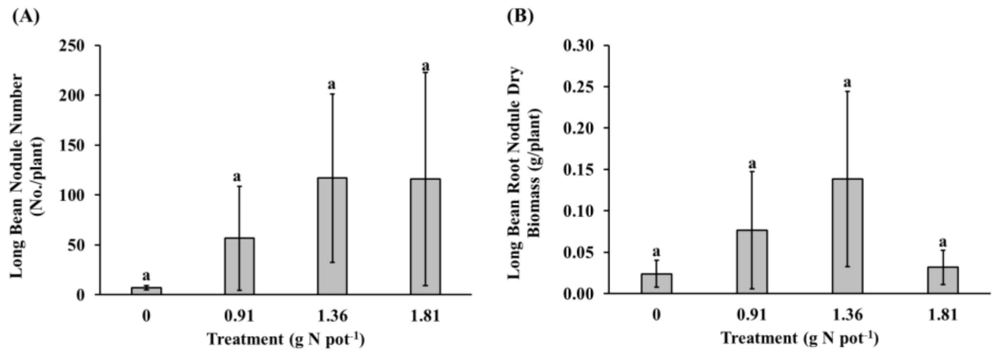

Figure 6. Long bean (**A**) nodule number and (**B**) root nodule dry biomass. Letters indicating statistically significant differences should be interpreted within a specific date and crop only. Different letters indicate statistically significant differences between the N rates for the crop.

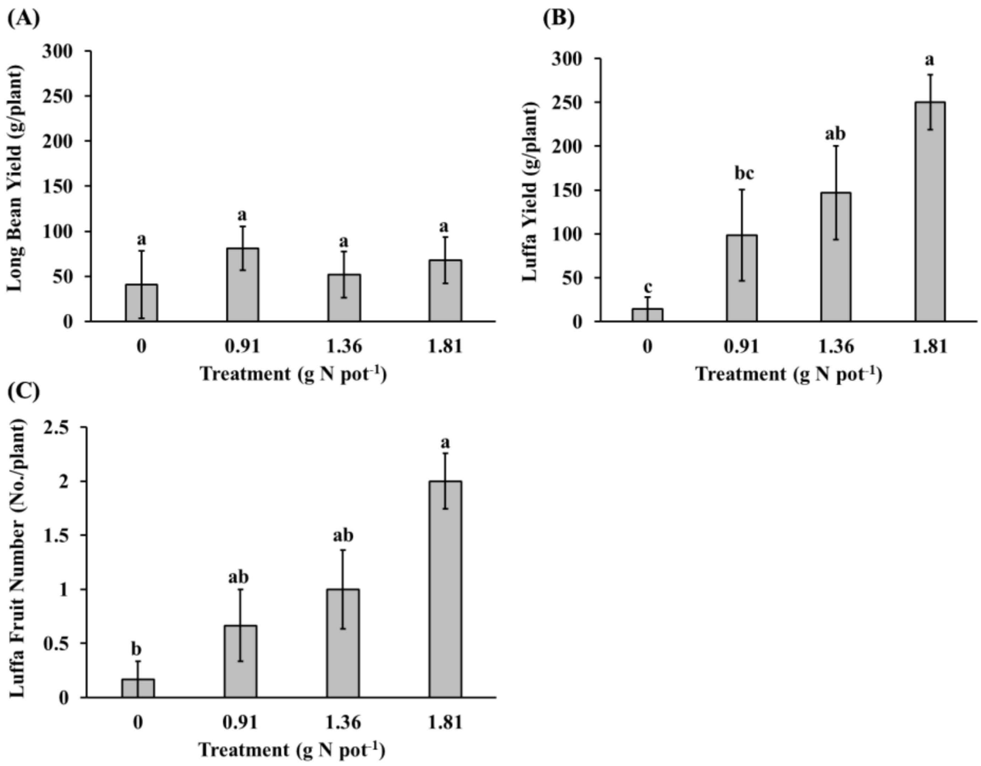

Figure 7. Cumulative yield (g/plant) of (**A**) long bean and (**B**) luffa, and (**C**) cumulative fruit number of luffa. Each bar represents the mean with a standard error of six pots per treatment. Different letters indicate statistically significant differences between the N rates for the crop.

3.4. Nitrogen Use Efficienct (NUE)

Nitrogen treatments did not affect NUE significantly for long bean or luffa, and NUE was twice as large for luffa compared to long bean (Figure 8).

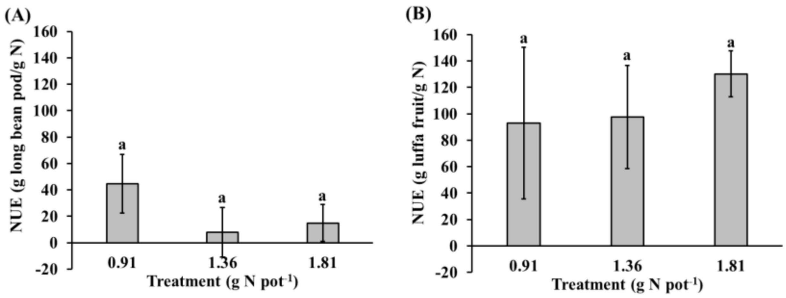

Figure 8. Nitrogen use efficiency (NUE) of long bean (**A**) and luffa (**B**). Each bar represents the mean with a standard error of six pots per treatment. Different letters indicate statistically significant differences between the N rates for the crop.

4. Discussion

4.1. Nitrogen and Carbon Partitioning and Plant Growth

Leaf greenness reflects the nitrate level of plants [18,22,23], and the SPAD meter can reliably estimate plant N level and the yield of crops like cucumber and rice [24,25]. In this study, there was no correlation between SPAD readings and blade N concentration for both luffa and long bean (result not shown), most likely because measurements of blade total N were made two months after the last SPAD measurement. These two types of measurements were not paralleled at the same time because there were not enough leaves to sample for leaf N measurements without affecting crop health. The leaf blade N concentration was determined after the harvest was completed and it was similar among treatments, likely because of plant senescence after harvesting. At harvest, most nutrients were withdrawn from the vegetative organs to the fruits [26]. This is consistent with the decreasing trend in leaf SPAD readings observed during the reproductive stage for both crops fertilized with N. SPAD can be the diagnostic tool for N status in cucumber [27]. Mature leaves become the source for new growth when there is a nutrient deficiency [28]. In the late growth stage, SPAD readings were decreasing (Figure 2), which may be explained by nutrient withdrawal from the leaves to the fruits.

Higher N inputs increased long bean shoot biomass and root volume relative to plants receiving the low N rate, although it did not increase yields. This is consistent with a previous study on soybean [29] and our observation of long bean new shoots and vines developing even at the end of harvest. This may be due to the indeterminate growth habit of long bean, where vegetative parts compete for carbohydrate sinks with reproductive parts [30]. There was no significant difference in the R/S ratios among treatments, which is contradictory with Ryle et al. [31], who reported that white clover had a greater R/S ratio with no N input than with N inputs. This may be because root growth was constrained by the relatively small containers used in this study. Nitrogen had no significant effect on long bean pod yield, although there was a non-significant declining trend in NUE at higher N inputs, which could indicate a reduction or suppression of N-fixation at high N inputs. Previous studies reported that BNF can meet 50–60% of N demand in soybeans [32], with a declining contribution of BNF as N inputs increase [33], and up to 94% higher yield relative to the non-inoculated controls [34].

Luffa yields increased with greater N inputs, consistent with reports by Hill et al. [17] for luffa and Patil et al. [35] for bitter gourd. Hill et al. [17] suggested that higher N bioavailability increases flower set, fruit number, and yield, though we did not measure flower set in this study. The trend of NUE to increase for luffa as N inputs increase could suggest insufficient N inputs to meet the production potential of luffa plants in this study. Meanwhile, there was no significant difference in shoot biomass among treatments, potentially because carbohydrates were allocated to reproductive parts in priority due to low N supply in this study.

4.2. Effects of N Inputs on Long Bean Nodules

Nitrogen bioavailability decreases *Rhizobium* infection in legume roots, the number and size of legume nodules, and nitrogenase activity per unit of mass, although nitrogenase activity can recover after lowering N inputs [36]. In this study, there was no significant effect of N inputs on nodule abundance or weight, suggesting that these N inputs did not affect nodule formation or growth. However, a non-significant decreasing trend in NUE at higher N rates and a lack of significant differences for pod yield with or without N inputs suggests a possible inhibition of N fixation activity at the highest N rates used in this study. This could be due to late N applications that would inhibit nitrogenase activity [37,38]. In addition, using split N applications might have enhanced the inhibitory effect of N inputs on fixation, as small but frequent nitrate inputs increase nitrate absorption [39]. However, it remains unclear if BNF was indeed inhibited in this study, and if so, which specific inhibition mechanism was at play.

Nitrate is the N form most found in soils [40], and soil nitrate is often linked to N fixation inhibition [36]. On the other hand, low N inputs can be beneficial to plants and nodules, as low N inputs can lead to more vigorous plant growth [41], enhanced diversity of *Rhizobium* strains in nodules [42], and yields reaching their full potential [43–45]. This is consistent with the numerically higher nodule mass of long bean plants with medium N inputs in this study, although the difference with other treatments was not statistically significant. Furthermore, N speciation in soils can affect nodule formation, although reports on the effects of nitrate vs. ammonium on nodule formation are contradictory [46,47]. Hence, future studies focusing on a few N sources with a single type used at each time at different rates may better determine the effects of N sources and rate on BNF in long bean, allowing the maximization of N inputs from BNF and ultimately reducing fertilizer N inputs.

5. Conclusions

In this high tunnel study, two Asian vegetable crops, long bean and luffa were investigated. N inputs enhanced vegetative growth of long bean, potentially inhibited N fixation and had no significant effect on reproduction within the range of N inputs used. In contrast, luffa allocated N and carbohydrates to reproductive parts in priority when N supply was insufficient, within the range of N rates applied. These results provide important insights on which N appication rate is best for long bean and luffa production in controlled environments, while also informing N management for field production of these two crops. Among the four N rates studied, 1.81 g/plant N had significantly greater fruit yield and number of fruits than the control or lower N rates for luffa. This N rate can be considered as the best N input for potted luffa production in high tunnel conditions. As an effective nitrogen fixer, long bean did not have any significant difference in pod yield between the four N rates. However, the potential benefits of lower N inputs for long bean and higher N inputs for luffa should be investigated further in future studies.

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