Journal of Tropical Crop Science Vol. 12 No. 1, February 2025
www.j-tropical-crops.com

Received 21/11/2024; Revised 30/01/2025; Accepted 05/02/2025
https://doi.org/10.29244/jtcs.12.01.116-123

# Effect of Nitrogen on Intercropped Radish (*Raphanus sativus* L.) and Spinach (*Spinacia oleracea* L.) Productivity under Greenhouse Conditions

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## Abstract

Due to a rapidly increasing global population and limited arable land, there is a need to increase crop productivity per unit area and optimize resource utilization by plants. One strategy to achieve improved resource utilization is intercropping, whereby two or more crops are grown in mixed or alternating patterns within a block of land. This study aimed to evaluate the effect of nitrogen on radish (*Raphanus sativus* L.) and spinach (*Spinacia oleracea* L.) intercropping on crop growth parameters and yield. Intercropped radish and spinach grown in the greenhouse resulted in only one radish-spinach intercrop ratio (50:50) with the same or greater productivity as radish and spinach monocultures, and it did not include an addition of nitrogen. The LER value associated with this treatment was 1.06. However, adding fertilizer resulted in significantly different ($P<0.05$) leaf numbers, chlorophyll content, and dry biomass of intercropped radish and spinach compared to treatments without fertilizer, except for leaf number in radish. Radish and spinach's replacement series response curves are concave and convex, respectively, in fertilizer and non-fertilizer treatments. The response curves imply that radish is more aggressive than spinach in intercropping systems. In the only intercrop treatment with an LER>1.0 (50:50 without fertilizer), radish was 2.9 times more competitive than spinach.

Keywords: chlorophyll content, crop productivity, dry biomass, intercrop, leaf number

## Introduction

Due to a rapidly increasing global population and limited arable land, there is a need to increase crop productivity per unit area and optimize resource utilization by plants. One strategy to achieve improved resource utilization is intercropping, whereby two or more crops are grown in mixed or alternating patterns within a block of land (Deb et al., 2022; Yousif, 2023; Ngairangbam, 2024). Intercropping is receiving growing interest for its potential to improve biodiversity, crop productivity, and farm income (Głowacka and Flis-Olszewska, 2022; Yang et al., 2022; Tamburini et al., 2020). Intercropping has also shown potential for improved pest, disease, and weed management (Ling et al., 2022; Liu et al., 2023). The improved efficiencies seen in intercropping are attributed to the complementary resource utilization created by the diversity of the intercrop components (Boetzl et al., 2023; Li et al., 2023). One measure of improved efficiency created by intercropping is the land equivalent ratio (LER), defined as the amount of land required in mono-crops to attain the same yield as in intercrops at the same management level (FAO, 1985). A global meta-analysis of 939 intercropping observations found an LER of 1.30 in irrigated or non-irrigated systems, regardless of fertility management or intercropping pattern. This study also found that intercropping increased gross income by 33% compared to monoculture (Martin-Guay et al., 2018). However, resource use and time to maturity must be considered for each species to achieve optimal crop productivity and quality in intercropping systems.

Spinach (*Spinacia oleracea* L.) is a nutrient-dense, cool-season leafy vegetable from the Amaranth family. Spinach seeds germinate from 2°C to 30°C,

Received 21/11/2024; Revised 30/01/2025; Accepted 05/02/2025
https://doi.org/10.29244/jtcs.12.01.116-123

while growth is optimal at 15°C to 18°C, and are capable of withstanding temperatures as low as -9°C (Koike et al., 2011). These growth characteristics allow productive cropping seasons to be extended early in the spring or late into the fall, particularly with greenhouses or high tunnels. High tunnels require a fraction of the investment of a greenhouse and can extend a growing season as much as four weeks in the spring and eight weeks in the fall (Wells and Loy, 1993). Spinach has demonstrated viability as a component in several intercropping systems. Intercropping spinach has resulted in LER values of 1.66 with groundnut (Monim et al., 2010), 2.29 with ginger and pointed gourd (Islam et al., 2015), and 1.78 with potato (Singh et al., 2016). These findings indicate the potential of spinach for complimentary resource utilization with various other crops.

Radish (*Raphanus sativus* L.), another important vegetable crop, is an open-pollinated root plant in the Brassica family. The minimum, maximum, and optimum range of soil temperature conditions for radish seed germination are 4°C, 35°C, and 18 - 29°C, respectively (Pothour, 2017). These growth attributes allow radish seeds to be planted in spring and fall. Growing should be suspended at the peak of summer, when hot temperatures can cause radishes to bolt, rendering them unviable for consumption. The need thus arises for more controlled environmental conditions in certain regions, such as a greenhouse. Pinheiro et al. (2019) evaluated the response of collard green and radish crops in monoculture and intercropping under different spatial arrangements, reporting that spatial arrangements did not affect the growth and development of radish or collard green and that all arrangements resulted in high LER the greatest being 1.69. The effect of paired row planting of radish intercropped with vegetable amaranths on yield components of radish in sandy regosol was investigated by Brintha and Seran (2010). The study resulted in high LER values in all tested intercropping treatments. In addition to high LER values associated with intercropping radish, the genetic diversity of different radish varieties could improve cadmium absorption and biomass of radishes of some varietal intercropping combinations (Lin et al., 2014).

Nitrogen (N) is commonly the most limiting nutrient in crop production in field conditions and greenhouse settings (Waladi, 2023; Ma et al., 2022). Spinach responds significantly to increased N fertility with increases in leaf number, leaf area, and total yield (Mola et al., 2020; Gülüt and Şentürk, 2024). Like spinach, Jilani et al. (2010) found that higher N levels resulted in higher leaf number, length, and biomass; root length, diameter, and weight; and yield in radish compared to treatments without N. Therefore, supplemental N fertilizer could be a key spinach productivity variable when intercropping with other vegetables, such as radish. Considering the potential productivity improvement offered by both intercropping and greenhouse production, this study aimed to evaluate the N effect on intercropped radish and spinach on crop growth parameters and yield. Research that explicitly examines the effect of N on radish-spinach intercropping is still limited - thus, there is value in conducting this study. It is hypothesized that at least one radish-spinach intercropping ratio will have the same yield as radish and spinach monocultures. The N addition will produce at least one radish-spinach intercropping ratio with the same yield as N-fertilized radish and spinach monocultures.

## Materials and Methods

*Greenhouse Experiment*

A greenhouse experiment was conducted in Spring 2020 at the University of Nebraska-Lincoln, Department of Agronomy and Horticulture, Weed Science Research Greenhouse, Lincoln, NE. The study was a random complete block design with four replications, two nutrient levels (addition of urea and no urea) as the main plot effect and five intercropping ratios of spinach and radish as the subplot effect. Spinach variety "Bloomsdale" and radish variety "Cherry Belle" were purchased from a local garden store, Earl May (Lincoln, NE), as seed packages. Spinach and radish seeds were planted on January 23, 2020, at a depth of 2.5 cm in a replacement series of 100:0, 75:25, 50:50, 25:75, and 0:100 (radish: spinach (R:S)). Seeds were sowed into 51 cm x 36 cm x 12 cm (length x width x depth) flats filled with a soilless mix of a 1:1:1 mixture (w/w) of peat moss, perlite, and vermiculite for optimal drainage and water retention. To mimic interplanting, spinach or radish seeds were sowed in four rows, with four plants per row (Figure 1). Each flat contained a total of 16 plants. All four rows were radish or spinach in the monoculture flats (100:0 and 0:100 R:S). In the intercrop flats, 75:25 or 25:75 (R:S) treatments represent three rows of radish and one row of spinach or one row of radish and three rows of spinach, respectively. Flats with 50:50 (R:S) treatments were planted with two alternating rows of spinach and radish each. All spinach and radishes emerged by January 27, 2020, and on February 4, 2020, each row was thinned to 4 plants per row according to its respective replacement series treatment.

On February 13, 2020, granular urea (46-0-0 NPK) from Pro-AP (Wawaka, IN) was applied to the N (N) fertilizer treatments at an optimal rate of 2 ppm per

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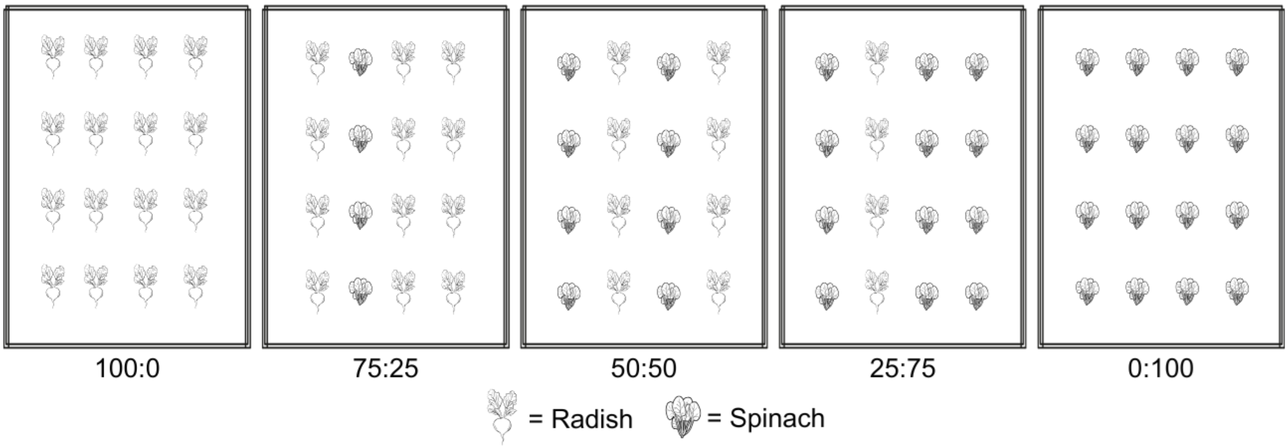


Figure 1. Replacement series of 100:0, 75:25, 50:50, 25:75, and 0:100 for radish and spinach in the flatted tray.

flat. Greenhouse conditions were set at minimum and maximum temperatures of 21.1°C to 26.7°C during the day and 18.3°C to 21.1°C at night, respectively. The supplemented light was on from 6:00 to 20:00 to mimic optimal growth conditions, and high-pressure sodium bulbs were used with a peak-light irradiance of 1200 W.m$^{-2}$. All treatments were watered once a day until the end of the study to avoid drought stress.

*Data Collection*

Leaf chlorophyll content was measured on February 14, 21, and 28, 2020, using a MINOLTA SPAD-502 (SPAD) chlorophyll meter. Leaf numbers per plant were also recorded for each flat. Upon harvest, spinach was cut at the soil level, and radish was harvested without the fibrous root attached to the taproot. Each crop corresponding to the flats was harvested and placed in a paper bag. All plants were dried to constant weight in an oven set at 60°C. The dry biomass of spinach or radish per flat was then divided by the number of plants of each crop per intercrop treatment to get the average yield per plant. Intercropping productivity was measured by land equivalent ratio (LER) using the following equation:

$$LER_{R,S} = LER_R + LER_S = \frac{Y_{R,S}}{Y_{R,R}} + \frac{Y_{S,R}}{Y_{S,S}}$$

where $LER_R$ and $LER_S$ are the land equivalent ratios of radish and spinach monocultures, respectively, $\frac{Y_{R,S}}{Y_{R,R}}$ is the yield ratio of intercropped radish over radish monoculture and $\frac{Y_{S,R}}{Y_{S,S}}$ is the yield ratio of intercropped spinach over spinach monoculture. The competitive ratio (CR) of each crop at each intercrop density was also calculated using the following equation:

$$CR_{R,S} = \frac{LER_R}{LER_S} * \frac{X_{S,R}}{X_{R,S}}$$

where $\frac{LER_R}{LER_S}$ is the ratio of radish and spinach LER and $\frac{X_{S,R}}{X_{R,S}}$ is the ratio of planted areas of radish and spinach.

*Statistical Analysis*

Data was analyzed as a general linear model using PROC GLIMMIX in SAS (Version 9.4, SAS Institute Inc., Cary, NC). Mean separation was performed using Fisher's least significant difference at $P < 0.05$.

## Results and Discussion

Intercropped radish and spinach grown in the greenhouse resulted in only one radish-spinach intercropping ratio with the same productivity as radish and spinach monocultures, and it did not include an addition of N. Statistical analysis showed there is no interaction between fertilizer and intercrop density on all collected data parameters (Table 1). However, the addition of fertilizer resulted in significantly different ($P<0.05$) leaf numbers, chlorophyll content, and dry biomass of intercropped radish and spinach compared to treatments without fertilizer, except for leaf number in radish (Table 2). The average radish leaf number with added fertilizer and without was 5.7 and 5.4, respectively. Conversely, Spinach resulted in a significantly higher leaf number of 6.5 with fertilizer and 5.0 without fertilizer. These results are similar to those reported by Lekgoathi et al. (2022), where the leaf area of spinach increased with the application of N in the well-watered treatment.

Chlorophyll content was measured using the SPAD chlorophyll meter, which outputs the 'greenness' of leaves in SPAD units. A higher SPAD unit correlates to the higher chlorophyll content of leaves, demonstrated by studies of tomato and pepper seedlings (Massimi et al., 2023), muskmelon (Azia and Stewart, 2001), and butterhead lettuce chlorophyll content using SPAD-502 meters (Leon et al., 2007). Results of this study show that adding fertilizer significantly increased chlorophyll content in both radish and spinach

 

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compared to treatments without fertilizer. Radish and spinach treated with fertilizer had chlorophyll contents of 36.6 and 71.5 SPAD units, respectively, while radish and spinach without fertilizer had chlorophyll contents of 26.8 and 29.5 SPAD units, respectively. These values are expected given that N plays a key role in leaf chlorophyll development - adding N results in higher leaf chlorophyll (Ansari et al., 2020).

Dry biomass was also significantly affected by the addition of fertilizer. Both radish and spinach had higher dry biomass of 1.8 and 0.2 g per plant with fertilizer, respectively, compared to dry biomass of 1.0 and 0.1 g per plant without fertilizer. Mirjalili and Poorazizi (2019) reported that higher nitrogen application significantly increased dry weight and total nitrogen content in spinach; Gazoulis et al. (2021) support the notion that increased nutrient availability, including nitrogen, positively influences spinach biomass. Moreover, Yousaf et al. (2021) found that nitrogen application significantly improved the growth and yield of radishes. Araujo (2023) reported that using organic inputs in radish cultivation led to a notable increase in root diameter and overall productivity.

Our results showed a significant interaction in intercropping density on dry biomass per radish and spinach plant. Radish had a significantly higher dry biomass per plant in intercropping ratios of 25:75 and 50:50 (R:S) compared to radish monoculture (Table 3). Conversely, spinach had significantly lower dry biomass per plant in all intercropping ratios than spinach monoculture (Table 3). These results indicate high intraspecific competition between radishes and significant interspecific competition between spinach and radishes. Cousens and O'Neill (1993) attributed these replacement series results as a qualitative change in outcome with increasing density, where one species is dominant at low density and the other is dominant at high density.

Regarding total LER, our results showed that an R:S intercrop ratio of 50:50 without fertilizer resulted in the highest LER of 1.06 (Figure 2). This LER value indicates that a 50:50 (R:S) intercropping system has a higher resource use efficiency over radish or spinach monocultures. No other intercrop ratio with or without fertilizer resulted in an LER>1, indicating that no other intercrop ratio attained the same yield as radish and spinach monocultures. The yield response curve for each crop can explain this phenomenon. In

Table 1. Significance levels of variance analysis on leaf number, chlorophyll content, and dry biomass per plant

| Treatment | Leaf number | Chlorophyll content (SPAD units) | Dry biomass (g per plant) |
|---|---|---|---|
| Fertilizer | * | * | * |
| Density | ns | ns | * |
| Fertilizer*Density | ns | ns | ns |

Notes: *Significant at α = 5%, ns = not-significant according to Fisher's least significant difference at P < 0.05.

Table 2. Effect of additional N fertilizer on leaf number, chlorophyll content, and intercropped radish and spinach dry biomass

| Fertilizer | Leaf number | | Chlorophyll content (SPAD units) | | Dry biomass (g per plant) | |
|---|---|---|---|---|---|---|
| | Radish | Spinach | Radish | Spinach | Radish | Spinach |
| +Nitrogen | 5.7 a | 6.5 a | 36.6 a | 50.6 a | 1.8 a | 0.2 a |
| No Fertilizer | 5.4 a | 5.0 b | 26.8 b | 29.5 b | 1.0 b | 0.1 b |

Notes: LS-means with the same letter in the same column are not significantly different according to Fisher's least significant difference at P < 0.05.

Table 3. Effect of plant density on dry biomass of intercropped radish and spinach

| Density | Dry biomass (g per plant) | |
|---|---|---|
| | Radish | Spinach |
| 100% | 1.11 b | 0.24 a |
| 75% | 1.19 b | 0.17 b |
| 50% | 1.58 a | 0.14 bc |
| 25% | 1.63 a | 0.11 c |

Notes: LS-means with the same letter in the same column are not significantly different according to Fisher's least significant difference at P < 0.05.

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Figure 2, the response curve of radish is concave, and the response curve of spinach is convex in both fertilizer and non-fertilizer treatments. According to replacement series outcomes, the response curves imply that radish is more aggressive than spinach in intercropping systems (Swanton et al., 2015), and intercropped radish and spinach reduce total yield compared to radish and spinach monocultures.

The aggressivity of radish can also be thought of in terms of its CR - a measurement of intercrop competition that indicates the number of times by which one component crop is more competitive than the other (Willey and Rao, 1980). According to Table 4, under 75:25 (R:S) intercropping, radish was 27.1 and 20.3 times more competitive than spinach with fertilizer and without, respectively. In the only intercrop treatment that had an LER>1.0 (50:50 without fertilizer), radish was 2.9 times more competitive than spinach, compared to 2.4 times more competitive than spinach with added fertilizer (Table 4). As the competitiveness of radishes decreased with decreasing density, the competitiveness of spinach increased with increasing density for fertilizer and non-fertilizer treatments, as illustrated in Figures 1 and 2 A.

## Conclusions

Our results indicate that the highest land productivity occurred in 50:50 intercropped radish and spinach without fertilizer. Considering the benefits that added N provides radish and spinach development, we were surprised that an intercrop ratio with fertilizer also did not result in an LER>1. According to these results, added N did not affect the measured parameters. Future research regarding intercropped radish and spinach productivity should ensure consistent management strategies, such as fertilizer application (dose and time) across all treatments. The number of treatment replications should also be increased for increased statistical support of our original hypotheses.

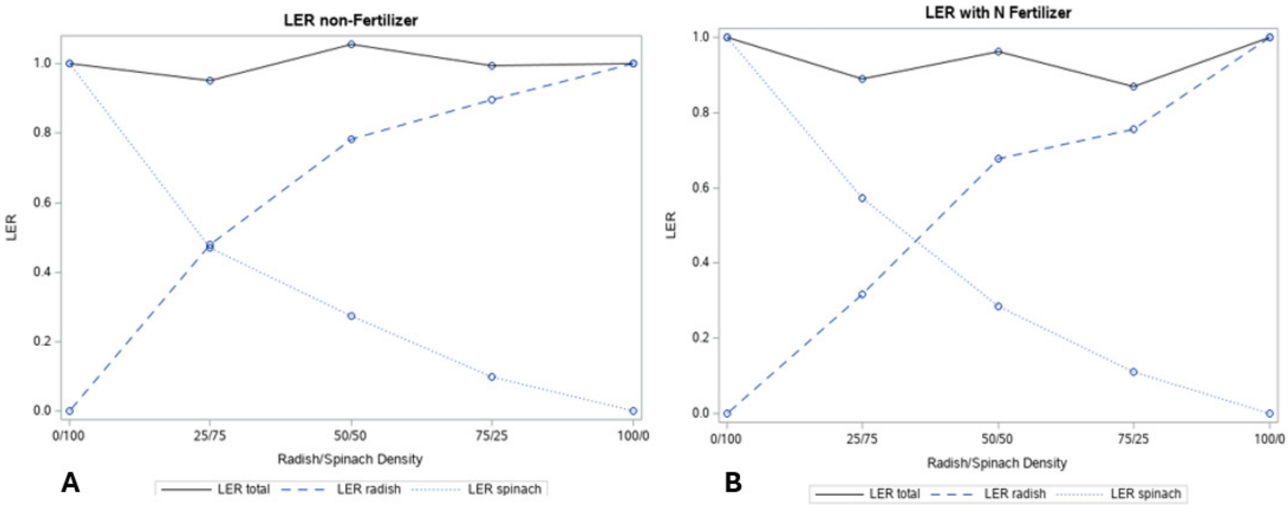


Figure 2. LER of intercropped radish and spinach without fertilizer (A) and with fertilizer (B).

Table 4. Competitive ratio values of intercropped radish and spinach

| Intercropping combination (R:S) | Competitive Ratio of radish | Competitive Ratio of spinach |
|---|---|---|
| No Fertilizer | | |
| 75:25 | 27.08 | 0.04 |
| 50:50 | 2.87 | 0.35 |
| 25:75 | 0.34 | 2.94 |
| With Fertilizer | | |
| 75:25 | 20.25 | 0.05 |
| 50:50 | 2.38 | 0.42 |
| 25:75 | 0.18 | 5.44 |

Received 21/11/2024; Revised 30/01/2025; Accepted 05/02/2025
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## Acknowledgments

Professor John Lindquist provided the opportunity to conduct this research for the 2020 Interplant Competition course at the University of Nebraska-Lincoln. Associate Professor Amit Jhala provided the extra greenhouse space needed to perform the last repetition of our study.

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Received 21/11/2024; Revised 30/01/2025; Accepted 05/02/2025
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