



Article

Optical Characteristics of Greenhouse Plastic Films Affect Yield and Some Quality Traits of Spinach (*Spinacia oleracea* L.) Subjected to Different Nitrogen Doses

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Abstract: Light and nitrogen strongly affect the growth, yield, and quality of food crops, with greater importance in green leafy vegetables for their tendency to accumulate nitrate in leaves. The purpose of this research was to explore the effect of two greenhouse films (Film A and B) on yield, and quality of spinach grown under different nitrogen regimes (not fertilized—N0%; sub-optimal N dose—N50%; optimal N dose—N100%). Film A and Film B were used as clear and diffused light films, with 75% and 87% thermicity, and 85% and 90% total transmittivity, respectively, where only Film B had a UV-B window. Film B elicited an increase in yield (22%) and soil–plant analysis development (SPAD) index (4.6%) compared to the clear film, but did not affect chlorophyll a, b, and total chlorophyll content. In addition, the diffuse film significantly decreased ascorbic acid in the crop but had no effect on lipophilic antioxidant activity and phenols content, but decreased ascorbic acid content. Finally, nitrate content was strongly increased both by nitrogen dose (about 50-fold more than N0%) and greenhouse films (about six-fold higher under diffuse light film), but within the legal limit fixed by European Commission. Therefore, irrespective of N levels, the use of diffuse-light film in winter boosts spinach yield without depressing quality.

Keywords: greenhouse clear film; greenhouse diffuse-light film; spinach yield; nitrate content; antioxidant activity; ascorbic acid



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1. Introduction

Spinach (*Spinacia oleracea* L.) is a major nutrient-dense leafy vegetable. It contains high amounts of Fe, K, Mg, vitamins (A, B6, C, K, E), antioxidants, chlorophylls [1,2] and it is rich in fiber, but very low in calories. Due to its special nutritional properties, about 1.7 million hectares are dedicated to spinach cultivation over the world [3]. Europe accounts for about 39,000 ha [3], mainly concentrated in the Mediterranean basin countries where the environmental conditions (light and temperature) are optimal to achieve the best response in terms of both yield and quality. In Italy, about 6300 ha are dedicated to spinach cultivation [4], of which 8.0% are under controlled conditions (greenhouses or tunnels).

One of the major factors that affects the high nutritional quality of spinach, and other green leafy vegetables is the nitrate content of the leaves, which shows adverse effects on human health [5–7]. Nitrate accumulation in plant tissues directly depends on (i) nitrogen (N) fertilization doses [6,8,9], (ii) type of N fertilizers [6,9–12], (iii) root N uptake [5] and (iv) N metabolism [13,14]; the latter includes both N mobilization from

the root to the shoot or N assimilation. This assimilation depends on the activity of the nitrate-reductase enzyme that is reported to be regulated by light through activation of the gene codifying it [15], and it is regulated by nitrate on the transcription and post-translation level. Therefore, there is a clear interactive light * N fertilization effect on the nitrate content of plants [5,6,16,17]. Regardless of N fertilization, light intensity is inversely correlated with the nitrate content of plant tissues [5,6,13,17–21], therefore nitrate content can be usefully reduced by increasing light intensity. Nevertheless, light is positively related to several other quality components like minerals, vitamins and antioxidants [17,21–23], including chlorophylls. Chlorophylls are responsible for leaf greenness that contributes to the esthetical value of several green-leafy vegetables including spinach [24]; in addition, they are involved in preventing mutant DNA from proliferating, that is responsible for some forms of cancer [25].

Light intensity changes across the seasons, and it is higher during spring/summer (maximum values in June/July) than autumn/winter period (minimum values in December) [26]. The nitrate content of green leafy vegetables is generally higher when they are grown during winter than during spring [19,20]. In addition, the Rd/Rg ratio (Diffuse radiation/Global radiation) also changes across the seasons. The Rd/Rg ratio is higher during autumn/winter than spring/summer period, due to the increasing cloud cover and precipitations [27]. Marpaung and Hirano [27] reported maximum and minimum Rd/Rg of 0.68 in October and 0.51–0.52 in June and July. It is well known that diffuse radiation determines beneficial effects on plant productivity [26], which are frequently reported as diffuse radiation fertilization effects [28] since they enhance the radiation use efficiency of plants. Global radiation (short-wave radiation) consists of direct solar radiation and diffuse radiation resulting from reflected or scattered sunlight due to several factors/compounds, such as air molecules, water vapor, clouds, dust, pollutants, forest fires, and volcanoes; overall, atmospheric conditions can reduce direct radiation by 10% on clear and dry days and by 100% on thick and cloudy days [29]. Similarly, the glass or plastic cover of a greenhouse intercepts a part of solar radiation, creating light conditions different from open field conditions [30]. The amount of daylight received may be reduced around 30% by the glasshouse structure [31,32]. On the other hand, cultivation under a protected environment allows off-season production, resulting in a better price for farmers; in addition, the adoption of greenhouses or tunnels, combined with intensive production methods, makes it possible to reach higher yield than the same vegetable crops grown in open field conditions [33].

About half of greenhouse spinach cultivation areas in Italy are concentrated in the Campania region (Southern Italy), where radiation is not a limiting factor. Nevertheless, also in this optimal condition, in a context of energy sustainability, it is important to optimize light penetration into the greenhouses by using photo-selective plastic films as covering material [34], particularly during the winter period to balance both light and temperature. Various commercial products are available in the market, that differ by several factors like thickness, light diffusion, solar radiation transmission, mist control, thermicity, anti-drip, and anti-dust effect. However, the optical properties of the plastic cover film of greenhouses/tunnels diversely affect yield and quality traits of vegetable crops.

We previously tested two of these products on lamb's lettuce grown during spring, a season with a generally high light intensity but a potentially low Rd/Rg, and fertilized with increasing rates of N [35]. By contrast, the present study was conducted on spinach grown during winter in two greenhouses covered each with a different plastic film that has distinct optical properties (low and high Rd/Rg ratio). The scope of the current study was to explore the influence of light conditions on the productivity and quality of spinach cultivated under diverse nitrogen levels.

2. Materials and Methods

2.1. Experimental Set-Up and Design, and Plastic Films Properties

The “Platypus RZ” F₁ spinach (RIJK ZWAAN) was cultivated during winter 2019 in large pots (0.38 m² area and 60 cm height) placed in plastic tunnels, at the Department of Agricultural Science (Portici, Naples, Italy; N40° 48.870'; E14° 20.821'; 70 m a.s.l.). Pots were filled with sandy soil (91% sand, 4.5% silt, and 4.5% clay), with good fertility (P₂O₅ 253 ppm, K₂O 490 ppm, organic matter 2.5% and total N 0.09%), pH 7.4 and electrical conductivity 0.151 dS m⁻¹.

The experimental design provided a split-plot factorial combination between two greenhouses covered each with a different film (Film A and Film B) and three N levels: not fertilized control (N0%), sub-optimal N dose (25 kg ha⁻¹—N50%), and optimal N dose (50 kg ha⁻¹—N100%) under each film. Each of the three fertilization treatments was replicated three times and completely randomized in the plastic tunnels, for a total of nine experimental units greenhouse⁻¹ (18 in total).

The plastic films used to cover the two tunnels, have different optical characteristics. Film A, supplied by Lirsa srl (Ottaviano, Naples, Italy; commercial name LIRSALUX), is a thermal and clear plastic film, 150 microns thick, with anti-drip effect (an additive is added to the film formulation, which flattens the water droplets into a layer of water that runs down the sides of the greenhouse). Film B, manufactured by Ginegar Plastic Products and supplied by Polyeur srl (Benevento, Italy; commercial name SUNSAVER), is a thermal and diffused light film, 150 microns thick, with a light diffusivity of 58% and anti-drip effect. Transmission measurements on the two plastic films were realized using an UV/VIS Spectrophotometer (JASCO V-650 (JASCO Corporation, Tokyo Japan), accuracy 0.5 nm, range 190–900 nm) with an Integrating sphere (JASCO ISN-722, inside diameter 60 mm, range 200–870 nm), which allows the estimation of the total light transmission.

The total transmission UV-Vis spectrum of the plastic Film A and Film B are reported in Figures S1 and S2, respectively. Film A does not transmit UV radiation, identically to most traditional films present in the market and used for greenhouse covering. The thermicity of this film is 75% and the total transmittivity (direct plus diffused components of light transmitted) in photosynthetically active radiation (PAR) is about 85%.

In Figure S2, the spectrum of film B in the same range, is reported and it notes a UV-B “window”. This means that from 270 to 330 nm (UV-B range: 280–320 nm) the film partially transmits. The value of the thermicity of this film is 87% while its total transmittivity in PAR is 90%. The optical characteristics of the two plastic films (Transmittivity [%], Radiation [Lux], Light intensity [$\mu\text{mol m}^{-2} \text{s}^{-1}$]) were monitored constantly during the trial.

2.2. Plant Management

Spinach was sown on 17 January with a plant density of 340 seeds m⁻², resulting in 130 seeds pot⁻¹. N was added as calcium nitrate (26%) and it was given in a single solution on 13 February due to the short cycle length. The irrigation was managed accurately to avoid any leaching risk. Experimental pots were irrigated with an amount of water equal to the evapotranspiration, which was calculated by the Hargreaves formula. The harvesting occurred on 12 March.

2.3. Photosynthetically Active Radiation and Temperature Measurements

During the growing period, the light intensity in the photosynthetically active radiation (PAR-400 to 700 nm wavelength range) and temperatures were monitored continuously. Light intensity was recorded by a WatchDog A150 data logger (Spectrum Technologies Inc., Aurora, IL, USA) placed at canopy level, and was expressed as micro-moles of light energy m⁻²s⁻¹. Temperatures were measured with probes (Vantage Pro2, Davis Instruments) placed in the pots at canopy level and distributed randomly across the greenhouses. Both data were reported as hourly day mean divided into 15-day intervals over the growing period (60 days) starting from sowing up to final harvest.

2.4. Yield Measurements, SPAD Index and Color Parameters

At harvest, the yield was determined by cutting the whole pot surface, and was expressed as $t\ ha^{-1}$. For each treatment replicate, a representative leaf sample of each pot/replicate was collected and oven-dried at $70\ ^\circ C$ for 72 h in order to assess dry matter percentage, and subsequently utilize the dry material for the assessment of nitrate concentration. SPAD index measurements were conducted on a representative vegetable sample (ten undamaged young fully expanded leaves) per replicate, via a chlorophyll meter SPAD-502 (Konica Minolta, Tokyo, Japan). The CIELAB (Commission internationale de l'éclairage) color parameters (L^* ; a^* ; b^*) were measured by a Minolta CR-300 Chroma Meter (Minolta Camera Co. Ltd., Osaka, Japan).

2.5. Qualitative Parameters Assessments

On each dried sample of leaves, nitrate concentration was assessed by Foss FIAstar 5000 (FOSS Italia S.r.l., Padova, Italy) continuous flow Analyzer. On the fresh sample (leaves), after freezing and lyophilizing, ABTS antioxidant activity (ABTS) and total phenols were assessed, whereas total ascorbic acid content (AsA) and chlorophyll a, and b measurements were assessed on fresh samples (leaves) before harvest.

ABTS antioxidant activity was determined by 2,2-azinobis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS) method [36]. Antioxidant compounds inhibit $ABTS^+$ radical, proportionally to their concentration; therefore they are indirectly measured by UV-Vis spectrophotometry (ONDA V-10 Plus (Giorgio Bormac s.r.l., Carpi, Italy) at 734 nm. The data are presented in $mmol\ of\ Trolox\ eq.\ 100\ g^{-1}\ dw$.

Total ascorbic acid concentration was evaluated following the Kampfenkel et al. method [37], which is based on the reduction of Fe^{3+} to Fe^{2+} by ascorbic acid; Fe^{2+} with 2,2-dipyridyl form a complex, of which the quantitation was performed at 525 nm ONDA V-10 Plus (UV-Vis spectrophotometry, ONDA V-10 Plus, Giorgio Bormac s.r.l., Carpi, Italy). Results were expressed as $mg\ 100\ g^{-1}\ fresh\ weight\ (fw)$.

Total phenols concentration was determined by Folin–Ciocalteu method [38]; absorption was appraised at 765 nm through a UV-Vis spectrophotometer (ONDA V-10 Plus, Giorgio Bormac s.r.l., Carpi, Italy). Data of total phenol were expressed in $mg\ gallic\ acid\ eq.\ 100\ g^{-1}\ dw$.

As for chlorophyll a, and b, after extraction in 99% acetone and centrifugation at $3000 \times g$ for 5 min, pigments content was determined by a Hach DR 2000 spectrophotometer (Hach Co., Loveland, CO, USA) at 662 and 647 nm, respectively. The extinction coefficients, used for the determination of chlorophyll a, b were described by Lichtenhaler and Wellburn [39].

2.6. Statistical Analysis

All data were examined with the SPSS software package (SPSS version 22, Chicago, IL, USA), using a general linear model (GLM) for the Analysis Of Variance (ANOVA). The means were separated using the Duncan's Multiple Range Test (DMRT) test at $p \leq 0.05$.

3. Results

3.1. Environmental Conditions

The hourly day mean values of light intensity in the PAR (photosynthetic active radiation) and temperature during the growing period of spinach (15-day intervals) from sowing up to final harvest, are reported in Figures 1 and 2, respectively.

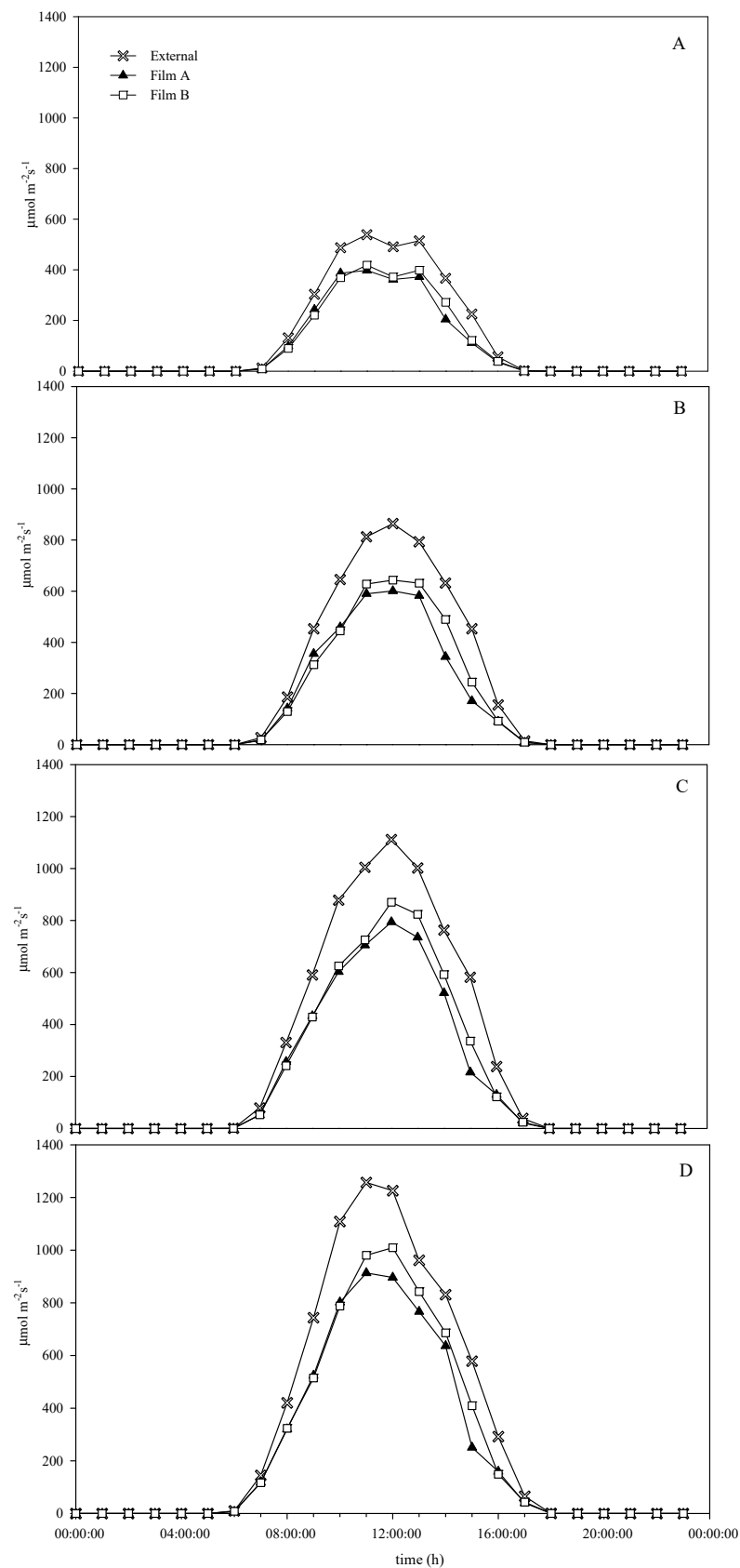


Figure 1. Hourly day mean value of light intensity in the PAR (photosynthetic active radiation) during the growing period of spinach referred to 15-day intervals starting from sowing (A = 1–15 days; B = 16–30 days; C = 31–45 days; D = 46–60 days).

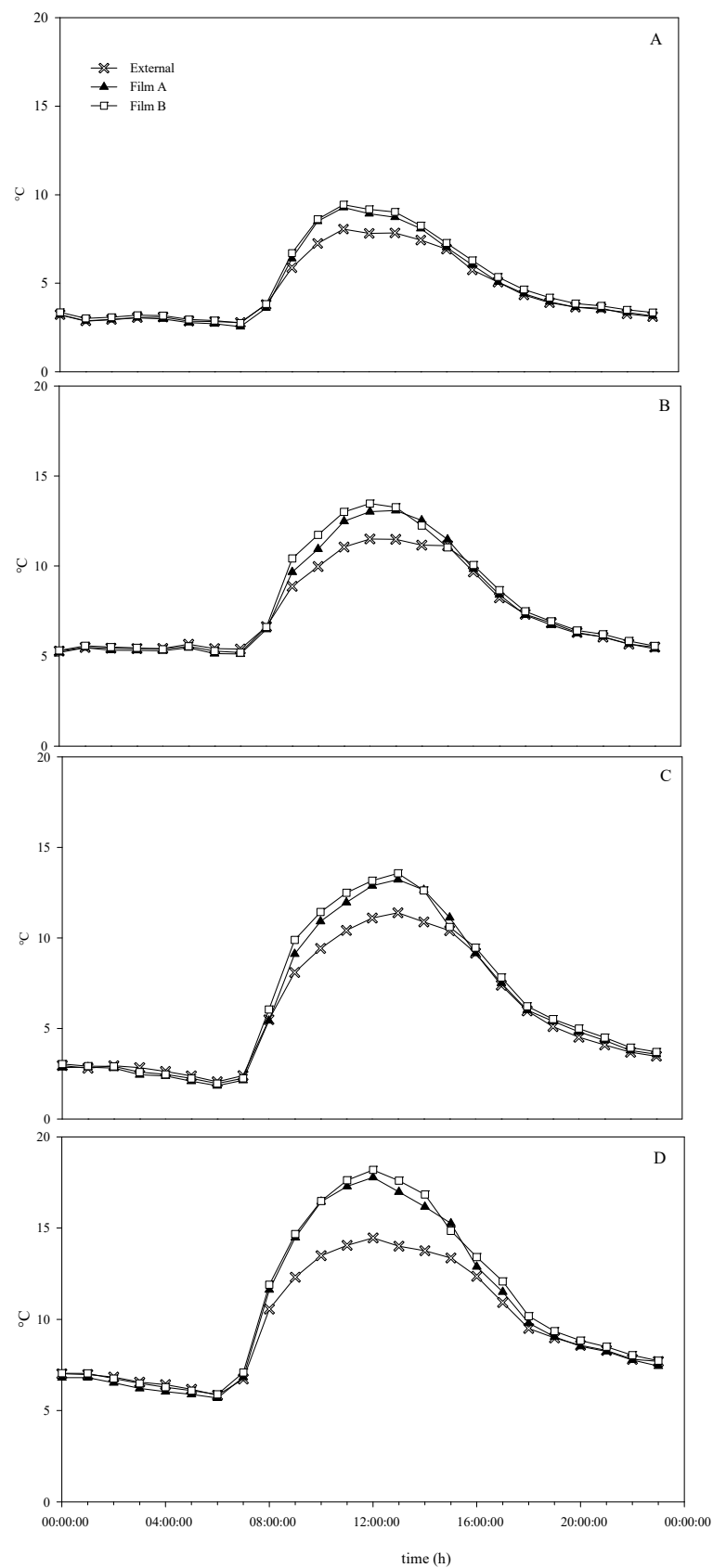


Figure 2. Hourly day mean value of temperature during the growing period of spinach referred to 15-day intervals starting from sowing (A = 1–15 days; B = 16–30 days; C = 31–45 days; D = 46–60 days).

The light intensity had an increasing trend in the four intervals, ranging on average from $\sim 230 \mu\text{mol m}^{-2} \text{s}^{-1}$ in the first interval to $\sim 570 \mu\text{mol m}^{-2} \text{s}^{-1}$ in the last interval; concomitantly it overcame 500 and $1200 \mu\text{mol m}^{-2} \text{s}^{-1}$ under external conditions, in the first and fourth interval, respectively (Figure 1). Film A and Film B decreased external light intensity by 31 and 26%, respectively. Differences between the two films were particularly evident starting from the second interval (16–30; Figure 1B), with a mean value of 7.7% (Figure 1B–D), with respect to 3.8% of the 1–15 day interval (Figure 1A).

The mean temperature inside the greenhouses was higher than that measured outside, increasing by 7.6% on average over the entire growing period (Figure 2). Notably, under Film B the mean temperature was always higher than that under Film A (7.59°C vs. 7.37°C); with the highest increase in the first 15 days interval (+4.1%) (Figure 2).

3.2. Yield and SPAD Index

The main effect of the two experimental factors (greenhouse cover film and N doses) on yield is reported in Figure 3. Plants yielded significantly more (+22.3%) under plastic Film B than under plastic Film A. The yield also had an increasing trend when N ranged between 0 kg ha^{-1} (N0%) to 50 kg ha^{-1} (N100%), but without a significant difference between the sub-optimal N dose (N50%) and optimal N dose (N100%) (Figure 3).

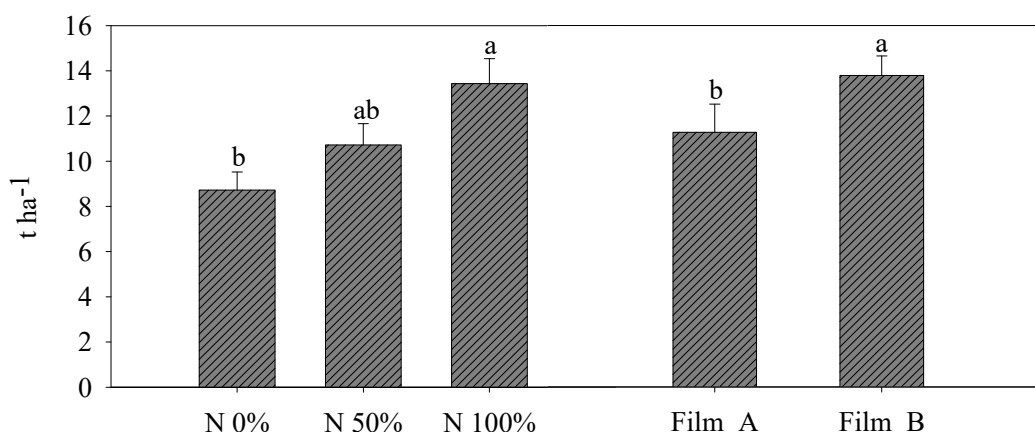


Figure 3. Effect of N fertilization (not fertilized = N0%; fertilized with 25 kg N ha^{-1} = N50%; fertilized with 50 kg N ha^{-1} = N100%) and plastic film (clear plastic film = Film A; light diffusion plastic film = Film B) on spinach yield (tons hectare^{-1} : t ha^{-1}). Diverse letters on the bars indicate significant differences according to Duncan's test ($p = 0.05$). Vertical bars designate \pm SE (standard error) of means.

Furthermore, in the case of SPAD index investigation, Film B equally elicited an increase of 4.6% over Film A (Figure 4). Similarly, the effect of N fertilization treatments on SPAD was less strong than the effect on yield, though significant, with a 6.4% and 11.3% increase, for sub-optimal and optimal dose compared to N0% fertilization, respectively.

3.3. Spinach Colorimetric Indices, Bioactive Qualities and Dry Matter Percentage

Regarding the CIELAB color parameters, the statistical analysis showed that only lightness (L^*) was significantly affected by both N doses and plastic films (Table 1). Notably, the two fertilized treatments were not different and they were significantly lower than control (-4.1%). Instead, Film B showed the lowest value of the L^* parameter (-3.1% as compared to Film A).

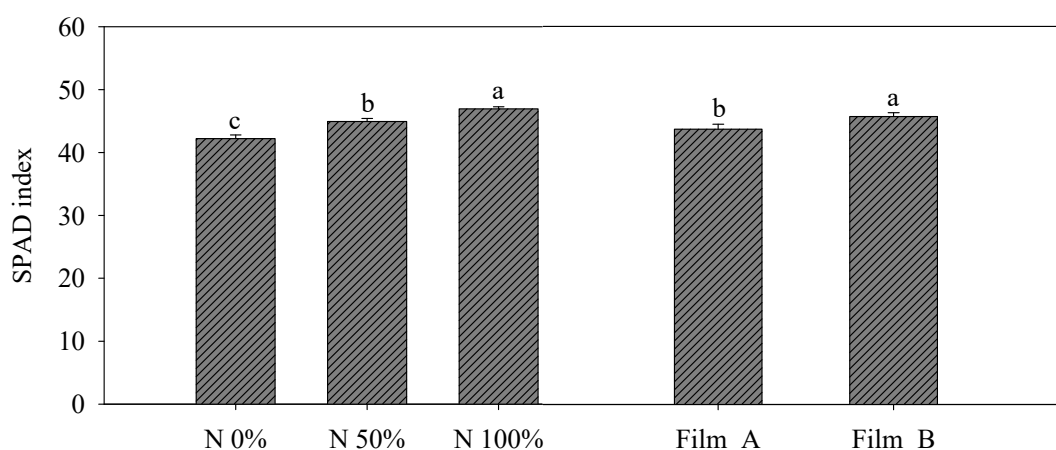


Figure 4. Effect of N fertilization (not fertilized = N0%; fertilized with 25 kg N ha⁻¹ = N50%; fertilized with 50 kg N ha⁻¹ = N100%) and plastic film (clear plastic film = Film A; light diffusion plastic film = Film B) on spinach SPAD (soil and plant development) index. Diverse letters on the bars indicate. Significant differences according to Duncan's test ($p = 0.05$). Vertical bars designate \pm SE (standard error) of means.

Table 1. Effect of N fertilization (not fertilized = N0%; fertilized with 25 kg N ha⁻¹ = N50%; fertilized with 50 kg N ha⁻¹ = N100%) and plastic film (clear plastic film = Film A; light diffusion plastic film = Film B) on color parameters (L^* = lightness, a^* = green/red coordinate, and b^* = blue/yellow coordinate) of spinach leaves.

Treatments	L^*	a^*	b^*
Fertilization			
N0%	38.99 \pm 0.63 a	-14.33 \pm 0.493	19.87 \pm 0.65
N50%	37.48 \pm 0.55 b	-13.52 \pm 0.35	18.44 \pm 0.44
N100%	37.31 \pm 0.76 b	-13.40 \pm 0.67	18.60 \pm 0.82
Film			
Film A	38.53 \pm 0.67 a	-13.92 \pm 0.47	19.42 \pm 0.82
Film B	37.32 \pm 0.51 b	-13.58 \pm 0.42	18.51 \pm 0.69
Significance			
Fertilization (N)	*	ns	ns
Film (F)	*	ns	ns
N \times F	ns	ns	ns

ns, *: non-significant or significant at $p \leq 0.05$, respectively. All data are expressed as mean \pm SE (standard error), $n = 3$. Diverse letters within each column indicate significant differences according to Duncan's test ($p = 0.05$).

There was no effect of N fertilization on ABTS, and AsA, whereas total phenols decreased significantly with increasing N fertilization rates, but only the N100% treatment was significantly different from the other two treatments (-18.8% with respect to their mean value) (Table 2). Greenhouse cover film affected only AsA, which was significantly lower (-61.2%) in plants grown under Film B than plants grown under Film A (Table 2). There was no change in ABTS antioxidant activity and total phenols content due to different films used (Table 2). Finally, a significant higher value of leaves dry matter percentage was recorded in N0% treatment, with about a 15% increase over the mean value of the two fertilized treatments; also, Film A elicited a 9.3% leaves DM increase over the Film B value (Table 2).

Table 2. Effect of N fertilization (not fertilized = N0%; fertilized with 25 kg N ha⁻¹ = N50%; fertilized with 50 kg N ha⁻¹ = N100%) and plastic film (clear plastic film = Film A; light diffusion plastic film = Film B) on ABTS antioxidant activity (ABTS), total phenols, Total Ascorbic Acid (AsA) and dry matter (DM) of spinach leaves.

Treatments	ABTS	Total Phenols	AsA	DM
	mM Trolox eq. 100g ⁻¹ dw	mg Gallic Acid g ⁻¹ dw	mg 100 g ⁻¹ fw	%
Fertilization				
N0%	22.77 ± 1.70	3.22 ± 0.18 a	33.49 ± 4.93	9.9 ± 0.6 a
N50%	21.90 ± 1.29	2.88 ± 0.17 a	27.45 ± 7.33	8.8 ± 0.2 b
N100%	20.55 ± 1.72	2.48 ± 0.15 b	23.62 ± 3.81	8.3 ± 0.3 b
Film				
Film A	22.10 ± 1.91	2.95 ± 0.17	40.62 ± 6.71 a	9.4 ± 0.5 a
Film B	21.42 ± 1.23	2.78 ± 0.16	15.75 ± 5.33 b	8.6 ± 0.2 b
Significance				
Fertilization (N)	ns	**	ns	*
Film (F)	ns	ns	**	*
N × F	ns	ns	ns	ns

ns, *, **: non-significant or significant at $p \leq 0.05$ and 0.01 , respectively. All data are expressed as mean ± SE (standard error), $n = 3$. Diverse letters within each column indicate significant differences according to Duncan's test ($p = 0.05$). dw: dry weight, fw: fresh weight.

Chlorophyll a, chlorophyll b and total chlorophylls significantly increased with N fertilization up to 50 kg ha⁻¹ (Table 3). Furthermore, nitrate content increased linearly ($y = 43.348x - 11.784$, $R^2 = 0.9974$) with N dose (Table 3):

Table 3. Effect of N fertilization (not fertilized = N0%; fertilized with 25 kg N ha⁻¹ = N50%; fertilized with 50 kg N ha⁻¹ = N100%) and plastic film (clear plastic film = Film A; light diffusion plastic film = Film B) on chlorophyll a, chlorophyll b, and total chlorophylls, and nitrate of spinach leaves.

Treatments	Chlorophyll a	Chlorophyll b	Total Chlorophylls	Nitrate
	mg g ⁻¹ fw	mg g ⁻¹ fw	mg g ⁻¹ fw	mg kg ⁻¹ fw
Fertilization				
N0%	0.905 ± 0.034 b	0.547 ± 0.045 b	1.452 ± 0.079 b	52.3 ± 20.8 c
N50%	0.976 ± 0.044 ab	0.716 ± 0.105 a	1.692 ± 0.146 a	1968.4 ± 650.7 b
N100%	1.015 ± 0.025 a	0.786 ± 0.075 a	1.800 ± 0.099 a	3205.5 ± 537.5 a
Film				
Film A	0.976 ± 0.029	0.689 ± 0.075	1.665 ± 0.103	476.4 ± 134.4 b
Film B	0.954 ± 0.040	0.677 ± 0.076	1.632 ± 0.114	3007.7 ± 671.6 a
Significance				
Fertilization (N)	*	*	*	**
Film (F)	ns	ns	ns	**
N × F	ns	ns	ns	ns

ns, *, **: non-significant or significant at $p \leq 0.05$ and 0.01 , respectively. All data are expressed as mean ± SE (standard error), $n = 3$. Diverse letters within each column indicate significant differences according to Duncan's test ($p = 0.05$). fw: fresh weight.

There was no effect of different films on leaf content of chlorophyll a, chlorophyll b, and total chlorophyll (Table 3). By contrast, nitrate content was significantly affected by higher levels in spinach grown under Film B compared to Film A (Table 3).

4. Discussion

In the current research, the two plastic films with different properties, clear (Film A) and diffuse-light film (Film B), resulted in a different micro-climate. However, under Film B, the light intensity was higher than that recorded under Film A. Under both films conditions, the light intensity was lower than that of open-air conditions by 26% and 31%, for Film B and Film A, respectively.

Data reported in Figure 3 showed that the differences in light and temperature conditions resulted in differences in the yield of spinach, which was higher for plants grown under diffuse light film (Film B) than that of plants grown under clear film (Film A), specifically +22.3%. Our findings are consistent with the results of our previous research [34] on lamb's lettuce, where we found a similar increase in yield (by 22%) with a light diffused cover film. Diffuse light is reported as well to increase biomass yield of other vegetables like *Solanum lycopersicum* [40], and *Capsicum annuum* [41]. Kanniah et al. [28] reported that in conditions of high diffuse radiation, plant production increases as a result of a more efficient yield per unit of PAR, and this phenomenon is also known as the "diffuse fertilization effect". Roderick et al. [42] explained that on clear days, direct sunlight mainly reaches the upper canopy; whereas in the presence of diffuse radiation, the sub-canopy vegetation, usually shaded, is uniformly illuminated, since radiation comes from all directions and can penetrate deeper into the canopy. Hence, this produces higher light use efficiency and a higher yield, as photosynthesis is the primary factor driving plant productivity [43]. Additionally, some authors argued that plants cultivated under diffuse light suffer fewer stress events related to water and heat [44,45].

The yield increase reflects the trend of the SPAD index, which is a good indicator of the physiological and biochemical status of plants, and indirectly of chlorophyll content, which plays a key role in photosynthesis. The SPAD index of spinach plants grown under Film B increased by 4.6% with respect to that of spinach grown under Film A, although chlorophyll a, b, and total were not significantly affected by light conditions. Riga and Benedicto [46] found that plastic films affected leaf pigments (chlorophyll a, total, and carotenoids) of lettuce only in the harvests made from April to September, and no effects were recorded for winter harvest. The effect of Film B on the yield of the present experiment could be also ascribed to its partial UV-B transmission. Heuberger et al. [30] studied spinach subjected to different light conditions (no additional lighting, corresponding to standard growing practice; additional photosynthetically active radiation; and three different UV-B intensity, corresponding to field-grown condition; UV intensive, and UV permanent), and they found an increase in fresh and dry weight of spinach under additional PAR and intensive UV-B. The UV radiation (UV-A and UV-B) can modulate the growth and development of plants, but they vary depending on plant species [47,48]. In fact, growth was reduced by solar UV-B in lettuce [49], wheat and cotton [50], boosted in basil [51] and unaltered in other species like maize [52].

The yield and SPAD index were also affected by the N rate, with an increasing trend when the N dose increased from 0 to 50 kg ha⁻¹. Nonetheless, despite the stronger effect recorded for yield (+23.0% and +54.0%, for N50% and N100%, respectively), no significant difference was found between the sub-optimal and optimal doses. Instead, the increase in the SPAD index was only 6.4% and 11.3 %, for N50%, and N100%, respectively. Cozzolino et al. [34] reported an increase in SPAD index when the lamb's lettuce plants are grown under the diffuse light cover film and with increased N dose. Furthermore, the chlorophyll was positively affected by increased N doses, according to our previous research on spinach and lamb's lettuce [14,35,53,54].

Although there are few studies concerning the effect of light (clear and diffuse, high or low presence of UV-B) on crop quality, it is indisputable the importance that product quality assumes in the consumers' choices. The concept of quality differs with customer groups (consumers, food industry, participants markets, etc.) [33] and with the crop choice. Overall, size, color, consistency, shape, and freshness are the main components of external

quality; vitamins, minerals, bioactive compounds, carbohydrates, nitrate, residues, etc., are a part of the internal value of a product constituting its nutritional and health values [33].

In the present research, we investigated the effect of two experimental factors (light and N) on some traits of spinach quality, such as color, antioxidant activity (ABTS), total ascorbic acid, total phenols, and nitrate content. In regards to colorimetric parameters, only L^* was influenced by the tested factors. L^* decreased by 3.1% under Film B compared to A, and decreased equally in both fertilized treatments by 4.1% compared to unfertilized treatment. These outcomes contrast with those of Cozzolino et al. [34] on lamb's lettuce, where they observed an increase in a^* (greenness) and b^* values. It is likely that the different responses are due to different natural light conditions during the two growing periods: winter (low light intensity) in our research vs. spring (higher light intensity). Additionally, Kittas et al. [48] did not find differences in L^* and a^* when they cultivated eggplants at three different levels of UV transmission (5%, 3%, and 0%); they concluded that fruit color is primarily genetically determined, and then, it is influenced by environmental factors such as nutrients, temperature and light conditions.

Regarding nutritional and health benefits in the current research, no influence of cover film was recorded for ABTS antioxidant activity and total phenols content (Table 2). The results regarding the antioxidant activities did not confirm what was reported by Cozzolino et al. [34], who found a significant decrease of ABTS (−33.9% in lamb's lettuce) under the diffuse light film. These contrasting results could be due to different light conditions, as explained for color parameters. It seems that at a high light intensity, growth conditions are more favorable, therefore, plants do not induce the increase of antioxidant activity in contrast to what occurs under diffuse light conditions. In addition, Colonna et al. [55] reported a decrease of ABTS in several baby leaf vegetables, including spinach, when the plants were exposed to high light intensity.

Total phenols were not affected by greenhouse cover films, according to previously reported results in lamb's lettuce [34], spinach and rocket [55]. On the other hand, some authors [56,57] reported that UV-B induces the accumulation of secondary metabolites, which influence several physiological processes of plants. Irrespective of the greenhouse cover films, ABTS and AsA were not affected by N fertilization, however, total phenols significantly decreased at 50 kg ha^{−1} of N. This is probably due to the absence of nutritional stress conditions. Indeed, low availability of N may elicit the antioxidant system resulting in an increase of total phenolics, hydrophilic antioxidant activity and total antioxidant power in mustard [58].

Regarding the synthesis of ascorbic acid, it seems that it is influenced by the amount and intensity of light during the growing season, but it also is a cultivar-dependent trait [33]. Ascorbic acid concentration usually increases when the exposition to light increases, especially in leafy greens [59,60]. Our results are in line with the abovementioned ones, since total ascorbic acid concentration was significantly higher under clear than under light diffusion film, where the light intensity was higher. Heuberger et al. [30] suggested that slight oxidative stress due to UV-B exposure (1–2 kJ m^{−2}d^{−1} UV-B), as well as the addition of photosynthetically active radiation, increased the leaf ascorbate content, whereas the high oxidative stress leads to a reduction of the leaf ascorbate pool in spinach leaves. Instead, Colonna et al. [55] did not record any effect of light on ascorbic acid, probably because they evaluated the effect of different PAR only at harvest (two harvest times, corresponding to low and high PAR).

A deep discussion must be carried out on nitrate content in spinach. Indeed, this species has the characteristic to accumulate nitrate in its leaves. The nitrate accumulation depends on several factors: genetics, N fertilization, light conditions, and growing conditions (open field or protected environment, and autumn–winter or spring–summer periods) [6]. The interest in nitrate content in vegetables is strongly linked to their effects on human health. Bruning-Fann and Kaneene [61] reported that 5–7% of the total nitrate intake is converted to nitrite by oral bacteria and salivary enzymes, with a higher conversion rate in infants or patients with gastroenteritis. Although nitrate is relatively non-toxic, nitrite

and other reaction products of nitrate (nitric oxide and N-nitrous compounds) generally cause concern due to their adverse health effects. Nevertheless, results in this area are contrasting. In particular, several epidemiological studies did not confirm any direct correlation between nitrate concentration in food and the incidence of cancer [62,63]. However, the European Commission (EC; Regulation No. 1258/2011) [64] fixed a limit for nitrate content in leafy vegetables, which for fresh spinach is 3500 mg kg⁻¹ fw.

In the current research, nitrate content was about six-fold higher in spinach leaves grown under diffuse light film than that under clear film, though it was within the limit fixed by the EC, and the highest nitrate content was recorded in N100% treatment. The effects of light and N fertilization on nitrate accumulation are well known. In the present experiment, the high light intensity under the clear film presumably allowed to stimulate the nitrate reductase activity, since the nitrate accumulation was reduced. In addition, the N rate of 50 kg ha⁻¹ (N100%) was probably excessive since it caused an accumulation of nitrate in leaves, and also manifested no difference in yield when compared to N50% treatments. Proietti et al. [65] found that spinach grown at a photon flux density of 200 μmol m⁻² s⁻¹ (10/14 h photoperiod; light/dark), at five weeks stage showed more nitrate compared to plants grown at 800 μmol m⁻² s⁻¹. The activity of the main enzymes involved in the metabolism of nitrate and vitamin C increased under higher light intensity [66,67]; additionally, high light provides more energy to fix carbon dioxide to boost vitamin C synthesis and nitrate assimilation in plant leaves [68].

Irrespective of the greenhouse cover films, ABTS and AsA were not affected by N fertilization, however, total phenols significantly decreased at 50 kg ha⁻¹ of N. This is probably due to the absence of nutritional stress conditions. Indeed, low availability of N may elicit the antioxidant system resulting in an increase of total phenolics, and total antioxidant power in mustard [58].

5. Conclusions

Light and nitrogen are among the limiting factors for obtaining an optimal growth of plants, and they have greater importance in green leafy vegetables, such as spinach, as they strongly affect some quality traits such as nitrate content. Additionally, the optical properties of greenhouse cover films can have a great effect on light intensity.

Our findings, recorded in the Mediterranean area during the winter season, demonstrated that the cultivation of spinach under diffuse-light film resulted in an increase in yield and SPAD index, which is indicative of a better physiological and biochemical status of plants. The external quality of spinach, specifically color, as well as chlorophyll content, ABTS, and total phenols, were not influenced by the cover; instead, total ascorbic acid content decreased and nitrate content increased under the diffused light film. The optimal N dose boosted yield and increased pigments content but without a significant increase over sub-optimal dose; finally, phenols content decreased at optimal N dose whereas nitrate content increased. Therefore, it would seem that irrespective of the N levels, the use of diffuse-light film boosts spinach yield without depressing quality.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/horticulturae7070200/s1> Figure S1: UV-Vis transmission as a function of the wavelength of clear plastic film A (Lirsalux by Lirsa). Figure S2: UV-Vis transmission as a function of the wavelength of the light diffusion plastic film B (Sunsaver by Ginegar Plastic Products).

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