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RESEARCH ARTICLE

Effects of light-diffusing plastic film on lettuce production and quality attributes

Patrick Riga and Leyre Benedicto

NEIKER, Basque Institute of Agricultural Research and Development, Department of Plant Production. Parque Tecnológico de Bizkaia P. 812, 48160 Derio, Spain.

Abstract

In general, plants grown under diffuse light yield higher biomass than those grown under direct light as a result of a more uniform distribution of the light across the plant canopy. We compared the effects of a light-diffusing plastic film and a clear plastic film on growth of Batavia lettuce (*Lactuca sativa* L.) in two greenhouses during five growth periods. Lettuce grown under the light-diffusing film were smaller (up to 36%) than control plants grown under the clear film, due to the fewer leaves per plant (up to 22%) and lower mean values of individual leaf area (up to 29%). The photosynthetically active radiations use efficiency was sometimes lower (up to 23%) in lettuces grown under the light-diffusing film. The pigment contents tended to be lower in plants grown under the light-diffusing plastic. The total macroelement contents of the lettuces grown under the light-diffusing plastic were up to 10% higher than in the lettuces grown under clear plastic, mainly as a result of higher leaf K contents (up to 19% higher). In addition, use of the light-diffusing plastic tended to increase leaf nitrate contents (by up to 23%). The leaf solid soluble content and acidity values were higher in the lettuces grown under the light-diffusing plastic, while leaf pH values were lower than in the control plants. The findings showed that the light-diffusing plastic was detrimental to production of compact heads of lettuce, and to some quality parameters such as nitrate and pigment contents. Nevertheless, open-leaf cultivars would likely show a different response to the diffuse light

Additional key words: diffuse light; *Lactuca sativa*; light use efficiency; vegetable production.

Abbreviations used: Car (carotenoids); CC (construction cost); dw (dry weight); fw (fresh weight); LUE (light use efficiency); NR (nitrate reductase); PAR (photosynthetically active radiations); PV (photovoltaic solar panel); RH (relative humidity); SLA (specific leaf area); SSC (soluble solids content).

Authors' contributions: Conceived, designed, and supervised the work: PR. Field data acquisition and laboratory analysis: LB. Analysed the data and drafting of the manuscript: PR and LBE.

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Correspondence should be addressed to Patrick Riga: priga@neiker.eus

Introduction

Diffuse photosynthetically active radiation (PAR) increased gross primary productivity in different plant communities in both temperate (Cheng *et al.*, 2015) and Arctic (Williams *et al.*, 2014) ecosystems. In horticultural production systems, diffuse light increased the biomass yield of vegetables such as *Solanum lycopersicum* (Duek *et al.*, 2012), *Capsicum annuum* (Chun *et al.*, 2005) and also ornamental plants such as *Chrysanthemum* sp. (Markvart *et al.*, 2010) and *Anthurium* sp. (Li *et al.*, 2014b).

Increased ecosystem carbon uptake and light use efficiency (LUE) under diffuse light can be explained by at least three mechanisms. First, in the forest canopy, lower leaves are normally light-limited on a clear day when light is mostly direct, while diffuse light penetrates deeper into the forest canopy (Hollinger *et al.*, 1994; Oliphant *et al.*, 2011). Second, relative to direct, diffuse light is distributed across more leaves, leading to lower light saturation and photoinhibition in upper canopy leaves. This allows higher canopy LUE or photosynthesis (Gu *et al.*, 2002; Knohl & Baldocchi, 2008). Third, plants growing under diffuse light suffer fewer stress events related to water and heat (Steiner & Chameides, 2005; Urban *et al.*, 2012).

Li *et al.* (2014a) showed that in tomato plants (*Solanum lycopersicum*) grown under diffuse light, when global irradiance was high, leaf temperatures and photoinhibition at the top of the canopy were both lower than in control plants grown under direct light. The authors demonstrated that more uniform horizontal light distribution under diffuse light was the most important factor in the increased rate of photosynthesis.

Although the advantages of diffuse light for crop production are well established (Li & Yang, 2015 and refs therein), to our knowledge there are very few reports concerning the effect of diffuse light on vegetable quality attributes. Tani *et al*. (2014) showed that diffuse light reduced the ascorbic acid content in lettuce leaves compared to control plants. In addition no significant differences were observed between treatments for tenderness score and bitter taste intensity.

Lettuce is the most abundant leafy vegetable consumed in its raw form by humans. Worldwide lettuce production reached $24896 \cdot 10^3$ tonnes in 2013 (FAOSTAT, 2016). It is well known that light intensity and quality affect lettuce production and quality. Light intensity in the range of 400 to 932 μmol/m^2·s has been recommended for lettuce production (Knight & Mitchell, 1983; Fu *et al*., 2012). The possibility of improving lettuce production by using diffused light under roof mounted photovoltaic solar panel (PV) has been suggested (Tani *et al*., 2014). Relative to lettuce plants grown under transparent PV, plants grown under diffusing PV yielded higher values of dry biomass: 1.3- and 1.5- fold increases in summer and autumn respectively, but no significant difference in spring and winter.

The aim of the present study was to test the hypothesis that lettuce production and quality are enhanced by the use of light-diffusing plastic film as a greenhouse cover. Lettuce has a very different growth habit from that of other previously studied vegetables (*e.g.* tomato, pepper and cucumber plants, see above). In the variety considered here (Batavia type, cv Edurne, Syngenta), the leaves are arranged in a dense rosette, with all leaves reaching a similar height. The rosette develops into a compact head.

Material and methods

Plant material and growth conditions

Batavia lettuce plants (*Lactuca sativa* var. *capitata* L.) cv Edurne (Syngenta) were germinated and grown in cubes (27 cm^3) of peat substrate (Profi-sustrat tray 50/50, Gramoflor GmbH, Vechta, Germany) in a tunnel greenhouse located on a farm in the Basque Country, northern Spain (lat: 43º 17'N, long: 2º 52'W, alt: 65 m a.s.l.). The climate in this region is Atlantic temperate (the mean annual values of temperature (T) for 2015 were T = 16.3ºC, T_{max} = 20.8ºC, T_{min} = 11.7ºC and monthly rainfall = 53 mm). Seedlings at the 4-5 leaves stage were transplanted on 1 September 2014, 22 October 2014, 20 February 2015, 26 June 2015 and 13 August 2015 in soil in each of two greenhouses covered with the plastic films being evaluated. Plantation density was 11.11 plants/m^2. Soil was covered with black plastic mulch. Plants were irrigated by aspersion through a delivery line located at 2.50 m above the ground. After each harvest, the plastic mulch was removed and fertilizers were incorporated into the soil with a rotavator at: N, 100 kg/ha; P, 20 kg/ha and K, 200 kg/ha. The growing cycles were: 1 September-17 October, 22 October-15 January, 20 February-24 April, 26 June-30 July and 13 August-16 September.

Plastic films

We investigated the effects on lettuce production and quality of two commercially available plastic horticultural films: a clear film (Lumisol Clear AF, bpi.visqueen, British Polythene Industries, London) yielding < 35% light diffusion, and a light-diffusing film (Lumisol Diffused AF, bpi.visqueen) yielding > 90% light diffusion. The values of light transmission were ≥ 90 and ≥ 88% for Lumisol Clear and Lumisol Diffused respectively, while UV transmission and thermicity were identical for both of the films (manufacturer's data). Each plastic film was installed in a separate regular tunnel greenhouse (L = 44 m, W = 8.40 m and H = 3.10 m). Both greenhouses were aligned with the same longitudinal line in E-W direction, and each tunnel were spaced 7 m apart. Natural ventilation was made opening the doors manually according to the growers' experience.

Collection of climatic data

Global and diffuse photosynthetically active radiations (PAR, μmol photon/m^2·s) were recorded using BF5 Sunshine Sensors connected to a GP1 data logger (Delta-T Devices, Cambridge, UK). An external sensor was located at a height of 2 m above the greenhouses, and those located in the middle of each tunnel (one per greenhouse) were located at 2.60 m above the ground and above the sprinklers. Data were recorded every 10 minutes as integral values (μmol photon/m^2·s).

Air temperature was recorded every 10 minutes with HOBO U23 Pro v2 sensors (Onset Computer Corporation, MA, USA). An external sensor was located at a height of 2 m above the greenhouses and 3 sensors were placed along the centre of each greenhouse, spaced 4 m apart from each other at a height of 0.4 m above the ground.

Harvest

Lettuces were harvested on five dates: 16 October 2014, 12 January 2015, 22 April 2015, 30 July 2015, and 16 September 2015, to meet local market demands. At predawn and within one hour, 24 lettuces from the middle of each greenhouse were collected for analysis. Individual fresh weights were recorded and two sub-samples of 12 lettuces were obtained: one for determining growth parameters and another for chemical analysis. Lettuces were kept in a cold-room at 10ºC until analysis, carried out within a maximum of 4 hours.

Growth parameters and radiation use efficiency

The number of leaves > 5 mm was recorded in 12 lettuces. Batches of 6 adult leaves per lettuce were photographed with a digital camera (Olympus, mod. SP-600UZ, Tokyo, Japan) and the leaf area was determined using image analysis software ASSESS v. 2.0. The leaves were then dried at 70ºC in a ventilated oven for 72 h (until constant weight). The specific leaf area (SLA) was calculated by dividing leaf area (cm^2) by leaf dry weight (g). Light use efficiency (LUE, g/mol global PAR) was calculated as lettuce dry weight (g) divided by the sum of global PAR intercepted by a lettuce, assuming a constant lettuce surface area of 0.045 m^2 during the growth period [1 m^2 / (11.11 plants × 2)].

Chemical analysis

For analytical purposes, four batches of 3 lettuces per greenhouse were processed. Nitrates were quantified in 9 adult leaves from the inner of the lettuce per greenhouse (3 leaves per lettuce). Leaves were boiled in distilled water 1:6 (w:v) for 15 min and homogenized with a stick mixer (Princess, mod. The beast, Tilburg, The Netherlands) at 1000 W for one minute. The puree was boiled for another 15 min, with stirring, and filtered under vacuum through qualitative filter paper (Whatman nº 4, Healthcare, Buckinghamshire, UK). Distilled water was added to the filtrate to a final volume of 1 L. Nitrates were quantified with an ion-selective electrode (nº 9662, Crison, Barcelona, Spain) and a reference electrode (nº 5044, Crison) connected to a pH meter (Crison GLP22), following the manufacturer's instructions. Filtrates were diluted to yield final nitrate concentrations below 60 mg/L.

Soluble solids content, pH and acidity were measured in lettuce juice. For this purpose, 3 half lettuces per batch were homogenized in a professional food blender (Palson, mod. Baly, Barcelona) at maximal speed (1200 W) for 1 min. The puree was centrifuged (Sorvall Legend XTR, Thermo Fischer Scientific, Madrid) at 7600 g for 15 min to produce a clear juice. Soluble solids content (ºBrix) was determined directly in the juice with a digital refractometer (Optic Ivymen System, Barcelona). Titratable acidity was determined according to AOAC method 942.15 (AOAC, 1999) with a pH meter (model GLP22, Crison, Barcelona) and a Titrette® digital bottle-top burette (class A precision, Brand, Wertheim, Germany).

To determine the mineral contents, the remaining 3 half lettuces per batch were dried at 70ºC for 72 h (until constant weight). The dry material was ground and sieved through a 0.12 mm-mesh stainless steel. The ground material was homogenized, re-dried for at least 2 h at 80°C before weighing out 0.5 g samples for analysis. Samples of the dried homogenate were wet-digested in a mixture of 1% HNO_3 + 2% $HClO_4$ (85:15, v:v) under a temperature gradient ranging from ambient to 190ºC for 12 h. The mineral contents (K, Ca, Mg, Na, P, S, Fe, Mn, Cu and Zn) were determined and quantified by ICP-AES (Varian VISTA-MPX, Agilent Technologies, CA, USA). Calibration standards were prepared from Certipur® solutions (Merck, Germany) for all minerals.

The nitrogen and carbon contents of subsamples (100 mg) of the dried homogenate were measured in a TruSpec elemental analyzer (Leco, MI, USA). Calibration was carried out using standard reference material (orchard leaves, LECO®, Part. nº 50-055).

The organ construction costs were calculated as follows (Poorter, 1994):

$$CC = (-1.041 + 5.077\ C) \times (1\text{-}Ash) + (5.325\ N) \quad [1]$$

where CC is the total cost of producing one gram of plant biomass (g glucose per g dry weight), C is the carbon content of the organic biomass (g/g dw), and Ash and N are respectively the mineral and organic nitrogen contents of the total dry weight (g/g). Ash content provides a biased estimate of the mineral content because organic acids can be transformed into carbonates, and sodium, chloride or sulphur may also volatilize during burning of the organic matter at 450ºC (Masle *et al.*, 1992). We therefore replaced the ash values with the sum of the macro and microelements measured in the samples.

Carotenoids and chlorophylls were quantified in 4 batches of 3 lettuces per greenhouse. In each lettuce, 3 leaf discs of 3.96 cm^2 from individual leaf (1 disc per leaf, 3 discs per lettuce) were extracted (in DMSO) and quantified in duplicate. The test tubes were then incubated at 70ºC for 3 h in the dark. After cooling the extract in the dark at ambient temperature, a 3 mL aliquot was analysed at 480, 649 and 665 nm (spectrophotometer DU730, Beckman Coulter,

Table 1. Sum of global and diffuse photosynthetically active radiations (PAR), average daily air temperature and relative humidity (RH) outside and inside greenhouses covered by clear or light-diffusing (Diff.) plastic film, for each lettuce growth cycle

Lettuce growth cycle	Global PAR (mol/m²)			Diffuse PAR (mol/m²)			Mean air temperature (ºC)			Mean RH (%)		
	Outside	Clear	Diff.	Outside	Clear	Diff.	Outside	Clear	Diff.	Outside	Clear	Diff.
01 Sep-17 Oct 2014	1091	880	838	553	528	771	17.7	18.5	18.6 (ns)	80.5	83.6	82.8 (ns)
22 Oct-15 Jan 2015	874	664	626	464	448	571	11.0	11.8	11.9 (ns)	83.4	84.8	83.5 (ns)
20 Feb-24 Apr 2015	1379	1166	1022	666	697	903	11.5	13.4	13.2 (ns)	81.5	81.3	80.5 (ns)
26 Jun-30 Jul 2015	1231	943	852	560	600	755	21.9	24.2	24.2 (ns)	80.0	80.7	78.6 (ns)
13 Ago-16 Sept 2015	1059	847	720	467	498	643	19.4	22.0	22.1 (ns)	79.4	75.0	75.6 (ns)

Temperature and RH data inside greenhouses represent the mean values from 3 sensors. Maximum standard deviations were 0.08 for mean air temperature and 2.0 for RH across all planting dates. value. All data were recorded every 10 minutes. ns = not significantly different ($p < 0.10$) between clear and diffusing plastic.

California, USA). The chlorophyll *a*, chlorophyll *b* and carotenoids concentrations were determined according to the equation proposed by Wellburn (1994).

Statistical analysis

Data were subjected to one-way analysis of variance to determine any significant differences in the effects of the different plastic films. When ANOVA indicated a significant difference, a *t*-test for equality of means was used to identify differences between means. All statistical analyses were performed using SSPS 13 software.

Results

Effect of the plastic films on climatic conditions inside greenhouse

The sums of global and diffuse PAR for each treatment and growth period are shown in Table 1. The transmission coefficients for global PAR, as calculated by the ratio of inside to outside measured value of the parameter during each growth period, ranged from 76% to 85% for the clear plastic and from 68% to 77% for the light-diffusing plastic.

Lettuces grown under the light-diffusing plastic received between 5 and 15% less global PAR than those under clear plastic, depending on the growth period. The light-diffusing plastic yielded global PAR diffusion of between 88 and 92%, which represents an increase in the outside diffuse PAR of 1.7 to 2.0 times during the different growth periods. The clear plastic yielded global PAR diffusion of between 60 and 67%, which represents a 1.2 to 1.4-fold increase in the outside diffuse PAR.

On a daily basis, the amount of diffuse PAR transmitted inside the greenhouses by both plastics was dependent on global solar radiation (Fig. 1). On sunny days, with 30% of diffuse PAR outside, the light-diffusing plastic transmitted 85% PAR diffusion, whereas the clear plastic transmitted 43% of diffuse PAR. On cloudy days, diffuse PAR was very similar outside and

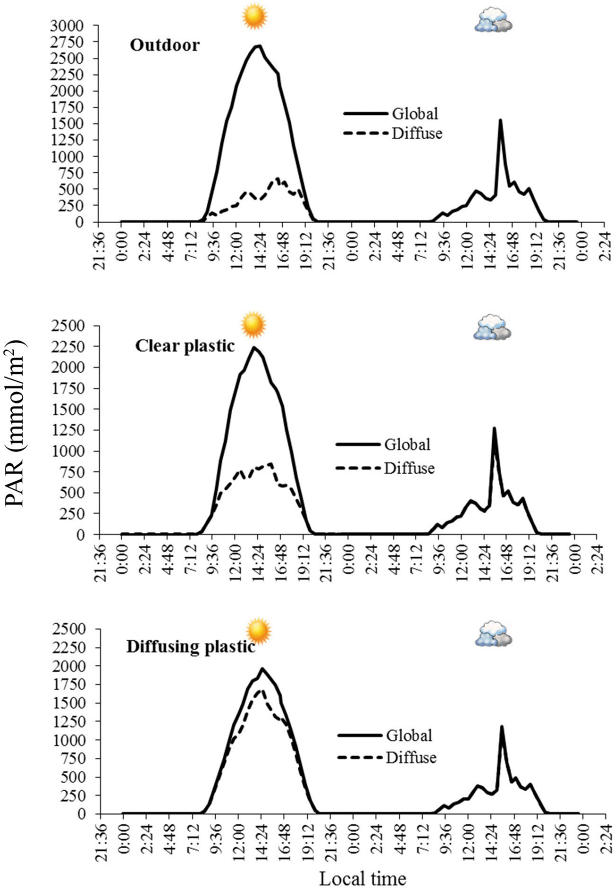

Figure 1. Example of daily variations in global and diffuse photosynthetically active radiations (PAR, mmol/m²) outside and inside greenhouses covered with clear or light-diffusing plastic films. Data were recorded every 10 minutes (as integral values) on 27 and 28 September 2014 using BF5 Sunshine Sensors. On the cloudy day, both lines were overlapped.

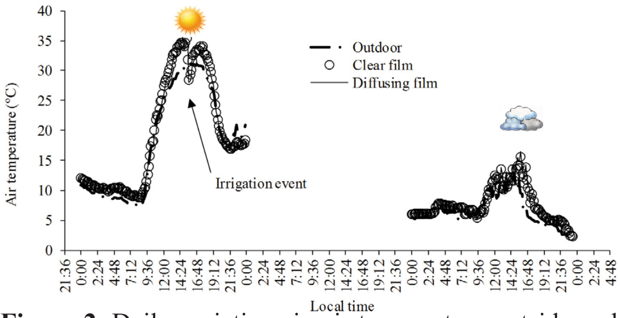

Figure 2. Daily variations in air temperature outside and inside greenhouses covered with clear or light-diffusing plastic films on sunny and cold days. Data were recorded every 10 minutes using HOBO U23 Pro v2 sensors.

inside both greenhouses (between 96% and 99%), irrespective of the type of plastic. This is attributed to the scattering of solar light by the clouds.

The mean daily air temperature and relative humidity (RH) inside the greenhouse during each growth period did not differ significantly in relation to the type of plastic (Table 1). Values of inside temperature and RH were similar or slightly different from outside values. Differences in minimum and maximum temperature between inside and outside ranged from 0.8ºC (22 Oct-15 Jan 2015) to 2.6ºC (13 Aug-16 Sept 2015) and for relative humidity, from -4.4% (13 Aug-16 Sept 2015) to 3.1% (01 Sept-17 Oct 2014). On sunny days, the night-time temperature inside the greenhouse was slightly higher than the outside temperature (difference between 0.8 and 1.9ºC), irrespective of the type of plastic (Fig. 2). During the hours of maximum solar radiation, the difference between temperatures inside and outside the greenhouses reached a maximum of 5ºC. On cold cloudy days, the temperature inside and outside the greenhouse was very similar.

Growth parameters and LUE

The mean fresh weight (fw) of lettuces was strongly dependent on the harvest date (Fig. 3A) owing to the climatic variation throughout the different growth periods and the fact that the days of harvest were selected to meet market demands. Irrespective of the type of plastic, the fw fluctuated between 712 ± 79 g and 254 ± 22 g. The light-diffusing plastic significantly reduced ($p < 0.001$) the fw by between 12 to 36% on almost all harvest dates, except during the February–April growth period, when plants received a greater amount of global PAR (1022 and 1166 mol/m^2 under light-diffusing and clear film respectively). Use of the light-diffusing plastic caused a significant increase in the dry weight (dw) of the lettuces between September 2014 and April 2015 (Fig. 3B), and in further growth periods, there were no differences in relation to the type of plastic film. The low fresh weights of lettuces grown under the light-diffusing plastic were due to the fewer leaves per lettuce and the smaller individual mean leaf areas (Fig. 3C and 3D). On almost all harvest dates, the number of leaves and the mean leaf areas were significantly lower (by up to 22% and 29%, respectively) in the lettuces grown under the light-diffusing plastic. However, the SLA values were generally similar under both types of film, although the SLA value was significantly lower in plants grown under the light-diffusing plastic and harvested in January, when the global PAR was lowest (Fig. 3E and Table 1).

The LUE in lettuces grown under the light-diffusing plastic was similar to or lower than in lettuces grown under the clear plastic (Fig 3F). The LUE decreased by up to 23 and 22% in the second and third growth periods respectively.

Leaf pigments

The type of plastic film significantly affected total chlorophylls (Chl *a*+*b*), Chl *a*, and carotenoids (Car) contents in lettuces harvested between April and September, while the Chl *b* content, Chl *a/b* ratio and Chl *a*+*b*/Car ratio were generally not affected (Fig. 4). Irrespective of the type of plastic, the chlorophyll content of the lettuces was higher during the winter harvest dates than during spring-summer dates. The Chl *a* content decreased from 15.4 µg/cm^2 on 16 Oct 2014 to 11.2 µg/cm^2 on 16 Sept 2015, and Chl *b* decreased from 6.3 to 3.3 µg/cm^2 between the same dates. The Chl *a*, Chl *a*+*b* and carotenoid contents of the lettuces grown under the light-diffusing plastic were similar to or lower than in lettuces produced under the clear plastic. The greatest reductions were 15% for Chl *a*, 8% for Chl *a*+*b* and 14% for carotenoids. Nevertheless, the reductions in pigment contents were not detectable by the human eye, and the light-diffusing film did not affect the visual appeal of the lettuces.

As values of the Chl *a*+*b*/Car ratio remained relatively constant, at around 4.5 µg/cm^2 on all harvest dates, the plants were not stressed.

Minerals, C, N and construction cost

Irrespective of type of covering plastic and harvest dates, total macroelements (sum of Ca, Mg, Na, K, N, S and P) ranged from 133 ± 8 to 180 ± 7 g/kg dw (Table 2). The major macroelements in lettuces were K and N, representing 52% and 28% of the sum of macroelements. The type of plastic film strongly affected the sum of macroelements on four harvest dates. Total macroelement contents in the lettuces grown under the light-diffusing plastic were up to 10% higher in January

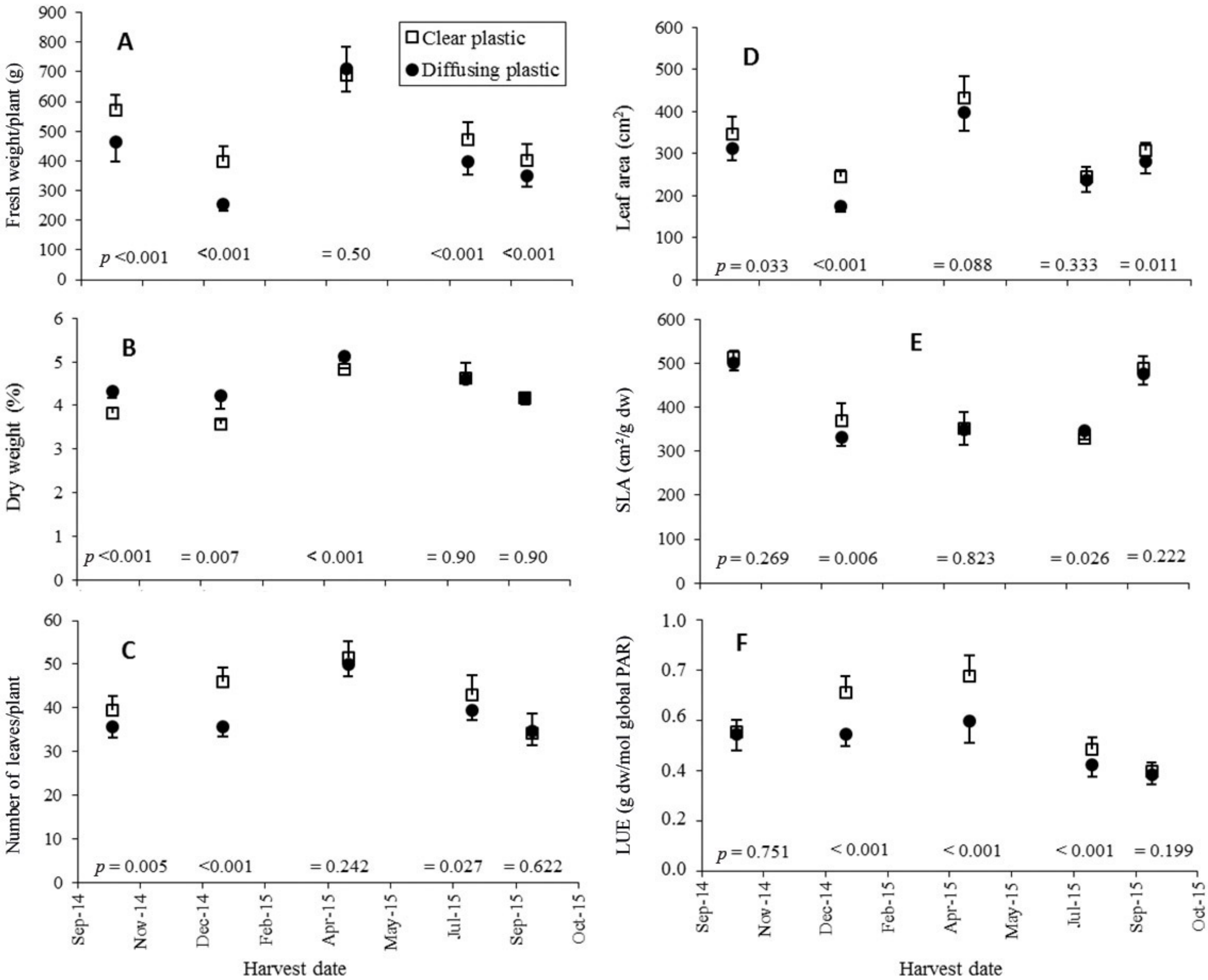

Figure 3. Effect of light-diffusing plastic on growth parameters and light use efficiency (LUE) for 5 harvests. Data are means of 12 replicates, except for LUE (n=6), and bars represent standard deviation. SLA: specific leaf area.

Table 2. Effect of clear and light-diffusing plastic films on macroelements content of lettuces

Date	Type of plastic film	P	Ca	Mg	Na	K	S	N	∑ Macro
		(g/kg dw)							
Oct 2014	Clear	5.25 a	18.6 a	4.70 a	4.25 a	86.2 a	3.09 a	46.1 a	168.1 a
	Diffusing	4.41 b	17.0 a	4.44 a	4.12 a	95.3 b	3.22 a	46.5 a	175.1 b
Jan 2015	Clear	6.02 a	14.7 a	3.87 a	4.04 a	88.3 a	2.67 a	43.8 a	163.3 a
	Diffusing	6.09 a	14.6 a	3.81 a	2.97 b	104.8 b	2.75 a	44.9 a	179.8 b
April 2015	Clear	5.19 a	9.9 a	3.01 a	2.85 a	74.1 a	3.47 a	39.8 a	138.3 a
	Diffusing	4.96 a	10.7 a	2.67 a	2.33 b	70.6 a	3.21 a	38.5 a	133.0 a
July 2015	Clear	5.00 a	25.6 a	6.20 a	7.06 a	65.6 a	4.11 a	45.2 a	158.8 a
	Diffusing	4.39 b	27.2 b	6.50 b	7.13 a	72.7 b	3.96 a	46.7 a	168.5 b
Sept 2015	Clear	5.55 a	20.3 a	6.14 a	5.68 a	66.7 a	3.37 a	45.2 a	153.0 a
	Diffusing	5.17 b	21.6 a	5.94 a	5.94 a	73.9 b	3.45 a	46.7 a	162.7 b
Max sd		0.47	2.77	0.70	0.83	7.23	0.38	2.19	7.98

Data represent the means of 4 independent replicates. Max sd represents the maximum standard deviation value over the 5 harvest dates. Different letters indicate significant difference ($p < 0.10$).

2015. The increase was mainly due to higher foliar K contents in the plants grown under the diffuse light (up to 19% higher for lettuces from the second harvest). By contrast, the P contents tended to decrease in lettuces grown under the light-diffusing plastic on the three harvest dates. Irrespective of type of plastic and harvest date, the Na and Mg contents fluctuated from 1.55 ± 0.23 to 3.46 ± 0.44 g/kg dw and from 2.56 ± 0.20 to 3.62 ± 0.34 g/kg dw respectively. The concentrations of the other macroelements in the lettuce were generally not affected by the type of plastic film.

Irrespective of treatments and harvest date, the sum of microelements (Fe, Cu, Zn and Mn) ranged from 148 ± 8 to 215 ± 19 mg/kg dw (Table 3). The major microelements in lettuces were Fe and Zn, representing about 54% and 28% of the sum of microelements. The type of plastic generally did not affect the microelement composition of leaves, although total microelement concentrations in lettuces grown under light-diffusing plastic were significantly lower on the last two harvest dates. These differences were due, in one case, to a reduction of 34% in the Zn content and in the other, a reduction of 16% of the Fe content.

Nitrate contents tended to be higher (up to 23%) in lettuces grown under the light-diffusing plastic than in those grown under clear plastic (Fig. 5). These differences were significant in lettuces grown in winter (harvested in December and April) with low light intensity. In all cases, the nitrate contents were below the maximum nitrate levels established for lettuce in EC Regulation No. 1258/2011 (EC, 2011): 5000 mg/kg fw for lettuces harvested between 1 October and 31 March, and 4000 mg/kg fw for harvests between 1 April and 30 September. Irrespective of the type of plastic cover and harvest date, leaf NO_3 content was strongly linearly correlated to the amount of global and diffuse PAR incident on the lettuces (Pearson coefficient, r = 0.65 and 0.67 respectively), while any consistent correlation

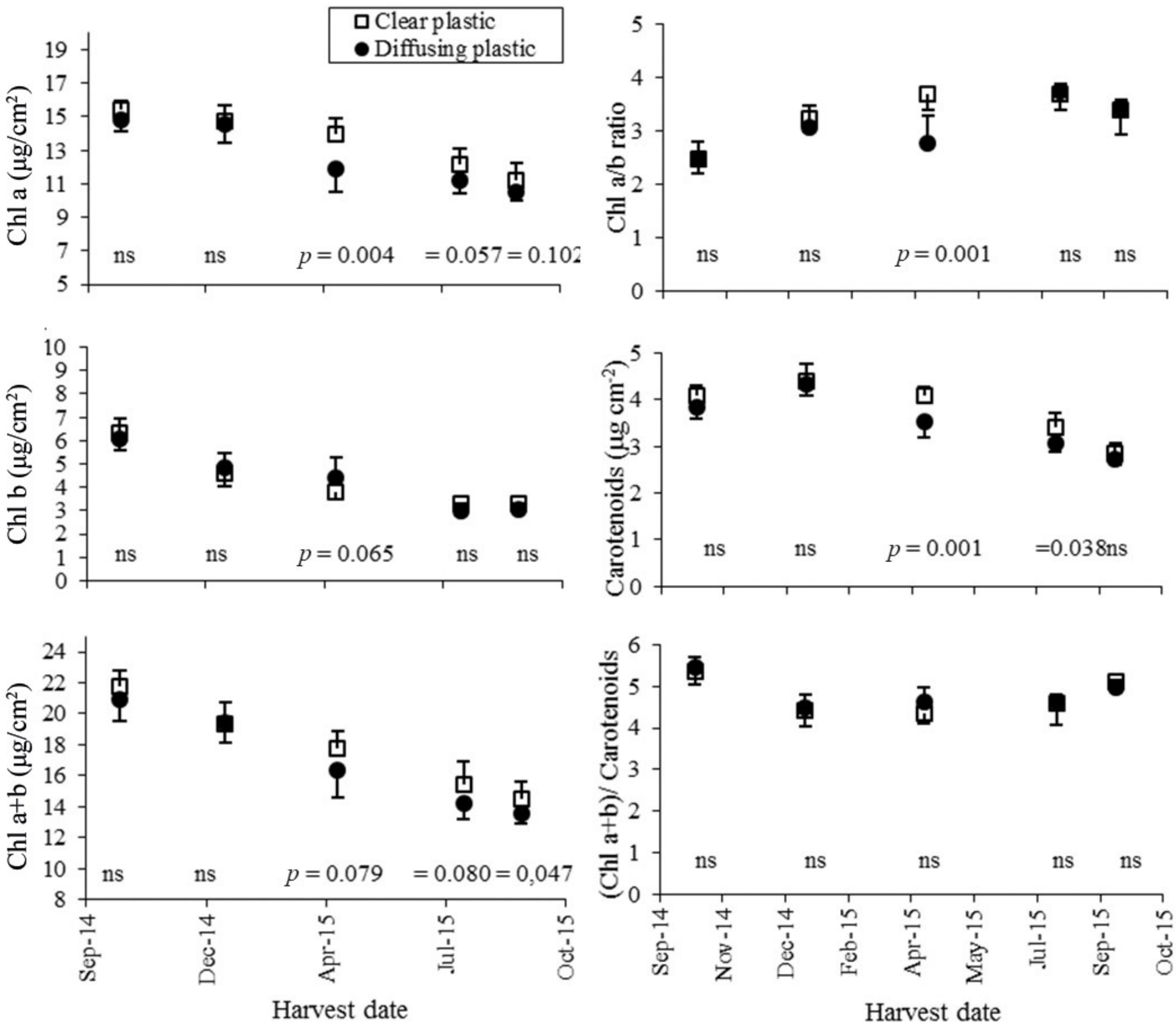

Figure 4. Effect of light-diffusing plastic on leaf pigments. Data are means of 8 replicates and bars represent standard deviations. ns = not significant, p> 0.10.

Table 3. Effect of clear and light-diffusing plastic films on microelements content of lettuces

Date	Plastic	Cu	Zn	Fe	Mn	∑ Micro	∑ Macro+Micro
		(mg/kg dw)					(g/kg dw)
Oct 2014	Clear	11.3 a	43.3 a	109.5 a	19.8 a	187.6 a	168.3 a
	Diffusing	10.9 a	45.9 a	111.2 a	19.8 a	191.6 a	175.3 b
Jan 2015	Clear	6.06 a	50.7 a	69.3 a	24.6 a	147.5 a	163.5 a
	Diffusing	6.45 a	53.5 a	76.3 a	29.0 a	164.1 a	180.0 b
April 2015	Clear	8.00 a	50.9 a	89.7 a	19.0 a	166.8 a	138.9 a
	Diffusing	10.1 a	49.1 b	92.2 a	19.6 a	166.8 a	133.2 a
July 2015	Clear	15.0 a	62.5 a	106.8 a	29.1 a	214.7 a	159.0 a
	Diffusing	14.3 a	41.5 b	106.6 a	27.6 a	188.6 b	168.7 b
Sept 2015	Clear	9.73 a	46.7 a	101.6 a	25.3 a	182.8 a	153.2 a
	Diffusing	9.53 a	44.6 a	85.62 b	21.9 b	161.8 b	162.8 b
Max sd		1.95	8.41	13.3	9.3	21.0	8.0

Data represent the means of 4 independent replicates. Max sd represents the maximum standard deviation value over the 5 harvest dates. Different letters indicate significant difference ($p < 0.10$).

was found between leaf NO_3 content and direct PAR (Fig. 6).

The type of greenhouse covering film did not affect the nitrogen content, carbon content, C/N ratio or the construction cost (CC) of lettuces. Irrespective of treatment and harvest date, the mean N content values ranged from 3.97% dw ± 0.16 to 4.66% ± 0.09, the mean C content from 36% dw ± 0.3 to 40% ± 0.9, the mean C/N ratio from 7.9 ± 0.2 to 10.0 ± 0.8 and the mean CC from 0.91 ± 0.01 to 1.08 ± 0.04 (g/g dw). The CC represents the amount of glucose used to provide the carbon skeletons, reducing power (NADH or equivalent) and chemical energy (ATP or equivalent) required for synthesis of one unit of dry biomass.

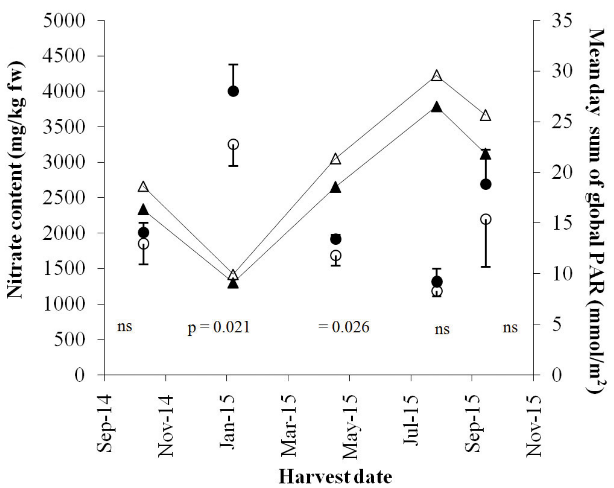

Figure 5. Effect of light-diffusing plastic on leaf nitrate content (●,○) and mean daily sum of global PAR (▲,△). Open symbols represent the control (clear plastic) treatment. Nitrate content values are means of 4 replicates and bars represent standard deviations. ns = not significant, $p >$ 0.10.

Soluble solids content, pH and acidity

Irrespective of the type of plastic film and harvest date, the soluble solids content (SSC) of the lettuce juice ranged from 2.93 ± 0.13 to 4.28 ± 0.10 ºBrix (Fig. 7). The type of plastic significantly affected the SSC on the first three harvest dates. The SSC was higher (by up to 16%) in lettuces grown under the light-diffusing plastic than in plants produced under clear plastic.

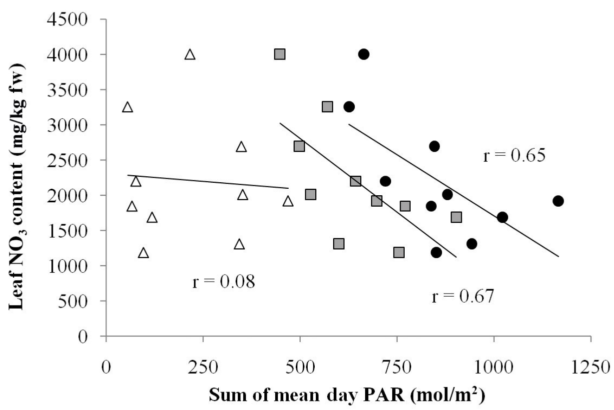

Figure 6. Relationships between leaf nitrate content and the sum of mean day radiationover each growth cycle (△: direct PAR, ■: diffuse PAR and ● global PAR) irrespective of the type of plastic cover and harvest date. Nitrate content values are means of 4 replicates. *r*= Pearson correlation coefficient.

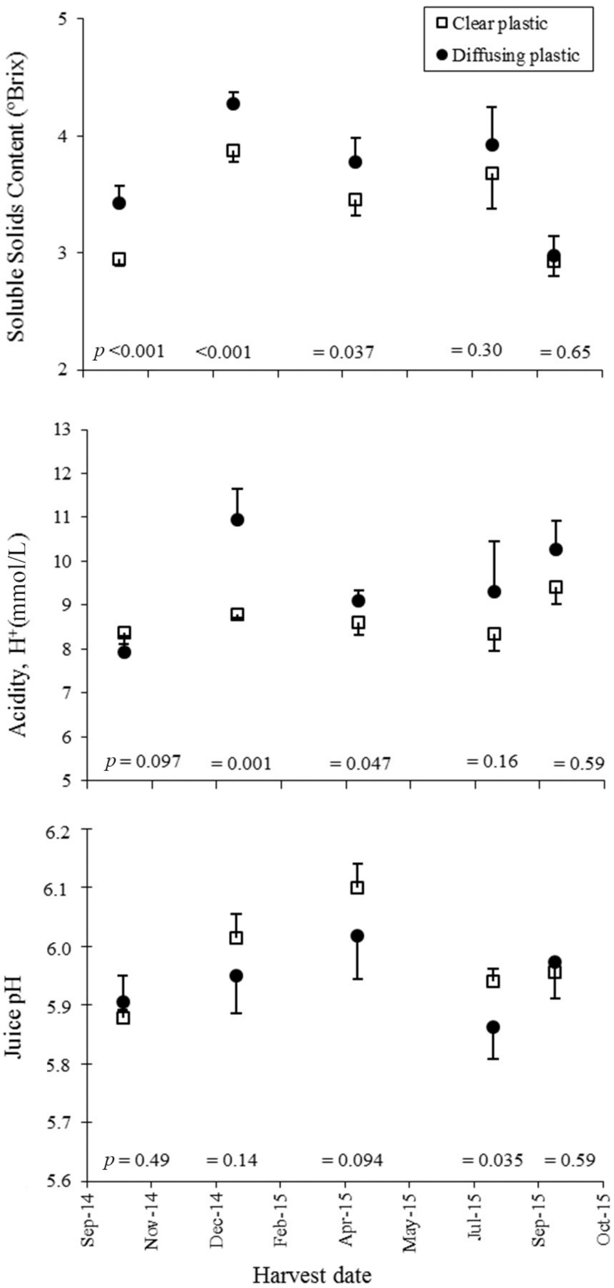

Figure 7. Effect of light-diffusing plastic on lettuce quality parameters. Data are means of 4 replicates and bars represent standard deviations.

The acidity tended to be higher and the pH lower in the lettuces grown under the light-diffusing plastic than in those grown under the clear plastic (Fig. 7).

Discussion

Although lettuce growth was enhanced by light diffusion under roof-mounted solar photovoltaic panels leading to uniform irradiations compared to plants grown under direct but fluctuating light (Tani *et al.*, 2014), use of the light-diffusing plastic evaluated in the present study reduced the fresh weight of the lettuce, due to the fewer and smaller leaves than in plants grown under the clear plastic. This is inconsistent with previous findings, which have demonstrated that diffuse light increased biomass production in tomato (Duek *et al.*, 2012), pepper (Chun *et al.*, 2005), *chrysanthemum* (Markvart *et al.*, 2010) and *Anthurium* (Li *et al.*, 2014b) plants. The increase in biomass is attributed to the more efficient use of diffuse light than of direct light by these other crops (Gu *et al.*, 2002; Li *et al.*, 2014a). In tomato plants, diffuse light enhances photosynthesis, mainly because horizontal and vertical distributions of the photosynthetic photon flux density within the crop was more uniform under diffuse light (Li *et al.*, 2014a). Nevertheless, the Batavia lettuce grown in the present study displays a very distinctive growth habit, with leaves forming a dense rosette that develops into a compact head in which all leaves reach a similar height. This growth habit implies that the outer and middle leaves were the ones strongly affected by light conditions and the effects due to the vertical distribution of the photosynthetic photon flux density were very limited.

As biomass production of the lettuce was correlated with the sum of the global PAR incident on the plant (r = 0.89, n = 10), irrespective of the type of plastic cover and harvest date, the loss of lettuce production should be related to the reduction (of between 5 and 15%) in the global PAR transmitted under the diffuse plastic relative to the clear plastic. However, the difference in the global PAR for each growing period was not related to the reduction in the fresh weight of the lettuces (see Table 1 and Fig. 3A), because plants grown under the diffuse light displayed a lower capacity to convert solar energy into biochemical energy (*i.e.* lower values of LUE). The photosynthetic apparatus of lettuce plants became acclimated to the diffuse light, thus reducing carotenoids and Chl (a+b) contents mainly as a result of a decrease in Chl a during spring, summer and autumn. There are some conflicting reports on the effects of diffuse light on Chl contents: *Anthurium* plants grown under light-diffusing glass were less green (measured as CIELAB values, p = 0.07) than those grown under clear glass (Li *et al.*, 2014b), whereas lettuces grown under diffusive roof-mounted solar photovoltaic panels showed similar SPAD values to those grown under non-diffusive panels during winter, spring and autumn, and higher values in summer harvests (Tani *et al.*, 2014). The Chl content and Chl *a/b* ratio increased in tomato plants grown under diffuse light (Li *et al.*, 2014a).

In the present study, the values of the Chl a/b ratio remained between 2.5 and 3.8, as expected (Lichtenthaler *et al.*, 1981). Lettuce plants did not show any differences in the Chl (a+b)/Carotenoids ratios in relation to the type of plastic covering. As carotenoids play an important role in protecting the photosynthesis apparatus from photodamage by energy dissipation (Ort, 2001), we can expect that the plants did not suffer an excess of photosynthetic photon flux density under either type of plastic films.

The lowest Chl contents found in lettuces plants grown under light-diffusing plastic cannot be related to the N content, as the type of plastic did not affect leaf N content. This contrasts with the findings of Li *et al*. (2014a), who reported higher N contents in tomato plants grown under diffuse light. However, in the present study the use of the light-diffusing plastic tended to increase the nitrate contents in leaves, and thus diffuse light seems to reduce N assimilation. The results showed that leaf NO_3 content was reduced concomitantly with the increase of the amount of global PAR incident on the lettuces. It is well known that light stimulates de novo synthesis and activation of plant nitrate reductase (NR) at the transcriptional level. NR catalyses the reduction of nitrate to nitrite using NADPH as electron donors, and represents the first step in the pathway of nitrogen assimilation from nitrate into organic compounds (Krouk *et al*., 2010). Curiously, in the present study leaf NO_3 content was more related to the amount of diffuse PAR incident on the plant than to direct PAR. To the best of our knowledge no information is available about this phenomenon and further in-depth researches were needed to elucidate the effect of diffuse light on nitrogen assimilation.

Although C content was not affected by the light-diffusing plastic, the SSC (measured in lettuce juice) was higher in plants grown under diffuse light. The SSC values were highly positively correlated with sugar contents (mainly glucose, fructose and sucrose) in fruits. Assuming this is also true for lettuce leaves, diffuse light seems to affect the accumulation of the end-products from photosynthesis. In addition, lettuce grown under the light-diffusing plastic were more acidic than lettuce harvested under the clear plastic, indicating that leaves contained more organic acids and/or amino acids, thus contributing to the establishment of titratable acidity. Further research is needed to elucidate how diffuse light affects nitrogen and carbohydrate metabolism in lettuce.

Relative to the clear plastic, the light-diffusing plastic induced an increase of up to 10% in total macroelement contents of lettuce leaves. This increment was mainly due to higher values of leaf K content (of up to 19%). To the best of our knowledge, data relating the effect of diffuse light on plant mineral composition are lacking, with the exception of N, as discussed above. The effect of diffuse light on the total macroelement contents of lettuce was statistically significant for all harvest dates, except April 2015. Comparison of these data with those relating the effect of diffuse light on the plant fresh weight (Fig. 3A) shows that the fresh weight of the lettuces grown under both types of plastic and harvested in April were similar. Altogether these results suggest that the lower mineral contents of lettuces grown under clear plastic may be due to a dilution effect caused by the increase in biomass. Nutrients are diluted because the amount of biomass produced is higher than the amounts of mineral absorbed and translocated to leaves (Bates, 1971).

In conclusion, our findings showed that use of the light-diffusing plastic film was detrimental to production of compact heads of lettuce. However, open-leaf cultivars would likely show a different response to the diffuse light. The loss of biomass was due to the fewer, smaller leaves produced. Nevertheless, the foliar mineral contents (mainly K), soluble solids content and acidity were higher in lettuce grown under the light-diffusing plastic than in lettuce grown under the clear plastic.

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