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ARTICLE

Effects of Low Nitrogen Supply on Tomato (*Solanum lycopersicum*) Fruit Yield and Quality with Special Emphasis on Sugars, Acids, Ascorbate, Carotenoids, and Phenolic Compounds

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The objective of this study was to determine the impact of lowering nitrogen supply from 12 to 6 or 4 mM NO_3^- on tomato fruit yield and quality during the growing season. Lowering nitrogen supply had a low impact on fruit commercial yield (−7.5%), but it reduced plant vegetative growth and increased fruit dry matter content, improving consequently fruit quality. Fruit quality was improved due to lower acid (10−16%) and increased soluble sugar content (5−17%). The content of some phenolic compounds (rutin, a caffeic acid glycoside, and a caffeic acid derivate) and total ascorbic acid tended to be higher in fruit with the lowest nitrogen supply, but differences were significant in only a few cases (trusses). With regard to carotenoids, data did not show significant and univocal differences related to different levels of nitrogen supply. Thus, reducing nitrogen fertilization limited environmental pollution, on the one hand, and may improve, on the other hand, both growers' profits, by limiting nitrogen inputs, and fruit quality for consumers, by increasing tomato sugars content. It was concluded that primary and secondary metabolites could be affected as a result of a specific response to low nitrogen, combined with a lower degree of vegetative development, increasing fruit irradiance, and therefore modifying fruit composition.

KEYWORDS: Acids; ascorbate; carotene; flavonoids; fruit irradiance; fruit quality; gap fraction; nitrogen; polyphenolics; *Solanum lycopersicum*; sugars; tomato

INTRODUCTION

Nitrogen (N) is one of the main nutrients required for plant growth and is therefore applied to crops in large amounts to ensure big yields. Nitrogen fertilizer was often used in excess in the past; as a consequence, soil and water were subject to heavy pollution. This problem has now been addressed, and fertilizing practices are being revised to limit pollution and fertilizer costs (*1*). Until recently, nitrogen recommendations for tomato greenhouse production on rockwool were approximately 10 or 13−15 mM (*2*, *3*) depending on the growth stage and the country. However, those recommendations can be improved. Dumas et al. (*4*) showed that 6 mM NO_3^- in nutritive solution was sufficient to optimize young tomato growth from seedlings to early bloom. Complementary studies demonstrated that nitrogen supply was positively correlated to fruit yield, up to a threshold value above which the overfeeding of plants did not increase yield (*5*−*7*). Additionally, the impact on marketable fruit yield should be considered instead of the impact on total fruit yield. A previous study had already demonstrated that total fruit yield was affected by increasing nitrogen supply from 50 to 250 kg/ha, whereas the marketable fruit yield was not (*8*). From an environmental point of view, it thus seems necessary to optimize nitrogen use efficiency by the plant and to limit its loss in the environment. However, the consequences of reducing nitrogen supply on fruit quality are poorly known.

Visual appearance and firmness are the only traits available to the consumer for assessing fruit quality when buying

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tomato (*9*). Fruit color was reported to be unaffected by nitrogen supply (*10*, *11*), whereas fruit firmness varied (*12*−*14*). Reducing nitrogen supply could thus have a limited impact on crop yield and the visual appearance of the fruit, but its effects on tomato fruit quality are still not clear. Aziz (*15*) found higher sugar content in fruits harvested from plants grown under low N supply (1 mM) compared to fruits harvested from plants receiving higher nitrogen levels (12.9 and 15.8 mM). Simonne et al. (*10*) reported decreased titratable acidity when the nitrogen supply was increased from 0 to 392 kg ha^{-1}. However, increasing the nitrogen supply from 2.25 to 36 mM was reported to increase sugar and acid contents, thus improving tomato quality (*14*). These differences might be linked to the fact that lowering the nitrogen supply in young plantlets has a strong impact on plant vegetative development and, consequently, carbon import to the fruit and fruit metabolism. Thus, the impact of reducing nitrogen supply probably depends on the developmental stage of the plant.

More attention is now focused on promoting the health benefits of the regular consumption of fruits and vegetables because fruits contain a wide assortment of antioxidant molecules (carotenoids, phenolics compounds, and ascorbate) that contribute to fruit nutritional quality. Nevertheless, the role of tomato consumption in cancer or cardiovascular disease is difficult to establish and needs more investigations. To date, the number of clinical studies is limited, and the results are sometimes contradictory (*16*−*23*).

Only a few studies are available on the impact of nitrogen supply on fruit antioxidant content (*24*, *25*), and more information is needed. Studies on leaves have revealed that nitrogen supply has wide-ranging effects on primary (*26*−*28*) and secondary (*29*, *30*) metabolism. Tomato leaf polyphenolic compounds were shown to increase in response to low nitrogen supply (*31*, *32*), but no significant variations were observed in tomato fruit (*33*). A decrease in ascorbic acid content in tomato fruit was reported in several studies when the nitrogen supply was increased (*24*, *34*). On the other hand, β-carotene content seemed to increase with increased nitrogen supply; however, the impact of nitrogen supply on lycopene content is more controversial (*24*, *25*). Nitrogen supply could affect fruit primary and secondary metabolism. It could also affect plant vegetative development, triggering changes in fruit irradiance and, consequently, fruit metabolism, as was suspected for increased ascorbate content under low nitrogen supply (*24*).

In the present study, we focused on the impact of lowering the nitrogen supply on fruit compounds. To do so, nitrogen treatments were initiated 2 months after sowing and continued until the last harvest. The aim of this study was to determine the impact of lowering the nitrogen supply during fruit development on the yield and quality of greenhouse-grown tomato fruits, with special emphasis on the fruit visual aspect and fruit content of primary and secondary metabolites. This study was carried out over a whole cultural cycle while maintaining the normal practices used by growers. The hypothesis of an indirect effect of nitrogen supply on vegetative growth was tested by following the growth of the different plant organs. In addition, the fruit microclimate was characterized by the gap fraction to determine whether or not nitrogen supply had an impact on fruit irradiance. Changes in primary and secondary metabolites are discussed in relation to previous data on the effects of nitrogen supply or changes in fruit microclimate.

MATERIALS AND METHODS

Plant Material, Nitrogen Treatments, and Growth Conditions. The tomato plants (*Solanum lycopersicum* L. cv. Clotilde, Syngenta Seeds SAS, France) were grown in a greenhouse at the Ctifl Research Station (Bellegarde, southern France, 43° 45 N). On October 23, 2004, seeds were sown on rockwool rolls covered with vermiculite (20 × 27 mm, Grodan BV, Roermond, The Netherlands). After 8 days, seedlings were transferred to larger rockwool cubes (65 × 75 × 75 mm) and, finally, on November 28, 2004, plants with three−four true leaves were transplanted onto rockwool blocks (two plants per block, 100 × 15 × 25, Grodan BV). Planting took place in three modules of a glasshouse of 240 m^2 each, with a planting density of 2.5 plants/m^2.

Throughout plant development, three complete types of solutions were used to fertilize plants depending on the plant developmental stage (**Figure 1**). The final nitrate concentrations were 12 mM (a standard level in greenhouse production in 2005 when the experiment was realized) and two lower levels, 6 and 4 mM, in order to evaluate the consequences of reduced nitrate inputs and determine the optimal range of nitrate input. The first nutrient solutions, used from sowing to flowering of the second trusses (F2, December 16, 2004), were similar for the three treatments and contained 25 mM NO_3^-. From F2 to the flowering of the fourth truss (F4, January 4, 2005), three

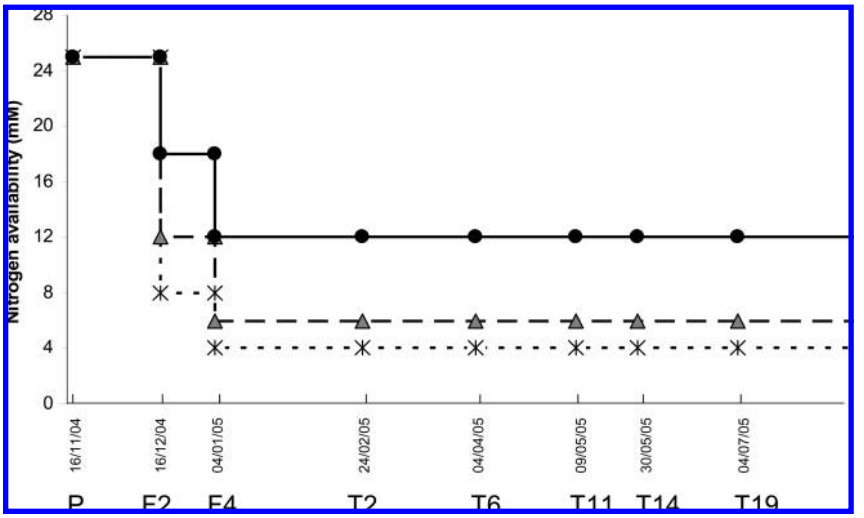

Figure 1. Cultural practices from planting (P) in November to the last harvest in July for truss 19 (T19). As of the flowering of the second truss (F2), three nitrogen levels were applied, and as of the flowering of the fourth truss (F4), 4, 6,and 12 mM NO_3^- were available for plants (stars, 4 mM; triangles, 6 mM; circles, 12 mM).

Table 1. Content in Macroelements of the Three Different Nutrient Solutions Used To Study the Impact of Nitrogen on Tomato Fruit Quality[a]

	nitrogen treatments		
	12 mM	6 mM	4 mM
pH	5.74	5.75	5.84
CE (mS cm^{-1})	2.4	2.4	2.4
HCO_3^- (mmol L^{-1})	0.5	0.5	0.5
Cl^- (mmol L^{-1})	3.0	8.9	11.2
SO_4^{2-} (mmol L^{-1})	2.1	2.5	2.3
NO_3^- (mmol L^{-1})	12.4	6.4	4.1
$H_2PO_4^-$ (mmol L^{-1})	1.8	1.9	1.7
Ca^{2+} (mmol L^{-1})	5.6	5.3	5.5
Mg^{2+} (mmol L^{-1})	1.6	2.0	1.8
Na^+ (mmol L^{-1})	0.9	1.0	1.0
K^+ (mmol L^{-1})	6.3	6.7	6.2
NH_4^+ (mmol L^{-1})	0.1	0.1	0.1

[a] Data correspond to the average composition applied to the plants from January to July.

Table 2. Climate during Fruit Development from Anthesis to Harvest for Each Truss Analyzed[a]

harvest date	truss	mean air temperature (°C)	maximal air temperature (°C)	global radiation (W/m^2)
Feb 24	2	17.4 ± 0.9	22.4 ± 1.7	746 ± 304
April 4	6	17.5 ± 0.8	23.9 ± 2.5	1220 ± 470
May 9	11	18.4 ± 1.2	25.0 ± 3.2	1327 ± 456
May 30	14	19.3 ± 1.5	25.6 ± 3.3	1242 ± 438
July 4	19	21.9 ± 2.5	28.7 ± 3.2	1317 ± 252

[a] Data were collected from meteorological stations within each glasshouse module; temperature (°C) was measured every minute and averaged over the fruit developmental period to obtain the mean air temperature. Maximal air temperature corresponded to the average of daily maximal temperatures from anthesis to harvest. Global radiation (W/m^2) was measured above the plants inside the glasshouse during fruit development. Data are means ± SD.

nitrogen levels were applied: 18, 12, and 8 mM. Then, from F4 until the end of growth, the plants were supplied with a third type of nutrient solution to reach the desired concentrations of 12, 6, and 4 mM NO_3^- (referred to as 12, 6, and 4 mM) at the root level. The three nitrogen treatments were repeated in each glasshouse module. Nutrient solutions were prepared from deionized water and a commercial mixture to obtain the desired mineral composition (**Table 1**). To maintain a constant cation/anion ratio, chloride was provided at 3, 9, and 11 mmol L^{-1}, respectively, for the 12, 6, and 4 mM media. Trace elements were provided by Oligonia (Agronutrition, France), formula T36 (0.04 mL/L), and Fe-EDTA was provided by Plantin Fer 250 (0.8 mg/L); the pH was adjusted to 5.7, and electrical conductivity was 2.4 mS/cm. The solutions were supplied using a drip irrigation system to maintain at least 30% drainage. Chemical pest and disease controls were in accordance with commercial practices. All plant side shoots were removed as they appeared; old leaves were removed every 15 days. Fruit load was set at five fruits per truss.

The mean air temperature measured from November 16 to July 31 was 21.6 °C during the day and 16.9 °C during the night, with relative humidities of 76 and 82.7%, respectively. There was no effect of the glasshouse module on the climatic conditions (data not shown). The mean climate during fruit development (from anthesis to fruit harvest) for each harvested truss is presented in **Table 2**. Air temperature was measured every minute and averaged from anthesis until harvest. Maximum air temperature corresponded to the average of daily maximum temperatures from anthesis until harvest. Global radiation (W/m^2) was measured above the plants (Pyranometer, Kipp and Zonen, Le Plessis Trevise, France) inside the glasshouse.

Characterization of Fruit Microclimate. The impact of nitrogen supply on fruit irradiance was investigated by measuring light transmittance from the top to the bottom of the canopy every two trusses. Light reaching the upper hemisphere of the fruits was estimated from upward-looking hemispherical photographs on April 7. At this date, the 16th truss (T16) of the plant was at anthesis at the top of the canopy, and the fruits of T6 had been harvested 3 days earlier. Ten upward-looking hemispherical photographs were taken per treatment with the hemispherical lens positioned at the first fruit of trusses 6, 8, 10, 12, and 14 (Nikon, 4500, fisheye lens). For each truss number and nitrogen supply, the gap fractions, that is, the transmittance of light through the canopy, considering the vegetation elements as opaque, was estimated using Can-Eye software [http://www.avignon.inra.fr/can_eye (*35*)]. This software makes it possible to compute the gap fraction from the color images by separating the green elements (i.e., vegetation) from the gaps (i.e., other elements present in the images such as the sky and the greenhouse structures). The percentage of mixed pixels was $< 3\%$. The bidirectional gap fraction was estimated over a range of zenith angles (0−60°) with a zenith angular resolution of 2.5° and an azimuth angle angular resolution of 5° (for azimuth ranging between 0 and 360°) (see Supporting Information). The gap fraction was then integrated over the azimuth and zenith directions and subjected to an analysis of variance to determine the impact of the fruit position and the nitrogen supply.

Fruit and Plant Sampling, Physical Trait Measurement, and Sample Preparation. We decided to compare different fruit harvests to follow the impact of lowering nitrogen on a long-term basis, from February to July. Every month, red ripe fruits were harvested: each harvest corresponded to a different truss number, T2, T6, T11, T14, and T19, harvested on February 2, April 4, May 9, May 30, and July 4, 2005, respectively.

Physicals traits (external coloration, firmness, and mean weight) were measured on 20 ripe fruits per module and per treatment (60 fruits per treatment). External color was characterized using the Hunter Laboratory color space on the fruit equatorial perimeter with a chromameter (Minolta Chromameter CR300, Minolta, France SA): *L* corresponds to lightness, *a* ranges from green to red, and *b* ranges from blue to yellow. Two firmness readings per fruit were made on the fruit equatorial perimeter with a penetrometer (Durofel, COPA-Technologie SA, St Etienne du Grès, France), using a 5 mm diameter probe. The index varied from firm (100) to soft (0).

Sugar and acid contents were determined on six subsamples (6 × 10 fruits) per treatment (using two of the opposite fourths). Means shown under Results thus correspond to the average of six independent samplings, extractions, and analyses. Antioxidant content was determined on three subsamples of 10 fruits per treatment. Antioxidant results thus corresponded to the mean of three independent samples (of 10 fruits) per treatment. Two of the opposite fourths were immediately frozen in liquid nitrogen and maintained at −80 °C until they were blended together in liquid nitrogen to determine antioxidant content (polyphenolic compounds, ascorbate, and carotenoids). An aliquot of the frozen powder was lyophilized prior to the polyphenolic analysis.

From February to July, plant growth was characterized from above ground dry weight measurements on six plants per month and per treatment (two plants per glasshouse module). Laminas, rachis, stems, peduncles, and fruits were isolated and put in an oven set at 80 °C for 1 week before being weighed to determine their dry mass (DM). The fruit DM content was estimated on a sample fraction by the difference in weight before and after lyophilization. Leaves removed during regular deleafing and regularly harvested fruits from these plants were also oven-dried, and their weight was added to lamina, rachis, or fruit dry weight.

Four plots of six plants were designed per glasshouse module and per treatment to calculate tomato crop yield. Fruits were harvested twice a week from February to July. The commercial

yield corresponds to the total fruit weight that can be sold, and the cluster yield to the weight of tomatoes that can only be sold as cluster-type tomatoes.

Chemical Analyses. Sugar and acid contents were determined by HPLC, in the same run. Tomatoes were ground in a Warring blender; the homogenate was then centrifuged at 14000 rpm (5 °C) for 5 min. The supernatant was recovered and diluted 20 times with water. Prior to injection, the extract was filtered through a cellulose acetate filter, and 10 μL of extract was injected into the HPLC. Samples were analyzed using a Varian ProStar instrument consisting of a Varian ProStar 401 autosampler linked to a Varian ProStar 330 UV detector, set at 210 nm for acid quantification, and to a Varian ProStar 350 RI refractive index detector for sugar quantification. Chromatographic separations were performed at 30 °C on a cation exchange column (7.8 × 300 mm, Transgenomic, San Jose, CA), fitted with a guard column (ICSep ICE-GC-801/C, Transgenomic). The mobile phase consisted of sulfuric acid (20 mN H_2SO_4) at a flow rate of 0.4 mL/min for 15 min. Quantification was based on peak area, referred to as a standard curve. Compounds were expressed as milligrams per gram of DW. Standards were purchased from Sigma Aldrich (Saint Quentin-Fallavier, France).

Phenolic compounds were analyzed using the method of Fleuriet (*36*) and modified as follows (see also ref *37*). All steps were carried out under cold conditions, either in a cold chamber or on ice. One hundred and twenty-five milligrams of dried tomato powder was extracted with 5 mL of 70% cold (−20 °C) aqueous ethanol. Fifty microliters of taxifolin (2 mg/mL methanol, Extrasynthèse, Lyon, France) was added as an internal standard. The mixture was blended for 1 min and homogenized for 30 min. After centrifugation, the supernatant was evaporated to dryness under vacuum. The residue was dissolved in a mixture of methanol (700 μL) and water (300 μL); prior to injection (20 μL) into the HPLC apparatus, the extract was purified by 5 min of centrifugation at 1000 rpm. The HPLC apparatus and analytical conditions were previously described in Gautier et al. (*37*). Rutin content was expressed as milligrams of rutin per gram of DW; other compounds (caffeic derivative compounds and naringenin chalcone) were expressed as equivalents of chlorogenic acid (mg of chlorogenic acid/g of DW). Rutin and chlorogenic acid standards were purchased from Sigma (Saint Quentin-Fallavier, France). Caffeic derivative compounds (CAG, caffeic acid glycoside; CAD1, -2, caffeic acid derivates) and naringenin chalcone (NC) were identified by their absorption spectra (**Figure 2**).

A spectrofluorometric technique was used to assay vitamin C content from 5 g of frozen powder ground from tomato fruit using the method of Deutsch and Weeks (*38*) and automated by Edberg et al. (*39*) with a fluorometer (Jenway model 6200 fluorometer, Felsted, U.K.). The method relies on the complexing of oxidized vitamin C with phenylenediamine to produce a fluorescent quinoxaline and was previously described (*37*, *40*).

Pigments were extracted with acetone and petroleum ether, and the contents of lycopene and β-carotene were estimated from the absorbance measurement at 503 and 451 nm, respectively, with a Shimadzu, UV-1605 spectrophotometer according to the method of Lime et al. (*41*).

Statistical Analyses. Fruit traits (yield, color, weight, firmness, fruit composition) were subjected to a two-way analysis of variance considering the effects of "nitrogen supply", "date of harvest", and the interaction between them using the XLSTAT statistical software (XLSTAT, Addinsoft, France). Mean comparison was performed with a Newman−Keuls test (α = 5%). The least significant difference (LSD) was calculated, and significant differences according to Newman−Keuls test are indicated by different letters in the tables and figures.

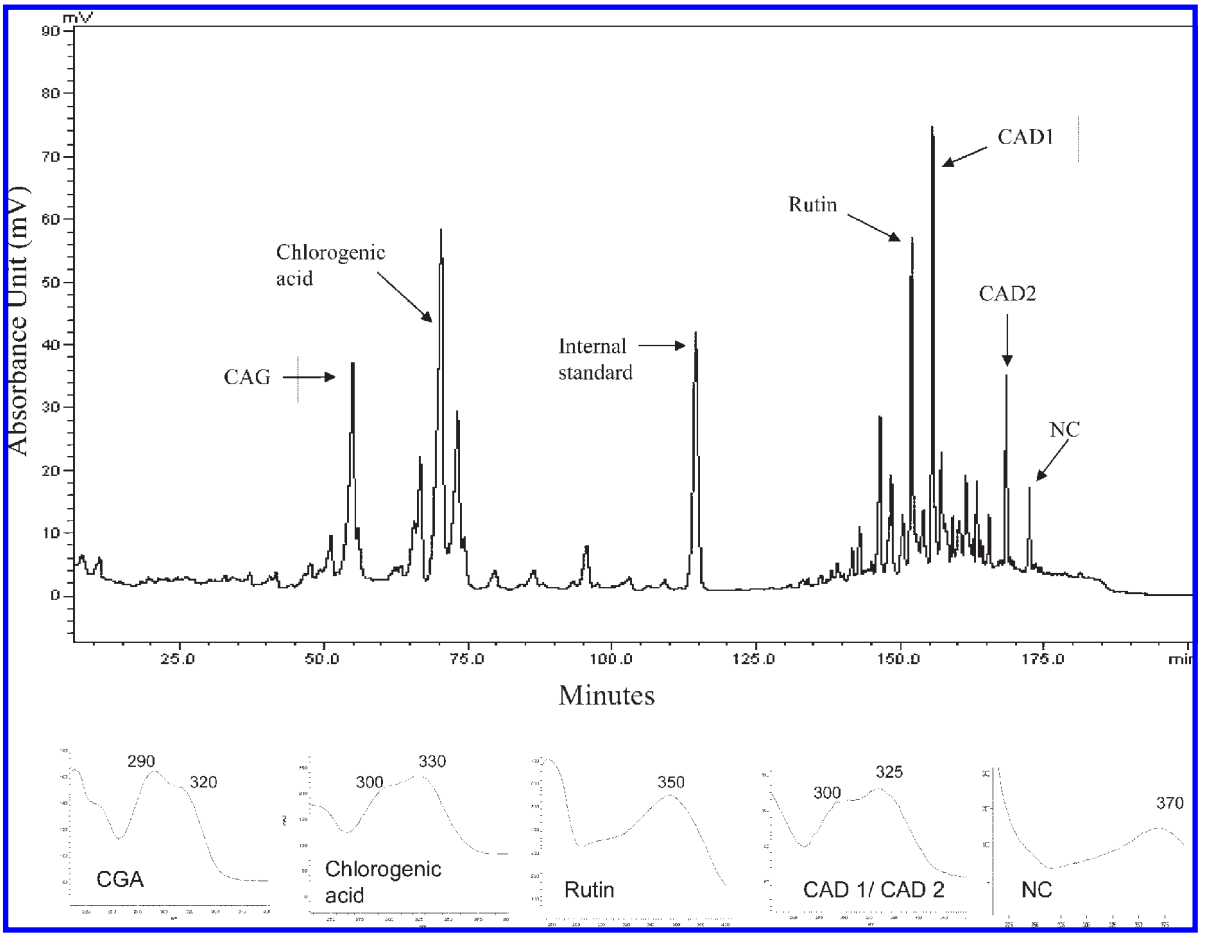

Figure 2. Chromatogram and UV spectra of phenolics found in deep red tomatoes. Detection was at 330 nm.

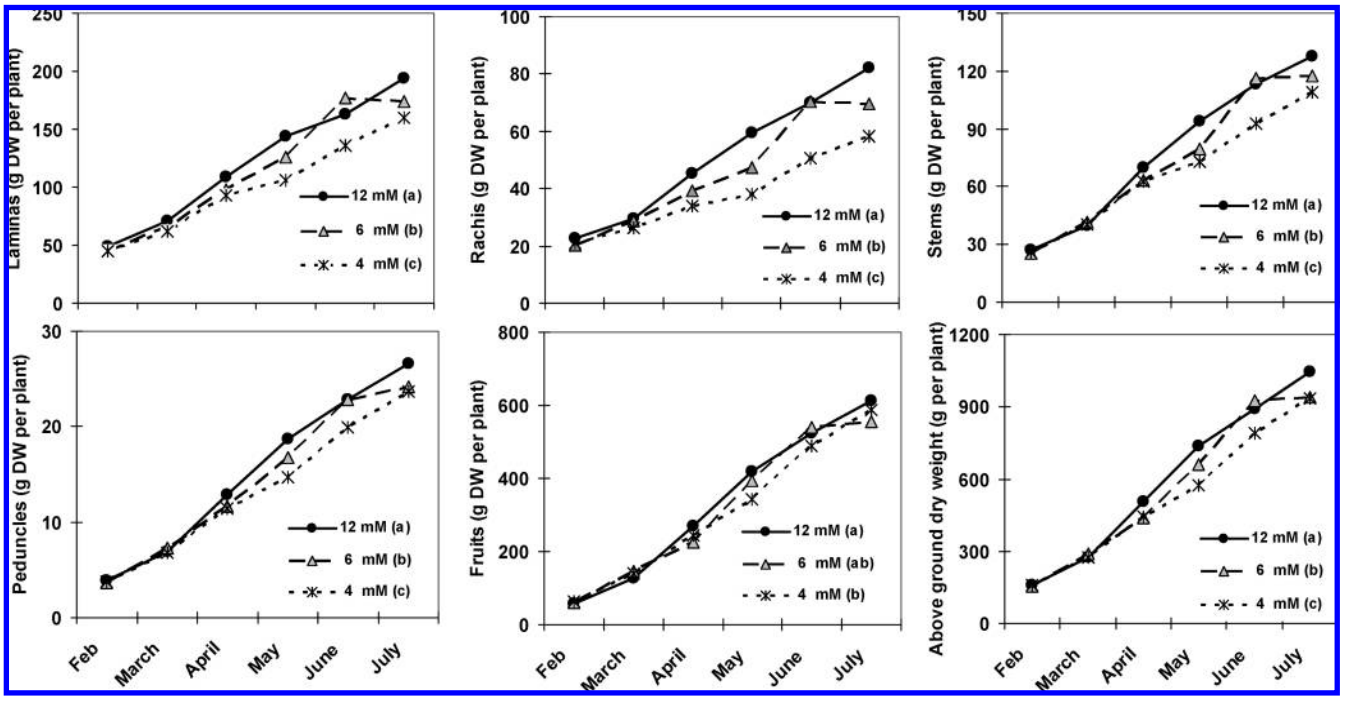

Figure 3. Impact of nitrogen supply on the increase in the dry mass (DM) of laminas, rachis, stems, peduncles, and fruits and aerial dry mass from February until July. Six plants were harvested per date and per treatment. The impact of the time and of the nitrogen supply was assessed by a two-way analysis of variance. Time and nitrogen availability were always highly significant ($P < 0.001$), and the difference among treatments was assessed by a Newman−Keuls test: different letters indicate significant differences at 5% between nitrogen supplies.

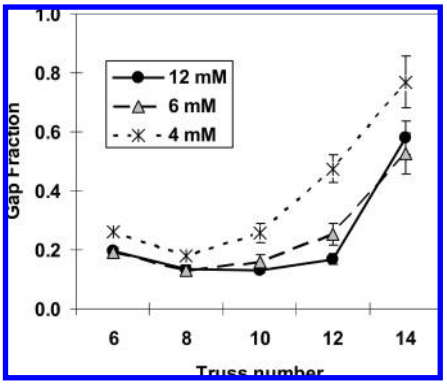

Figure 4. Impact of nitrogen supply on fruit microclimate. For each truss number and nitrogen supply, the gap fraction, that is, the transmittance of light through the canopy to the fruit, was investigated from upward-looking hemispherical photographs and estimated using Can-Eye software. Data are mean ± SD (see Supporting Information).

The visualization of the impact of reducing nitrogen supply on fruit composition was obtained by performing a principal component analysis on fruit composition data at different dates of harvest using XLSTAT statistical software.

RESULTS

Impact of Nitrogen Supply on Plant Dry Weight and Fruit Irradiance. Plant aboveground dry weight significantly decreased when nitrogen supply was reduced from 12 to 4 mM NO_3^- (**Figure 3**). In July, the lower nitrogen supply (4 mM) led to a 10% decrease in plant dry mass, compared to 12 mM. This was due to decreased vegetative dry mass, especially rachis (−29%), laminas (−17%), and stems (−15%). The peduncle dry mass was also reduced by 11%, whereas the fruit dry mass was reduced by only 4%.

These changes in dry mass also induced changes in fruit irradiance, according to the measurement of the gap fraction (**Figure 4**). Light transmittance decreased from the top to the bottom of the canopy, but lowering nitrogen levels had a significant impact on the gap fraction profile; it increased light transmittance in younger (T14), expanding (T10 and T12), and ripening fruits (T6 and T8).

Impact of Nitrogen Supply on Fruit Yields and Fruit Traits. The present data showed strong seasonal variations (**Tables 3** and **4**). The reduction in nitrogen supply from 12 to 4 mM NO_3^- slightly reduced commercial fruit yield by 7.5% (**Table 3**), but the cluster yield was poorly affected ($P < 0.1$) and was around 25 kg/m^2. A transient decrease was observed for the 4 mM nitrogen treatment in May, which was reversed in June, but a significant decrease was observed only in July (6 months after the beginning of lowering nitrogen) when the temperatures were no longer optimal for tomato growth. Thus, in the present study, lowering nitrogen did not have a strong impact on fruit yield. Similarly, fruit external color was not modified regardless of the nitrogen supply (**Table 4**). In contrast, fruit weight slightly decreased (significant in only T11) as fruit firmness (significant in only T19). In addition, fruit dry matter content increased when nitrogen supply was reduced (**Table 4**, significant in only T14 considering the Newman−Keuls test).

Sugars and Organic Acids Content. Both nitrogen supply and truss number had an impact on fruit sugar and acid contents, with a significant interaction between them except for sucrose, citric acid, and total acid (**Table 5**).

Sucrose content did not significantly vary with the nitrogen supply but decreased during the season (from T2 to T19). In contrast, glucose and fructose contents increased from T2 to T19. Moreover, reducing nitrogen supply (4 mM) also increased hexose content, especially in fruits from T2 to T11, whereas it was no longer significant in fruits harvested later. Consequently, total soluble sugars increased when the nitrogen supply was reduced, and the maximum increase of 18% (from 260 to 306 mg/g of DW) was observed on T6.

Table 3. Impact of Nitrogen Supply on Fruit Yields[a]

	N (mM)	cluster yield (kg/m^2)	commercial yield (kg/m^2)
February	12	0.900 e	1.072 d
	6	1.089 e	1.271 d
	4	1.013 e	1.182 d
March	12	3.592 d	4.096 c
	6	3.282 d	3.825 c
	4	3.396 d	3.793 c
April	12	3.585 d	4.154 c
	6	3.658 d	4.156 c
	4	3.391 d	4.000 c
May	12	3.730 a	6.960 a
	6	6.802 a	6.900 a
	4	5.810 bc	5.943 b
June	12	5.609 bc	6.119 b
	6	5.643 bc	5.982 b
	4	6.085 ab	9.349 ab
July	12	4.993 c	5.504 b
	6	5.303bc	5.642 b
	4	4.199 d	4.542 c
LSD		0.72	0.62
nitrogen		(*)	*
truss		***	***
nitrogen × truss		(*)	*

[a] Yields were calculated from fruits harvested twice a week from February to July. LSD, least significant difference; NS, not significant, i.e., $P > 0.1$; (*), $P < 0.1$; *, $P < 0.05$; ***, $P < 0.001$. Means were compared using a Newman−Keuls test with a confidence interval of 95%. Different letters per column indicate significant differences ($P < 0.05$).

Nitrogen effect on malic acid was weaker than the truss effect (which was especially due to its very low content in T2); malic acid content was only significantly reduced in T19 under the lower nitrogen supply (4 mM). Citric acid content significantly decreased under the lower nitrogen supply and during the season (with higher value in T2). Consequently, total acid content decreased by 10−16% under the lower nitrogen supply. The ratio between sugar and acid contents (a quality trait highly correlated to the sensing of fruit gustative quality by consumer) significantly increased during the season and even more when the nitrogen supply was reduced.

Phenolic Compounds, Carotenoids, and Ascorbate Content. For the antioxidant compounds, the effect of nitrogen supply was less significant than the truss effect and, surprisingly, interactions were not often observed (**Table 6**).

In deep red tomato, chlorogenic acid, rutin, a caffeic acid glucoside (CAG), two caffeic acid derivates (CAD1, CAD2), and naringenin chalcone were the major compounds detected (**Figure 2**). CAG, rutin, and chlorogenic acid were the most abundant phenolic compounds observed in Clotilde fruit, and their content varied during the season. CAG and chlorogenic acid content were at their peak in T14 (0.5 and 0.4 mg/g of DW, respectively), whereas rutin content was at its maximum in T19 (slightly lower than 0.3 mg/g of DW). The impact of nitrogen supply on phenolic compounds was not significant for the first truss harvested; significant effects of lowering nitrogen could be observed on only T14 or T19 but only for some phenolic compounds. According to the results of the Newman−Keuls test, significant increases were observed for rutin only on T14, for chlorogenic acid only on T19 (but with overlapping of groupings), for CAG only on T14, and for CAD1 only on T19, whereas for CAD2 and naringenin chalcone no significant differences between nitrogen treatments were observed. Consequently, fruits harvested on plants grown with the lowest nitrogen supply tend to have the highest phenolic content.

Carotene (b-car, β-carotene; lyc, lycopene) content increased during the growing period, but it decreased later, with T14 having significantly higher β-carotene and lycopene contents (0.3 and 1.0 mg/g of DW, respectively). Reducing the nitrogen supply had a poorly significant effect on carotene levels (significant at $P < 0.05$ for β-carotene and at $P < 0.1$ for lycopene). Moreover, results of the Newman−Keuls test show a significant reduction of lycopene content only in T14 and a poorly significant increase (with overlapping of groupings) of β-carotene content only in T19.

Total ascorbate content increased by 28% from T6 to T19 (from 2.6 to 3.3 mg/g of DW). The oxidized ascorbate content decreased from T2 to T11 and then increased. The global effect of nitrogen levels was not significant on the oxidized ascorbate and was significant at $P < 0.05$ for total and reduced ascorbate; nevertheless, lowering the nitrogen supply on total ascorbate level was not a clearly significant effect ($0.05 < P < 0.1$) despite the tendency observed in some trusses of an increase in ascorbate content when the nitrogen supply is lowered. Consequently, the ascorbate redox ratio (reduced ascorbate per total ascorbate content) was only significantly increased when the nitrogen supply was lowered in T2 and T11.

Synthetic View of the Impact of Nitrogen Supply on Fruit Composition. The impact of nitrogen supply on fruit composition was visualized by performing a principal component analysis (PCA) on primary and secondary compounds data (**Figure 5**). T2, T6, and T19 were clearly differentiated on the PCA plane, whereas T11 and T14 were not. The first component of the PCA, which explained 32% of the variability, coincided with the separation of the three nitrogen treatments within each truss; the higher abscissa corresponded to the lower nitrogen treatment and the lower abscissa to the higher nitrogen treatments (**Figure 5A**). The impact of nitrogen was the most pronounced on the T6. T2 and T19 were well separated by the *x*-axis: fruits on T2 contained more citric acid and sucrose but less glucose and fructose; they therefore had a lower sugar/acid ratio compared to fruits on T19 (**Figure 5B**). Consequently, PC1 discriminated changes in primary compounds (sugars and acids). In contrast, PC2 (which explained 24% of the variability) was correlated to antioxidant content; positive ordinates were correlated to increased phenolic compounds such as CAD1, rutin, and CAD2, but reduced CAG content, and to a lower ascorbate redox state. Antioxidant content was dependent on the truss number: T2 and T19 had positive ordinates, whereas T11 and T14 had negative ordinates, and T6 was intermediate.

DISCUSSION

Within the framework of changing cultural practices, the present experiment, carried out in a greenhouse while maintaining the normal practices used by growers, confirmed that nitrogen supply may be reduced from 12 to 4 mM NO_3^- without any significant changes in the commercial yield. As already observed by Warner et al. (*11*), fruit visual quality

Table 4. Impact of Nitrogen Supply on Fruit Parameters: Fruit Weight, Dry Matter Content (Grams of Dry Weight per 100 g of Fresh Weight), Firmness, and Color (*L*, *a*, *b* Indices)[a]

						color		
harvest date	truss	N availability (mM)	fruit wt (g)	fruit DM (%)	firmness	*L*	*a*	*b*
Feb 24	2	12	121.43 de	4.96 bc	81.11 a	40.30 a	18.07 d	17.83 d
		6	113.92 e	5.03 bc	79.78 ab	40.31 a	17.88 d	17.95 d
		4	114.20 e	5.38 ab	79.03 abc	39.63 abc	17.43 de	17.14 d
April 4	6	12	133.35 bcd	4.60 cd	80.24 ab	38.82 cd	16.85 de	17.88 d
		6	140.81 ad	4.75 cd	78.03 abcd	38.53 d	16.02 e	17.46 d
		4	139.19 adc	5.10 bc	80.40 ab	38.49 d	13.97 f	17.17 d
May 9	11	12	150.65 a	5.00 bc	78.20 abcd	38.79 cd	17.30 de	19.46 c
		6	134.73 bcd	4.98 bc	76.24 cde	39.04 cd	18.18 d	19.96 bc
		4	130.71 bcd	5.34 ab	77.26 bcd	39.30 bcd	18.52 d	20.08 bc
May 30	14	12	140.20 ab	5.05 bc	75.58 def	38.83 cd	19.76 c	19.90 bc
		6	141.97 ab	5.08 bc	74.06 efg	38.71 d	19.93 c	20.27 bc
		4	134.57 bcd	5.56 a	73.30 fg	38.94 cd	20.19 c	20.83 b
July 4	19	12	128.36 bcd	4.66 cd	71.68 g	40.23 a	21.82 a	22.73 a
		6	129.16 bcd	4.34 d	67.26 h	39.97 ab	22.12 b	22.46 a
		4	123.06 cde	4.38 d	67.41 h	40.33 a	23.77 b	23.02 a
LSD			10.42	0.32	2.09	0.59	1.23	0.82
nitrogen			*	***	***	NS	NS	NS
truss			***	***	***	***	***	***
nitrogen × truss			*	*	(*)	NS	***	*

[a] Fruit traits were determined on fruits harvested on T2, T6, T11, T14, and T19. LSD, least significant difference; NS, not significant, i.e., $P > 0.1$; (*), $P < 0.1$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. Means were compared using a Newman−Keuls test with a confidence interval of 95%. Different letters per column indicate significant differences.

Table 5. Impact of Nitrogen Availability and Harvest Date on Tomato Fruit Sugar and Acid Contents[a]

harvest date	truss	N availability (mM)	sucrose	glucose	fructose	citric acid	malic acid	total sugar	total acid	sugar/acid
Feb 24	2	12	6.3 a	97.4 f	126.4 f	52.4 a	6.3 d	230.1 e	58.7 bcd	3.92 f
		6	6.1 a	100.4 f	131.7 ef	49.5 ab	6.0 d	238.2 e	55.5 def	4.3 e
		4	5.8 a	109.6 f	141.1 de	46.6 bc	6.2 d	256.5 d	52.9 efg	4.87 cd
April 4	6	12	6.4 a	114.8 de	139.4 de	50.1 ab	10.0 abc	260.6 d	60.0 abc	4.34 e
		6	5.8 a	120.3 cd	145.3 cd	43.9 cd	9.6 bc	271.4 cd	53.5 efg	5.07 cd
		4	5.9 a	136.6 ab	163.5 a	41.7 d	9.3 c	306.0 a	51.0 g	6.0 a
May 9	11	12	4.4 b	123.5 c	142.4 d	48.6 ab	9.5 bc	270.3 cd	58.1 bcd	4.68 e
		6	4.4 b	128.2 bc	148.8 bcd	46.7 bc	9.4 bc	281.5 bc	56.1 cdef	5.03 cd
		4	4.3 b	138.6 a	163.1 a	42.5 d	9.9 abc	306.0 a	52.4 fg	5.85 a
May 30	14	12	4.4 b	134.8 ab	153.0 abc	51.0 a	10.0 abc	292.2 ab	61.0 ab	4.79 cd
		6	4.2 b	138.5 a	157.2 ab	46.4 cd	10.4 ab	299.9 ab	56.8 cde	5.28 bc
		4	4.2 b	140.0 a	161.9 a	44.7 cd	9.9 abc	306.1 a	54.7 defg	5.61 ab
July 4	19	12	4.4 b	141.2 a	163.2 a	51.9 a	10.7 a	308.7 a	62.6 a	4.93 de
		6	3.5 bc	133.4 ab	158.1 ab	48.8 ab	10.0 abc	295.1 ab	58.7 bcd	5.03 cde
		4	2.9 b	136.3 ab	165.1 a	43.3 cd	9.2 c	304.3 a	52.6 fg	5.8 ab
LSD			0.7	6.7	8.0	2.5	0.6	14.6	2.7	0.3
nitrogen			**	***	***	***	*	***	***	***
truss			***	***	***	***	***	***	***	***
nitrogen × truss			NS	***	**	NS	**	***	NS	**

[a] Compounds are expressed in mg/g of DW. Fruit was harvested on trusses 2, 6, 11, 14, and 19, respectively, from February to July. LSD, least significant difference; NS, not significant, i.e., $P > 0.1$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. Data are means, compared using a Newman−Keuls test with a confidence interval of 95%. Different letters per column indicate significant differences.

(fruit external color and firmness) was not significantly modified by reduced nitrogen supply. Reducing nitrogen thus appears to improve growers' profits by limiting nitrogen inputs without affecting fruit yield and external quality. Nevertheless, the impact of this practice has never been fully studied on fruit composition. As a result, the present study

Table 6. Impact of Nitrogen Availability and Harvest Date on Tomato Micronutrient Content[a]

harvest date	truss	N availability (mM)	CAG	rutin	chloro	CAD1	CAD2	NC	beta	lyc	T-AsA	ox-AsA	r-AsA	redox
Feb 24	2	12	0.167 e	0.188 abc	0.204 cde	0.097 bcd	0.087 a	0.082 bc	0.186 c	0.883 bcd	2.810 ab	0.414 a	2.396 ab	0.852 e
		6	0.178 de	0.175 abc	0.196 cde	0.094 bcd	0.084 a	0.115 a	0.215 bc	0.996 ab	2.954 ab	0.395 a	2.559 ab	0.867 de
		4	0.183 de	0.135 c	0.154 e	0.088 cd	0.085 a	0.084 b	0.199 bc	0.980 ab	2.698 ab	0.299 b	2.399 ab	0.889 cd
April 4	6	12	0.199 de	0.166 bc	0.185 de	0.075 d	0.071 a	0.050 e	0.179 c	0.630 e	2.471 b	0.262 bc	2.209 b	0.894 bcd
		6	0.214 de	0.137 c	0.152 e	0.073 d	0.091 a	0.058 de	0.181 c	0.662 e	2.558	0.277 bc	2.281 ab	0.891 cd
		4	0.259 d	0.176 abc	0.125 e	0.107 bcd	0.079 a	0.057 de	0.208 bc	0.631 e	2.780 ab	0.266 bc	2.514 ab	0.904 bcd
May 9	11	12	0.366 c	0.134 c	0.285 bc	0.080 d	0.069 a	0.055 e	0.213 bc	0.904 bcd	2.507 ab	0.173 c	2.334 ab	0.929 b
		6	0.345 c	0.123 c	0.246 bcd	0.089 cd	0.073 a	0.055 e	0.235 b	0.901 bcd	3.030 ab	0.088 d	2.942 ab	0.972 a
		4	0.415 bc	0.148 c	0.316 b	0.098 bcd	0.080 a	0.061 cde	0.209 bc	0.767 cde	3.232 ab	0.049 d	3.183 a	0.985 a
May 30	14	12	0.456 b	0.149 c	0.416 a	0.077 d	0.075 a	0.062 bcde	0.302 a	1.132 a	2.629 ab	0.233 bc	2.396 ab	0.912 b
		6	0.528 a	0.149 c	0.392 a	0.081 d	0.077 a	0.071 bcde	0.302 a	1.024 ab	2.631 ab	0.228 bc	2.403	0.912 b
		4	0.572 a	0.267 ab	0.432 a	0.099 bcd	0.081 a	0.079 bcd	0.300 a	0.926 bc	3.039 ab	0.294 b	2.745 ab	0.903 bcd
July 4	19	12	0.150 e	0.236 abc	0.204 cde	0.124 b	0.083 a	0.082 bc	0.181 c	0.721 de	3.142 ab	0.399 a	2.743 ab	0.873 cde
		6	0.177 de	0.241 abc	0.210 cee	0.121 bc	0.078 a	0.070 bcde	0.209 bc	0.761 cde	3.333 ab	0.408 a	2.926 ab	0.877 cde
		4	0.165 e	0.294 a	0.269 bc	0.158 a	0.091 a	0.069 bcde	0.203 bc	0.732 de	3.498 a	0.428 a	3.070 ab	0.877 cde
LSD			0.059	0.067	0.059	0.022	0.016	0.014	0.025	0.125	0.569	0.076	0.537	0.024
nitrogen			**	*	NS	***	NS	*	*	(*)	*	NS	*	**
truss			***	***	***	***	NS	***	***	***	**	***	**	***
nitrogen × truss			NS	*	(*)	NS	NS	*	NS	(*)	NS	*	NS	*

[a] Fruit was harvested from trusses 2, 6, 11, 14, and 19, respectively, from February to July. Phenolics are expressed as equivalents of chlorogenic acid (chloro) in mg of chlorogenic acid per g of dry weight (DW), except for rutin content, which is expressed as mg of rutin per g of DW; carotenoids are expressed in mg/g of DW. Compounds studied: caffeic acid glucoside (CAG), chlorogenic acid, rutin, caffeic acid derivates (CAD1 and 2), naringenin chalcone (NC), β-carotene (beta), lycopene (lyc), total ascorbate (T-AsA), the oxidized ascorbate form (Ox-AsA), the reduced form (r-AsA), and the ratio of reduced form to total ascorbate (redox). LSD, least significant difference; NS, not significant; (*), $P < 0.1$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. Data are means, compared using a Newman−Keuls test with a confidence interval of 95%. Different letters per column indicate significant differences.

aims to determine the positive and negative impacts on fruit content in sugars, acids, ascorbate, phenolic compounds, and carotenoids.

The present experiment showed increased fruit sugar content under reduced nitrogen supply, in agreement with data reported by Aziz (*15*), and a significant decrease in total acid content. This is in agreement with the study of Simonne et al. (*10*), who also found a 10% decrease in titratable acidity in response to reduced nitrogen supply. In addition, changes in leaf sugar content have been reported on tomato leaves when plants were submitted to saturated, replete, or deficient media (*27*): the levels of sucrose, glucose, and fructose increased, and the levels of citrate, isocitrate, fumarate, and malate significantly decreased when plants were grown on the deficient medium. Consequently, the present data suggested that both tomato leaves and fruits may respond in a similar way to lower nitrogen levels: by increasing sugars and decreasing acids.

Nitrogen effects could be linked to changes in fruit microclimate. We observed that the vegetative growth was more significantly affected by lowering nitrogen than the reproductive growth. This was in agreement with results on tomato obtained after N withdrawal, for which source activity appeared to be more affected than sink activity (*42*). Thus, the distribution of dry matter to the fruit appeared to remain the first priority in the plant's development when the nitrogen supply was reduced. The increased gap fraction measured at the fruit level confirmed increased fruit irradiance (and fruit temperature also due to increased infrared radiation). Therefore, under low nitrogen supply, increased fruit irradiance combined with increased fruit temperature is likely to trigger changes in fruit composition. The impact of fruit microclimate has been previously reported by monitoring fruit temperature during fruit development (*43*) and fruit irradiance and temperature during ripening (*37*). Walker and Ho (*43*) reported that heating fruits from 25 to 35 °C increased carbon import and hexose content. On the other hand, increased fruit irradiance probably increases fruit photosynthesis and fruit sugar content. Carrara et al. (*44*) showed that tomato fruit had a consistent photosynthetic activity. Thus, increased fruit irradiance under low nitrogen supply may contribute to increased sugar content. The fact that increased fruit irradiance triggers increased fruit temperature may also explain the observed decreased acid content. Gautier et al. (*37*) observed a decrease in titratable acidity when fruit temperature increased from 21 to 26 °C. Consequently, the observed impact of lowering the nitrogen supply could be due to increased fruit irradiance and fruit temperature combined with a specific effect of decreased nitrogen supply on fruit primary metabolism.

The impact of lowering nitrogen on ascorbate and secondary metabolites was less pronounced. Lowering nitrogen levels had an impact on fruit ascorbate content, triggering a slight increase (from 11 to 29%) in total ascorbate; our finding was consistent with previous studies (*13*, *24*, *34*). For example, Simone et al. (*10*) reported a 25% decrease in ascorbate content when nitrogen supply was increased from 0 to 392 kg/ha. Increased ascorbate content could be related to increased fruit irradiance, as was previously suggested (*24*). The present study supports this hypothesis because it provides measurements of fruit irradiance expressed as gap fraction and clearly shows that reducing nitrogen modified the fruit microclimate by increasing fruit irradiance.

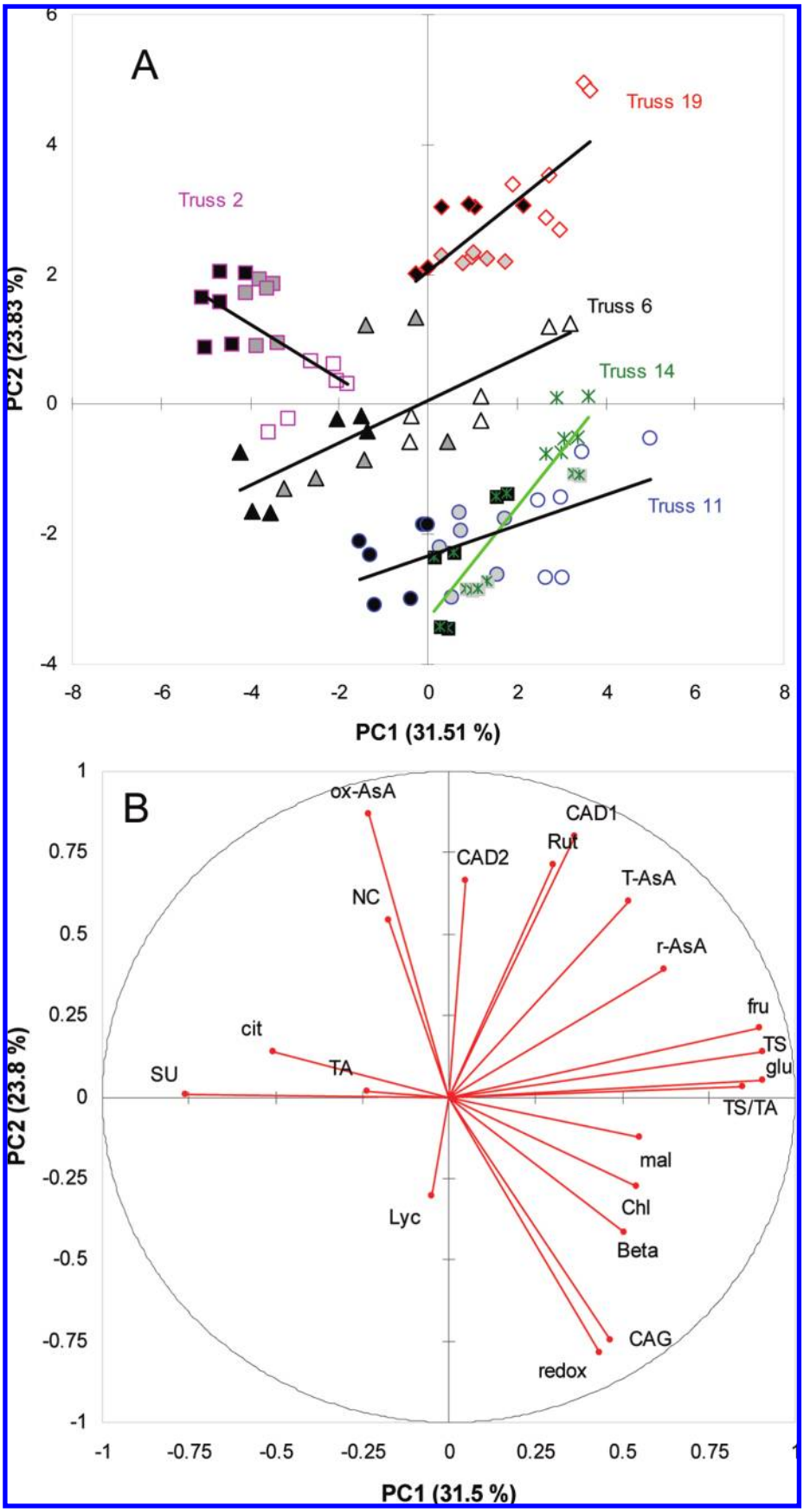

Figure 5. Principal component analysis (first principal component, PC1, versus second principal component, PC2) of the mature tomato fruit composition of trusses 2, 6, 11, 14, and 19 (T2, T6, T11, T14, and T19) harvested on plant receiving three different nitrogen levels 19 days after the flowering of T2 (open symbols, 4 mM NO_3^-; gray symbols, 6 mM NO_3^-; black symbols, 12 mM NO_3^-; squares, T2; triangles, T6; circles, T11; crosses, T14; diamonds, T19). PCA plot of the samples (**A**) and of the compounds analyzed (**B**) with an explained variance of 31.5% over PC1 and 23.8% over PC2. Compounds studied: SU, sucrose; glu, glucose; fru, fructose; TS, total soluble sugars; mal, malate; cit, citrate; TA, total acids; TS/TA, ratio TS/TA; CAG, caffeic acid glucoside; Chl, chlorogenic acid; Rut, rutin; CAD1 and 2, caffeic acid derivates; NC, naringenin chalcone; Beta, β-carotene; Lyc, ycopene; T-AsA, total ascorbate; ox-AsA, oxidized ascorbate; r-AsA, reduced ascorbate; redox, ratio r-AsA/T-AsA.

The present data confirmed the seasonal effect on carotene content in tomato fruit. β-Carotene content increased from February (harvest of T2) until the end of May (harvest of T14) and was correlated to increased temperature (r = 0.82, data not shown). Lycopene content also increased from the beginning of April (T6) until the end of May (T14) and was correlated to temperature (r = 0.85). However, at the beginning of July, both β-carotene and lycopene contents decreased; this may be due to the inhibition of the carotenogenesis pathway because of excessive temperatures (the maximum air temperature throughout the development of T19 was slightly lower than 29 °C). Tomes (*45*) had in fact shown that fruit temperature above 30 °C had an inhibitory effect on lycopene and β-carotene accumulation. The impact of lowering nitrogen on carotenes was not as obvious, except for the decrease in lycopene content observed in T14 under 4 mM. The same tendency was also observed in T11, whereas β-carotene was not significantly modified. This type of differential response between β-carotene and lycopene was previously described under increased fruit temperature (*24*, *37*). Moreover, the weak response to lowering nitrogen could be linked to the combination of (i) the response to increased irradiance that stimulates carotene synthesis and (ii) the response to a threshold temperature that does the reverse (inhibition of carotene synthesis). The combination of these opposite responses may explain why contradictory results have been reported in experiments designed to determine the impact of reducing nitrogen supply (*24*).

The phenolic content also showed variations from one harvest to another, in agreement with previous studies that reported a strong seasonal impact (*46*−*48*) and the influence of growth conditions (*24*, *49*). Regarding the levels of phenolics, the content of naringenin chalcone was relatively low when compared with other published data (*47*, *48*). This could be due partly to the different cultivars examined and to the use of a nonoptimal wavelength for detection of naringenin chalcone in the present study. On the other hand, we did not observe a general increase in phenolic compounds in response to reduced nitrogen supply, despite the fact that some compounds tended to increase. Stewart et al. (*33*) did not observe a consistent effect of reducing nitrogen nutrition on flavonol accumulation in tomato fruit either. In contrast, increased phenolic compounds had already been observed in response to reduced nitrogen supply in tomato leaves (*32*). On young tobacco leaves, Fritz et al. (*30*) observed a 4-fold increase in chlorogenic acid content and a 10-fold increase in rutin content. These increases are correlated to the stimulation of some key enzymes of the phenolic pathway. In response to reduced nitrogen supply, the induction of many enzymes of the phenylpropanoid pathway has been reported, including phenylalanine ammonia-lyase (PAL) and 4-coumarate: Coa ligase (4CL) in tobacco leaves (*30*) and enzymes involved in the flavonoid pathway such as chalcone synthase (CHS) and dihydroflavonol-4-reductase (DFR) in tomato leaves (*50*). The range of variation observed in this study on phenolic content seemed to be very small in comparison to previous data on leaves. There is no doubt that nitrogen plays an important role in polyphenolic synthesis, but tomato fruit seemed to be less affected and less reactive than leaves to nitrogen supply, perhaps because the fruit is a highly protected organ. The reduced nitrate supply may trigger a signal in the plant that leads to a complex regulation of nitrogen metabolism in the leaf (*26*, *29*), but maybe not in the fruit. The weaker response observed could also be due to the nitrogen level applied in the present experiment. A nitrogen supply of 4 mM did not put the plant in a state of nitrogen deficiency.

Nevertheless, rutin content increased when the nitrogen supply was lowered, which may be due to the putative role of rutin as a UV protector located in tomato peel (*51*). The increase in the concentration of this compound could be the result of an increase in both fruit irradiance and temperature. Further studies are needed to investigate changes in polyphenolic content in response to nitrogen supply on different organs within a plant. Nevertheless, the present data corroborated the fact that lowering nitrogen supply had no detrimental effect on fruit phenolic content and even tended to favor slight increases in these molecules.

Consequently, the present results suggest that moderate reduction in nitrogen supply can be used to improve fruit sugar content without affecting tomato fruit yield. Similarly, a moderate reduction in the nitrogen supply combined with increased fruit irradiance can be used to improve fruit nutritional quality by increasing the content of antioxidants in fruit, such as ascorbate and β-carotene, and, to a lesser extent, fruit phenolic compounds.

ABBREVIATIONS USED

CAD1, -2, caffeic acid derivates; CAG, caffeic acid glucoside; DM, dry mass.

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Supporting Information Available: Supporting information available showing the impact of nitrogen and truss position on the Bidirectional gap fraction (transmittance of light through the canopy to the fruit) as an indicator of the change in fruit microclimate triggered by lowering nitrogen. This material is available free of charge via the Internet at http://pubs.acs.org.

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