Yield and Quality of Three Cultivars of Dark Fire-Cured (Kentucky) Tobacco \textit{(Nicotiana tabacum L.)} Subjected to Organic (Compost) and Mineral Nitrogen Fertilization

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Abstract: A biennial experiment (2009 and 2010) was conducted at Calvi (Benevento, Southern Italy) to evaluate the effect of compost by organic fraction municipal solid waste (OFMSW), in combination with mineral nitrogen (N) fertilization, on yield and quality of three Dark Fire-cured (Kentucky) tobacco cultivars commonly cultivated at Benevento province (Campania region, Southern Italy). Six N fertilization treatments (N0 = soil N reserves available for plant growth; MIN = 135 kg ha$^{-1}$ of N applied as mineral fertilizer; C10 = 10 Mg d.w. ha$^{-1}$ compost; C10N = 10 Mg d.w. ha$^{-1}$ compost + 50% MIN; C20 = 20 Mg d.w. ha$^{-1}$ compost; C20N = 20 Mg d.w. ha$^{-1}$ compost + 50% MIN) were combined with the following cultivars: (i) Foiano, medium early maturing; (ii) Riccio Beneventano (local ecotype), medium maturing; (iii) SKL, medium maturing. Yield of cured leaves (Mg ha$^{-1}$) and growth components (number of leaves per plant, mean individual leaf area, leaf area per plant, specific leaf weight, stem diameter and height) and color parameters \((L^*, a^*/b^*)\) were measured. Leaf quality traits (nitrates, total N and alkaloids contents, score) and N use efficiency were also determined. The best growth and yield performance was reached in 2010 when plants were taller, developed both stems that were more robust and leaves having greater individual leaf area, and showed a higher leaf area per plant than in the first year. Regardless of the form of applied N (compost, mineral fertilizer, or a combination of both), tobacco plants appeared to be directly and positively influenced by increasing quota of readily available N received by each treatment, which was determined at the beginning of field growth by N soil balance and taking into account the percentage of N supplied by organic (compost) and mineral fertilizers. Results obtained with compost treatments, particularly when combined with mineral fertilizer (at C10N more than C20N), appeared comparable or sometimes better than those of full mineral fertilization although N fertilization by synthetic products was applied at very low doses.

Keywords: alkaloids; color; Foiano; nitrates; nitrogen use efficiency; organic fraction municipal solid waste; Riccio Beneventano; SKL; specific leaf weight

1. Introduction

Despite the no-smoking policies implemented in recent decades in many countries and in different continents, tobacco is still widely cultivated globally due to both the high income earned from its growth and the good prospect of challenging alternatives to simple smoking products [1–7]. Thus, today it is the most important non-food crop despite relevant reductions in cultivated areas in the last two decades (4.2 million ha in 2001 to 3.4 million ha in 2020; FAOSTAT [8]). In particular, in Italy, a decrease of 52% was recorded just in the last ten years (28,000 ha in 2010 to 13,378 ha in 2020; [9]).
Italy is the second-largest producer of tobacco in Europe, accounting for 25% of total European tobacco production [8]. There are several tobacco cultivation districts located in Northern (Veneto region), Central (Tuscany, Umbria and Lazio regions) and Southern (Campania region) Italian territories. Each district is characterized by types or cultivars greatly appreciated by manufactures (i.e., Light Air-cured Burley and Flue-cured Virginia Bright types from Campania, Umbria and Veneto regions for American blends; Dark Air-cured Havana and Fire-cured Kentucky types from Campania and Tuscany regions for cigars). In particular, the tobacco district of Benevento (Campania region, Southern Italy) is known for the cultivation of specific Dark Fire-cured (Kentucky) cultivars that are successfully used to manufacture particular types of TOSCANO® cigars, the TOSCANO® “Garibaldi”, which is lighter in color and sweeter in taste than TOSCANO® “Classico”.

Tobacco is grown around the world as an intensive crop because a large number of cultivation operations are generally undertaken during its field growth (it is a labor-intensive crop) and massive doses of resources (water, nitrogen, etc.) are commonly applied. Among agronomic factors, nitrogen (N) is the most important [10–12]. It modulates growth and development of plants [13–17], and is directly responsible for dry matter partitioning among different organs and yield response [13,14,18,19].

The quality of cured products is also largely influenced by N fertilization [10–12,14,16,17,20–22]. Sifola et al. [22] found that the brightness of cured leaves generally decreased with increasing N fertilization resulting in less bright and more opaque cured products, although the color intensity of cured leaves depended on the a*/b* ratio. In the same study, it was also reported that the fire-holding capacity was reduced only by rates of N higher than 170 kg N ha⁻¹ [22], presumably because of the closer structure of cured leaf tissues [10,11,23,24].

The chemical composition of cured leaves also markedly changes with N fertilization. Great amounts of N accumulate in the leaves when tobacco plants are grown under high rates of N fertilization [14,17]. Foliar N concentration appears to be positively correlated with the nicotine content of the cured leaves [10–12,14,16,20,21] but negatively with the leaf sugar content [10,11,16,25–27]. In particular, N accumulates in fresh leaves as nitrates when plants are grown under excess N fertilization [14,16,22,28,29] which, in turn, means a potential high undesirable tobacco specific nitrosamine (TSNA) content in commercial products after the curing period [16,30,31]. TSNAIs are potentially carcinogenic compounds [32,33]. Excess N fertilization also decreases the tobacco agronomic N use efficiency, and there is a negative relationship between the percentage amount of N recovered by plants and the increase in the N fertilization rate [14]. As a consequence, potential problems due to the loss of N in the environment are amplified [34].

Excess N fertilization is a widespread practice among tobacco growers around the world [16,17,22,35–38]. In Italy, according to N fertilizer recommendations of national and local extension services [39,40], the most suitable N rate for this crop usually ranges between 100 and 200 kg ha⁻¹, with differences depending on tobacco types and soils [14,16,22,38,40]. Since farmers of tobacco districts of Campania region often exceed these recommended N doses [18,21] it is urgent to test, including for tobacco, more sustainable N fertilization strategies involving, for example, the application of partial or full N doses as organic fertilizers so that losses of this nutrient in the environment may be successfully reduced.

Among organic fertilizers, compost has receiving great attention lately. The most evident advantages of its application in agriculture are I) the reduction in N fertilization by synthetic products, II) its role as a carbon sink, and III) the improvement in water retention capacity of soils [41–43]. The use of compost is currently recognized as a sustainable practice in itself because the frequently used materials derive from the recovery of selected organic matrices, whose disposal would be particularly complex and expensive [44]. In particular, when obtained by solid waste residues of cities (garbage, etc.), it also adds value via the disposal of, in a sustainable way, kinds of material that are otherwise very difficult to treat [45].
The compost from organic fraction municipal solid waste (OFMSW), properly treated in accordance with laws, shows an interesting improvement in plants' performance, particularly when combined with mineral fertilization. Application of OFMSW compost, alone or in combination with mineral N fertilizers, was tested in several crops with contrasting results [41–43,46]. On one hand, several studies showed a positive effect of OFMSW on yield and quality response mainly attributable to the best plant's nutrients uptake (N, P, K and Ca) under compost application [42,46–49]. On the other hand, others reported some negative effects [42,50]. In particular, in some cases, these negative effects appeared to be due to a prolonged vegetative growth promoted by compost that often can worsen crop yield and quality [51]. In other cases, however, they may be a consequence of a partial biological immobilization of N in the soil treated with compost, at least in the first year of compost application and particularly when compost C/N ratio is below 12 [46].

Although the mineral N fertilization of different tobacco types has been deeply investigated over time [7,13–16,19,25,28,52], very few studies have been conducted on the use of organic fertilization in general [53–55] and, as far as we know, regardless of different tobacco types, no previous studies have examined the use of compost from urban waste on the yield and quality of cured products. The present research aimed to fill this knowledge gap. With this aim, an experiment was conducted over two growing seasons in Southern Italy to evaluate the effect of compost from OFMSW, in combination with mineral N fertilization, on the yield and quality of some specific Dark Fire-cured Kentucky tobacco cultivars, commonly grown in Benevento province and greatly appreciated by cigar manufacturers. It is our opinion that its use could be the best opportunity and a good way to improve and to sustain the yield and quality of tobacco, and to increase the agronomic efficiency of N use, thus also reducing N losses to the environment.

2. Materials and Methods
2.1. Treatments, Crop Management and Weather Conditions

A biennial experiment (2009 and 2010) was conducted at a private farm in Calvi (Benevento, Southern Italy, 41°04' N, 14°52' E, 376 m a.s.l.) on a soil whose physical and chemical characteristics are reported in Table 1. Six N fertilization treatments (N0 = soil N reserves available for plant growth; MIN = 135 kg ha⁻¹ of N applied as mineral fertilizer; C10 = 10 Mg d.w. ha⁻¹ compost; C10N = 10 Mg d.w. ha⁻¹ compost + 50% MIN; C20 = 20 Mg d.w. ha⁻¹ compost; C20N = 20 Mg d.w. ha⁻¹ compost + 50% MIN) were combined with three cultivars of Dark Fire-cured (Kentucky) tobacco (Foiano, medium early maturing; Riccio Beneventano (local ecotype), medium maturing; SKL, medium maturing) with four replications (blocks). Seventy-two plots of 50 m² each were arranged each year. Compost product (Composta, GENESU Group, Perugia, Italy), whose chemical composition is reported in Table 2, were mixed into the top soil (25–30 cm) at each experimental dose at mid-April in both years.

Mineral N fertilizer (ammonium nitrate, 26% N) was applied as follows: 50% of dose at transplanting and 50% at side dressing, the latter split in two applications, at 10 (rosette phase) and 40 (beginning of stem elongation phase) days after transplanting (DAT). In particular, plots C10N and C20N received half of the mineral N fertilizer dose following the same scheduling (at transplanting and 2 times at side dressing). Accounting for the soil N reserves available for plant growth, the amounts of mineral fertilizer and/or compost applied, and the mineralization rates of organic N [42], the full doses of readily available N received by each treatment were: 18.5 (N0), 48.5 (C10), 116.0 (C10N), 78.5 (C20), 146.0 (C20N) and 153.5 (MIN) kg ha⁻¹. Before transplanting, 100 kg ha⁻¹ of P₂O₅ and 150 kg ha⁻¹ of K₂O were also added to the 0–20 cm top soil.
Table 1. Physical and chemical characteristics of soils in the experimental field.

| Texture      | I Year | II Year |
|--------------|--------|---------|
| Sand %       | 45.0   | 45.2    |
| Silt %       | 37.6   | 37.5    |
| Clay %       | 17.4   | 17.3    |
| Texture      | Loamy  | Loamy   |
| Total N g kg$^{-1}$ | 0.80  | 0.82    |
| P$_2$O$_5$ mg kg$^{-1}$ | 37.8  | 36.8    |
| K$_2$O mg kg$^{-1}$   | 250.8  | 275.9   |
| Organic matter % | 1.02  | 1.01    |
| NO$_3$-N mg kg$^{-1}$ | 6.0  | 5.2     |
| NH$_4$-N mg kg$^{-1}$ | 2.5  | 3.4     |
| pH           | 7.5    | 7.5     |
| Electrical conductivity dS m$^{-1}$ | 0.075  | 0.072   |

Total N (Kjeldahl method), P$_2$O$_5$ (Olsen method), K$_2$O (Tetraphenylborate method); Organic matter (Bichromate method).

Table 2. Chemical composition of COMPOST applied in both years.

| Dry matter | %        | 74.0 |
| Organic C | % d.w.   | 28.0 |
| Humic and fulvic acids | % d.w. | 14.2 |
| Total N   | % d.w.   | 2.1  |
| Organic N | % d.w.   | 2.0  |
| C/N       | 13.3     |
| P$_2$O$_5$ | % d.w. | 0.8  |
| K$_2$O    | % d.w.   | 1.8  |
| Cu        | mg kg$^{-1}$ | 67.2 |
| Zn        | mg kg$^{-1}$ | 146.0 |
| pH        | 7.9      |
| Salinity  | meq 100 g$^{-1}$ | 53.2 |

d.w., dry weight.

Tobacco seedlings were transplanted on 26 and 31 May in 2009 and 2010, respectively, at 1.0 m × 1.0 m distance (1 plants per square meter). Plants were furrow-irrigated in both years; seasonal irrigation volumes were 1500 and 1000 m$^3$ ha$^{-1}$ in 2009 and 2010, respectively. Irrigation volumes were empirically determined; in particular, in that area irrigation season is usually short and with few waterings (emergency irrigation). Leaves of the three cultivars were harvested, when ripe, from the central part of each plot (27.0 m$^2$) three times each year (18 August, 15 September, and 6 October 2009; 26 August, 20 September, and 12 October 2010), then fresh-weighed, manually or mechanically pierced, and finally threaded together and fire-cured in barns according to the standard procedure already reported in Sifola et al. [16].

Pest and disease controls, topping height, and sprouting controls were carried out according to standard practices on site.

The climate was typical Mediterranean with a mean temperature of 20.3 °C and 19.6 °C, for 2009 and 2010, respectively. Air temperatures were greater than 30 °C in the third ten-day period of June, and in the first ten-day period of July and in August in 2009 (Figure 1A), in the first and second ten-day periods in July and the third ten-day period of August in 2010 (Figure 1B). It is well known that temperatures higher than 30 °C greatly inhibit the growth and photosynthesis of tobacco [56]. During the growing season (end of May—mid October in both years), total rainfall amounted to 295 and 354 mm in 2009 and 2010, respectively, and was better distributed in 2010 than 2009 (Figure 1).
The climate was typical Mediterranean with a mean temperature of 20.3 °C and 19.6 °C, for 2009 and 2010, respectively. Air temperatures were greater than 30 °C in the third ten-day period of June, and in the first ten-day period of July and in August in 2009 (Figure 1A), in the first and second ten-day periods in July and the third ten-day period of August in 2010 (Figure 1B). It is well known that temperatures higher than 30 °C greatly inhibit the growth and photosynthesis of tobacco [56]. During the growing season (end of May—mid October in both years), total rainfall amounted to 295 and 354 mm in 2009 and 2010, respectively, and was better distributed in 2010 than 2009 (Figure 1).

Figure 1. Air temperature (maximum and minimum), and rainfall on a ten-day basis during the growing seasons at Benevento (A) = I year; (B) = II year).

2.2. Growth Components, Yield Measurements, Color Parameters, Leaf Quality Traits and N Use Efficiency

At same dates of commercial harvests, leaf number, leaf width and length, leaf dry biomass, stem height and diameter were measured on one plant per plot. Mean individual leaf area (leaf width × leaf length × 0.67; Ascione and Ruggiero [57], leaf area (LA, m² plant⁻¹) and specific leaf weight (SLW, kg leaf dry biomass m⁻² LA) were then calculated.

After the curing period, the yield of the cured product was determined at 19% standard moisture content. The commercial quality of cured products was determined by both expert evaluation (a score assigned to cured leaves samples by experts from tobacco companies) and color measurements (L*, a* and b* color parameters measured by a Chroma-meter CR-300. Minolta, Hannover, Germany) as previously reported [22]. N use efficiency (NUE, Mg yield per kg soil N) was calculated according the following formula [22]:

$$\text{NUE} = \frac{\text{Yield of cured leaves}}{\text{N applied by fertilizers} + \text{N soil}}$$  (1)
For cured leaves’ chemical compositions, a sample of 100 g of cured leaves was collected from each plot and prepared as reported in Sifola et al. [22]. The content of total N (Kjeldahl), nitrates and alkaloids was then determined [22].

2.3. Statistical Analysis

All results were subjected to analysis of variance (3-way ANOVA) using a general linear model. The SPSS software package (SPSS version 22, Chicago, IL, USA) was used. Means were separated by Duncan’s test at \( p < 0.05 \) and \( p < 0.01 \).

3. Results

3.1. Yield, Yield and Growth Components, and N Use Efficiency

The significance of factors and their interactions on yield, yield and plant growth components, and NUE is reported in Table 3. Regarding cultivar comparison, the SLK cv showed the best yield response, followed by Riccio Beneventano and Foiano (Figure 2), without any difference between years (i.e., \( Y \times C \) interaction was not significant; Table 3).

Table 3. Results of analysis of variance of yield, yield/plant growth components, and N use efficiency. For the explanation of indices, see Materials and Methods section.

| Significance       | Yield | Leaf nb | ILA | SLW | LA | Stem dm | Stem Height | NUE |
|--------------------|-------|---------|-----|-----|----|---------|-------------|-----|
| Years (Y)          | 0.01  | 0.01    | 0.01| 0.01| 0.01| 0.01    | 0.01        | 0.05|
| Cultivar (C)       | 0.01  | 0.01    | 0.01| 0.01| 0.01| 0.01    | 0.01        | 0.01|
| N Fertilization (F)| 0.01  | 0.01    | 0.01| 0.01| 0.01| 0.01    | 0.01        | 0.01|
| \( Y \times C \)   | ns    | 0.01    | 0.05| 0.01| ns  | 0.01    | 0.01        | ns  |
| \( Y \times F \)   | 0.01  | ns      | 0.01| 0.01| 0.01| 0.01    | 0.01        | 0.05|
| \( C \times F \)   | ns    | ns      | ns  | ns  | ns  | ns      | ns          | ns  |
| \( Y \times C \times F \) | ns | ns      | ns  | ns  | ns  | ns      | ns          | ns  |

nb.: number; ILA: individual leaf area; SLW: specific leaf weight; LA: leaf area; dm: diameter.

Figure 2. Effect of cultivar on yield of cured leaves. Different letters indicate significant differences according to Duncan’s test \( p < 0.05 \).

The effect of N fertilization treatments on yield was different in the two years (Figure 3). In particular, the increase in yield due to compost application with respect to the N0 treatment was greater in 2010 than 2009 and already evident at the lowest dose (C10; Figure 3). In addition, there was no significant difference in yield of plants grown with C10N and C20N treatments in both years (Figure 3). Finally, in the second year there was a significant positive effect on yield of C20N with respect to MIN treatment which was, in contrast, absent in 2009 (Figure 3).
Figure 3. Effect of Year I year: 2009; II year: 2010) × N Fertilization (N0 = soil N reserves; MIN = 135 kg ha⁻¹ of N mineral; C10 = 10 Mg d.w. ha⁻¹ compost; C10N = 10 Mg d.w. ha⁻¹ compost + 50% MIN; C20 = 20 Mg d.w. ha⁻¹ compost; C20N = 20 Mg d.w. ha⁻¹ compost + 50% MIN) interaction on yield of cured leaves. Different letters indicate significant differences according to Duncan’s test (p < 0.05).

In both years, plants of the Foiano cultivar produced the highest number of leaves per plant (Table 4) although they showed the lowest mean individual leaf area (Table 4) and SLW, the latter significantly only in 2009 (Table 4). In addition, in both years, the Foiano cultivar showed the highest stem height even though the diameter of the stems was not significantly different to that of Riccio Beneventano and SLK in 2009, and only significantly different to that of Riccio Beneventano in 2010 (Table 4).

Table 4. The effect of Year (I year: 2009; II year: 2010) × Cultivar, and Year × N Fertilization treatments (N0 = soil N reserves; MIN = 135 kg ha⁻¹ of N mineral; C10 = 10 Mg d.w. ha⁻¹ compost; C10N = 10 Mg d.w. ha⁻¹ compost + 50% MIN; C20 = 20 Mg d.w. ha⁻¹ compost; C20N = 20 Mg d.w. ha⁻¹ compost + 50% MIN) on leaf number, individual leaf area, specific leaf weight (SLW), leaf area (LA), individual leaf area (ILA), and stem diameter and height. For the explanation of indices, see Materials and Methods section. Different letters indicate significant differences according to Duncan’s test (p < 0.05).
There was a significant interaction \( Y \times F \) for individual leaf area, specific leaf weight (SLW, kg m\(^{-2}\)), leaf area per plant (LA, m\(^2\) plant\(^{-1}\)), and stem diameter and height (Tables 3 and 4). On the whole, all of these parameters, except for SLW were, on average, greater in the second than in the first year (Table 4). The minimum values of all parameters were recorded for the N0 treatment except for SLW in 2010 (C10; Table 4), whereas the maximum values of the same parameters were recorded from C20N in both years (Table 4).

Regarding N use efficiency, SLK showed the highest NUE with respect to both Foiano and Riccio Beneventano (Figure 4). NUE decreased with N fertilization treatment (Figure 5). The highest value was recorded for N0 (18.5 kg ha\(^{-1}\) of readily available N), and diminished, in both years, in the following order: C10 and C20 treatments (48.5 and 78.5 kg N ha\(^{-1}\) of readily available N, respectively), C10N and C20N (116.0 and 146.0 kg N ha\(^{-1}\) of readily available N, respectively), and MIN treatment (153.5 kg N ha\(^{-1}\) of readily available N) (Figure 5).
Figure 4. The effect of Cultivar treatment on NUE. Different letters indicate significant differences according to Duncan’s test \((p < 0.05)\). For the explanation of index, see Materials and Methods section.

Figure 5. The effect of Year (I year: 2009; II year: 2010) \times N Fertilization treatments (N0 = soil N reserves; MIN = 135 kg ha\(^{-1}\) of N mineral; C10 = 10 Mg d.w. ha\(^{-1}\) compost; C10N = 10 Mg d.w. ha\(^{-1}\) compost + 50% MIN; C20 = 20 Mg d.w. ha\(^{-1}\) compost; C20N = 20 Mg d.w. ha\(^{-1}\) compost + 50% MIN) on NUE. Different letters indicate significant differences according to Duncan’s test \((p < 0.05)\). For the explanation of index, see Materials and Methods section.

### 3.2. Quality Traits of Cured Products

The significance of factors and their interactions on cured leaf quality traits (nitrates, total N, alkaloids content, color parameters and score assigned by expert evaluators) is reported in Table 5.

Table 5. Results of analysis of variance of cured leaves quality traits. For the explanation of indices, see Materials and Methods section.

| Significance | Nitrates | Total N | Alkaloids | L* | a*/b* | Score |
|--------------|----------|---------|-----------|-----|-------|-------|
| Years (Y)    | ns       | ns      | 0.05      | 0.01| -     | 0.01  |
| Cultivar (C) | ns       | 0.01    | 0.05      | 0.01| 0.01  | 0.05  |
| N Fertilization (F) | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| Y × C        | ns       | ns      | ns        | ns  | ns    | ns    |
| Y × F        | 0.05     | 0.01    | 0.01      | 0.01| 0.01  | 0.01  |
| C × F        | ns       | ns      | ns        | ns  | ns    | ns    |
| Y × C × F    | ns       | ns      | ns        | ns  | ns    | ns    |

N tot = total nitrogen; L* = brightness, a* = green/red; b* = blue/yellow (color parameters); Score = expert evaluation of commercial quality.

The cultivar SLK accumulated the greatest quantity of nitrates, total N and alkaloids content, although differences were significant with respect to Foiano but not Riccio Beneventano (Table 6).

There was a significant interaction \(Y \times F\) for all parameters reported in Table 5. Nitrates, total N and alkaloids content increased significantly from the N0 treatment to the C20N treatment in both years but without any further increase in both parameters at MIN treatment in 2009 but with a significant decrease in alkaloids content in the 2010 (Table 6).

In both years, plants grown in C10N plots showed greater L* and score than those grown under other N fertilization treatments, whereas the highest \(a^* / b^*\) ratio was reached for the C20N treatment (Table 7). The lowest values of both parameters were always those of the N0 treatment (Table 7). Each cultivar reached the highest values of L* and the best score for the C10N treatment (Table 7), whereas the highest \(a^* / b^*\) ratio was always recorded for C20N (Table 7).
Table 6. The effect of Cultivar and Year × N Fertilization treatments on nitrates, total N and alkaloids content. Different letters indicate significant differences according to Duncan’s test ($p < 0.05$).

| Treatments | Nitrates | Total N | Alkaloids |
|------------|----------|---------|-----------|
|            | % d.w.   | % d.w.  | % d.w.    |
| Foiano     | 1.40 b   | 4.13 b  | 4.22 b    |
| Riccio     | 1.81 ab  | 4.28 a  | 4.37 ab   |
| SLK        | 1.85 a   | 4.36 a  | 4.48 a    |
| I year     |          |         |           |
| N0         | 0.64 d   | 3.53 e  | 3.61 g    |
| C10        | 1.49 bd  | 4.14 d  | 4.19 ef   |
| C10N       | 1.98 bc  | 4.17 cd | 4.77 ad   |
| C20        | 1.02 cd  | 4.19 cd | 4.25 df   |
| C20N       | 2.44 ab  | 4.74 ab | 5.02 ab   |
| MIN        | 2.27 ab  | 4.43 bd | 4.87 ac   |
| II year    |          |         |           |
| N0         | 0.54 d   | 3.18 e  | 2.76 h    |
| C10        | 1.14 cd  | 4.16 cd | 4.04 fg   |
| C10N       | 1.12 cd  | 4.45 bd | 4.63 ae   |
| C20        | 2.02 bc  | 4.61 ab | 4.47 cf   |
| C20N       | 2.34 a   | 4.92 a  | 5.12 a    |
| MIN        | 2.27 ab  | 4.55 ac | 4.54 bf   |

d.w.: dry weight.

Table 7. The effect of Year × N Fertilization treatments and Cultivar × N Fertilization treatments on color parameters ($L^*$, $a^*/b^*$) and score. For the explanation of indices, see Materials and Methods section. Different letters indicate significant differences according to Duncan’s test ($p < 0.05$).

| Treatments | $L^*$  | $a^*/b^*$ | Score  |
|------------|-------|-----------|--------|
|            |       |           |        |
| I year     |       |           |        |
| N0         | 22.71 f | 0.69 f    | 4.38 e |
| C10        | 24.68 e | 0.72 e    | 5.25 d |
| C10N       | 29.42 b | 0.82 c    | 6.63 a |
| C20        | 25.48 d | 0.90 ab   | 5.83 bc|
| C20N       | 25.40 de| 0.90 ab   | 6.04 b |
| MIN        | 27.44 c | 0.81 c    | 5.92 bc|
| II year    |       |           |        |
| N0         | 20.53 g | 0.62 g    | 3.42 f |
| C10        | 25.58 d | 0.75 d    | 5.29 d |
| C10N       | 30.38 a | 0.82 c    | 6.71 a |
| C20        | 25.93 d | 0.88 b    | 5.79 bc|
| C20N       | 25.56 d | 0.91 a    | 5.50 cd|
| MIN        | 28.79 b | 0.81 c    | 5.92 bc|
| Foiano     |       |           |        |
| N0         | 21.88 h | 0.64 i    | 3.88 f |
| C10        | 25.51 ef| 0.74 fg   | 5.19 e |
| C10N       | 29.91 b | 0.82 e    | 6.50 ab|
| C20        | 26.74 d | 0.88 d    | 5.75 cd|
| C20N       | 25.79 e | 0.87 d    | 5.63 ce|
| MIN        | 28.00 c | 0.81 e    | 5.75 cd|

| Riccio     |       |           |        |
| N0         | 20.61 h | 0.65 i    | 3.94 f |
| C10        | 24.41 g | 0.72 g    | 5.25 de|
| C10N       | 29.15 b | 0.82 e    | 6.75 a |
| C20        | 24.72 fg| 0.91 bc   | 5.94 c |
| C20N       | 25.16 eg| 0.94 a    | 5.69 ce|
| MIN        | 27.07 i | 0.81 e    | 6.00 bc|

| SLK        |       |           |        |
| N0         | 22.39 h | 0.68 h    | 3.87 f |
| C10        | 25.47 ef| 0.75 f    | 5.38 de|
| C10N       | 30.94 a | 0.82 e    | 6.75 a |
| C20        | 25.66 e | 0.88 cd   | 5.75 cd|
| C20N       | 25.49 ef| 0.91 b    | 6.00 bc|
| MIN        | 29.28 b | 0.81 e    | 6.00 bc|
There was no difference among cultivars in the range of variation of $L^*$, $a^*/b^*$ and score due to N fertilization treatments (Table 7) and, as previously reported for Year $\times$ N Fertilization interaction, $L^*$, $a^*/b^*$ and score of each cultivar was always the lowest at N0 treatment (Table 7).

4. Discussion

The present study aimed to evaluate whether compost obtained from urban waste can be used to improve and sustain the yield and quality of tobacco in a typical European tobacco cultivation area. It showed very interesting results. First of all, the best growth and yield performance was reached in 2010 when plants were taller, developed both stems that were more robust and leaves having a greater individual leaf area, and showed a higher leaf area per plant than in the first year (2009). This result was due to rainfall, which was better distributed in 2010 than 2009, particularly in June and July, which are the most important months for vegetative growth (plants were transplanted at the end of May in both years), confirming that the effect of N fertilization is mediated by the availability of water [13,14,58]. In fact, the latter directly determines the amount of N taken up by plants [14]. In particular, Sifola and Postiglione [14] in Light Air-cured (Burley) tobacco found that the N uptake rate was greater during stages of active growth.

The greater expansion of leaves, measured in 2010 with respect to the first year, lowered the SLW [59] because LA increased by 40% but leaf dry weight only increased by 14%. SLW is an important leaf quality trait and has often been associated with leaf thickness [16,32,60], which for tobacco crop is particularly relevant in determining commercial value [10,11]. In the present experiment, the lowered SLW negatively influenced the score assigned by expert evaluators, based on structure and texture, but also on elasticity, tissue integrity and color [16,22]. Indeed, it was significantly lower in 2010 than 2009 (5.4 vs. 5.7, respectively).

Regardless of the form of applied N (by compost, by mineral fertilizer or by a combination of both), overall, tobacco plants appeared to be directly and positively influenced by increasing the quota of readily available N received by each treatment, which was calculated at the beginning of field growth by N soil balance and taking into account the percentage of N supplied by organic (compost) and mineral fertilizers [42]. The order is the following: 18.5 (N0), 48.5 (C10), 78.5 (C20), 116.0 (C10N), 146.0 (C20N) and 153.5 (MIN) kg N ha$^{-1}$.

Interestingly, in most cases, maximum values were reached at N doses below that of the MIN treatment, confirming that 153.5 kg N ha$^{-1}$ is excessive for Dark Fire-cured (Kentucky) tobacco grown in that area [16]. In particular, yield, LA per plant, alkaloids content, $L^*$ and score in both years, together with individual leaf area, stem diameter and height in 2010, and SLW in 2009, were all influenced by N fertilization already starting from 116.0 kg N ha$^{-1}$ (C10N), where only 67.5 kg ha$^{-1}$ of N dose was applied as mineral fertilizer, and with no further significant increase at higher doses (C20N and MIN). In addition, as for individual leaf area and nitrates content in 2009, just 48.5 kg N ha$^{-1}$ (C10; about a full N dose applied as OFMSW) was sufficient to produce the greatest values. Finally, as expected, NUE decreased with increasing N fertilization rates [14,22] and the effect was significant up to C20N, and for the $a^*/b^*$ ratio in the second year and total N content in the first year.

The maximum values certainly represent the best results for yield, growth components (both individual leaf area and LA per plant, stem diameter and height), and also for SLW, as we demonstrated by considering the score assigned by evaluators. In any case, it should be considered that, as previously reported, the thickness of leaves is not the unique factor involved in the expert evaluation [22]. Regarding chemical compositions, maximum values of alkaloids content of cured leaves should be positive for a tobacco used to manufacture cigars because total alkaloids (90–95% of total alkaloids is nicotine) are responsible of the so-called “strength” of a cigar. This further quality parameter is, in fact, related to how much nicotine the cigar contains.

The maximum values should be, in contrast, the worst result for nitrates and total N (i.e., protein quota). It is well known that accumulation of N as nitrates is a signal of low
efficiency of N use [22] because they are a precursor for TSNAs, together with alkaloids. They are also highly dangerous in cured products, which can over-accumulate TSNAs during the storage period [61]. When we calculated the percentage quantity of nitrates in the total N content of cured leaves, we found that it increased with increasing N dose and, also in this case, regardless of the form of applied N (by compost, by mineral fertilizer or by a combination of both). In particular, they ranged between 18 (N0) and 51% (both C20N and MIN) in 2009 and 17 (N0) and 50% (MIN) in 2010, confirming results previously reported for a Light Air-cured (Burley) tobacco in Sifola et al. [22]. Finally, high total N content also generally means a potential high protein content, which could determine less-open cured leaf tissues [22,24] and, consequently, defects in the burning of cured products. Nevertheless, in the case of the present experiment, the highest values were recorded for C20 and C20N (2010 and 2009, respectively), which correspond to N doses of 70.5 and 146.0 kg ha$^{-1}$. In both cases, these values are lower than those reported by Sims et al. [24] and Sifola et al. [22] as being responsible for decreasing fire-holding capacity.

For both years, for C10N treatment, the $a^*/b^*$ values of cured leaves were similar to those of full mineral fertilization, which then showed the typical dark-brown color of Kentucky-cured leaves. Nevertheless, because the L* value at C10N was higher than that measured at MIN, the cured tissue from C10N showed a lighter but more brilliant dark-brown color [16] than that of the MIN treatment. It is worth noting that, in both years, the highest score was recorded for CN10. Nitrogen excess is reported to lower scores because of increased amounts of “green” cured tobacco following curing (delayed maturity in field) instead of the typical dark-brown color [24,62].

Regarding the cultivar comparison, SLK produced significantly more than Foiano, although with fewer leaves that were more expanded, but not more than Riccio Beneventano. However, compared to Foiano, it accumulated significantly higher quantities of nitrates (not a positive aspect), total N and alkaloids; the latter is a positive quality trait for the “strength” of cigars but not always in combination with nitrates. SLK showed the best NUE as compared with both of the other cultivars, presumably due to a sufficient N uptake which, in turn, offset a worst physiological efficiency, as shown by the content of leaf nitrates [14]. Both SLK and Riccio Beneventano are the most cultivated Dark Fire-cured (Kentucky) cultivars in the Benevento district. The $a/b$ ratio of all cultivars for C10N was not different from that recorded for MIN treatments. In addition, C10N had a higher value for brightness than MIN, and the greatest score.

On the whole, in the present experiment, the results obtained with compost treatments, particularly when combined with mineral fertilizer (C10N and, in a few cases, also C20N), appeared to be comparable with or sometimes better than those achieved using only mineral fertilization, in accordance with some previous work on different species [42,46,49,63].

5. Conclusions

In conclusion, this study evidenced that the response of plants to all compost treatments directly followed the available N dose calculated from the soil N balance. Interestingly, in most cases, the best results were obtained when N fertilization by synthetic products was applied at very low doses. As a consequence, it can be suggested to tobacco growers to use the OFMSW compost and fertilizers, reiterating that it is not necessary to exceed the extension services’ recommended doses; indeed, it is also possible to apply less than the recommended dose when using compost in combination with C10N and C20N. In this way, the beneficial effect of compost application on agronomic aspects can be appreciated. Further studies are also needed to evaluate the effect of OFMSW compost on the physical properties (i.e., water-retention capacity) or chemical characteristics (i.e., nutrient enrichment, etc.) of soils under tobacco crops.
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Abbreviations

nb.: number; IIA: individual leaf area; SLW: specific leaf weight; LA: leaf area; dm: diameter; N: nitrogen; d.w.: dry weight; OFMSW: organic fraction municipal solid waste.

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