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A Multipurpose Sustainable Farming System for Tobacco Crops in the Mediterranean Area

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Abstract: The present study aimed to test a multipurpose sustainable tobacco farming system allowing more efficient use of production factors (e.g., mineral N fertilizer) thanks to larger commercial yields, albeit diversified (smoke products, bioactive compounds for nutraceutical and cosmeceutical uses, energy), per unit of land area. Three tobacco types (dark air-cured, IBG; light air-cured, Bu; dark fire-cured, Ky) were grown in the field in 2021 on three different soils (sandy clay loam, SCL; sandy loam, SL; clay loam, CL). The total waste biomass (WB, kg dry weight, d.w. ha^{-1}) was measured. Commercial leaves yield (CLY, kg d.w. ha^{-1}), N agronomic efficiency (NAE, kg d.w. kg^{-1} N), total polyphenols content (TP, mg kg^{-1} d.w.), antioxidant activity (ABTS, DPPH and FRAP, mmol Trolox Equivalent, TE, kg^{-1} d.w.) and yield of polyphenols (PY, kg ha^{-1}) were determined. The calorific value (CV, MJ kg^{-1} d.w.), volatile matter (VM, %) and ash contents (%) were also measured, and biomass energy yield (BEY, GJ ha^{-1} yr^{-1}) was then calculated. Very high percentages (>40%) of total biomass produced by the different tobacco types were pre-harvest waste. NAE increased by 2- to more than 8-fold thanks to a greater potential commercial biomass produced with the same amount of N fertilizer used. Four main components were found in the tobacco polyphenols profile, namely 3-O-CQA, luteolin 7 rutinoside, rutin and quinic acid, which accounted for more than 80% of TP. BEY ranged between 122.3 GJ ha^{-1} yr^{-1} (Bu) and 29.9 GJ ha^{-1} yr^{-1} (Ky). Both polyphenols yield and energy potential per unit land area and/or per growing season appeared competitive with those from other herbaceous crops. The proposed multipurpose system appeared as a production circuit characterized by a virtuous and sustainable flow of resources.

Keywords: calorific value; *Nicotiana tabacum* L.; nitrogen agronomic efficiency; polyphenols; soils; waste biomass

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1. Introduction

In recent decades, agriculture changed to maintain a sufficiently high economic value. Its traditional activities (for food and fibers) were frequently supplemented by new ones aimed at providing new products or combinations of products. New services and functions were also offered with the aim to support (i) food security, (ii) biodiversity, (iii) rural development (particularly in marginal areas), (iv) landscape protection, (v) climate changes control, etc. [1,2]. The main results of these agricultural changes were the repositioning of the farms within their reference market, with the development of interesting new opportunities for specific supply chains [2].

New products, services and functions were made possible thanks to new knowledge. For instance, some of the latter allowed for developing plans for the valorization of waste materials deriving from agricultural crops, with the aim to produce bioactive compounds for nutraceutical and cosmeceutical uses or even to obtain energy [3–7]. These plans are generally steered towards sustainability, since they often offer additional services like soil protection [8], resource saving (e.g., water and N) [9], etc.

Sustainability in agriculture has been talked about for a long time. It is a very complex concept which can vary across regions of the world, according to different soils and climates, or crops. It must be analyzed from numerous points of view (i.e., social, environmental, economic), often related to each other [10–12], and needs to be adapted to each specific cropping and/or farming system. In agriculture, as well as in other contexts, it is urgent to quantitatively measure it. For this purpose, indicators were selected [11,13–15] which consider, as far as possible, most of interactions among economic, social and environmental aspects [16–18]. Among the indicators, diversification of products and profitability improvement were frequently reported as economic ones, while fertilizer use efficiency and soil, type or quality, as environmental indicators [18,19]. Thresholds for such indicators were also found, useful to evaluate sustainability efforts [13].

Sustainable agricultural processes, if any, must allow to obtain high-quality yields and adequate incomes. As already reported, they generally protect the cultivation environment through a more efficient use of resources. In this latter regard, the agricultural systems that propose the use of residual biomasses with high added value (i.e., extracts for the nutraceutical and cosmeceutical industries and/or biomasses for energy production) improve agronomic efficiency in the use of important resources (e.g., N fertilizer). This is possible thanks to the increased and diversified types of commercial products per unit land area. They, also, determine positive environmental or economic returns over a medium or long term [6,20].

Nitrogen agronomic efficiency (NAE) can be considered an important indicator of sustainability in agriculture [19,21–25]. The higher the NAE, the greater the agronomic, and economic, value of the N fertilizer unit. It is well known that in most crops, NAE is quite low, despite a great amount of N fertilizer generally being applied to obtain commercial yield [26–30], because commercial products generally represent a limited fraction of all biomasses produced. In sustainable agriculture, an inefficient use of N fertilizer should also be avoided since the carbon footprint of industrial production, distribution, field application and crop N release is quite high. It is estimated that the synthetic N fertilizer supply chain is responsible for 2–3% of the global emission of greenhouse gases [31,32], thus contributing to large social and environmental costs (i.e., the pollution of the air, soil and water) [15,33].

Tobacco is a cash crop widely cultivated over the world (3.13 million of hectares) [34], and it is still considered the most important non-food crop. About 78,400 ha are cultivated for tobacco in Europe [34]. In Italy, there are about 12,860 ha [35] distributed as follows: 3795 ha in the Veneto region (Northern Italy), 4410 ha in Umbria, 1501 ha in Tuscany, 252 ha in the Lazio regions (Central Italy) and 2850 ha in the Campania region (Southern Italy) [35]. The following tobacco varieties are currently cultivated in these Italian tobacco districts: dark air-cured IBG, light air-cured Burley, dark fire-cured Kentucky (Campania and Tuscany), flue-cured Virginia types (Umbria and Veneto). These different tobacco types are generally grown in different recommended types of soils whose physical and chemical characteristics greatly influence both the yield and quality of smoke products [36–38].

Tobacco cultivation usually produces large quantities of biomass, which are: (i) commercial leaves which often amount to less than 50% of the total aboveground dry matter [28,39–41] that, after curing, are profitably used by smoke industries, and (ii) pre-harvest waste materials, all the remaining aboveground parts (leaves and stalks) that, without any other treatment, in common practice are buried to pursue the dual advantage of eliminating, in a simple way, apparently worthless waste materials and of adding a certain amount of organic matter to the soil.

Although the burial of crop residual biomass was frequently suggested as a positive and sustainable practice by several authors [42,43], it might unfortunately produce contamination problems. Such residues are, in fact, reported as a potential source of pathogens [44], with particularly negative implications in the case of monoculture like that often practiced in tobacco farming. By contrast, different uses are well known and already reported for tobacco as well as for other species. They are: (i) polyphenols yield [6,45–48], (ii) energy production [3,4,49–52], (iii) biochar [7], (iv) proteins [53]. They may provide undisputed economic as well as environmental benefits.

The main aim of the present study was to test a multipurpose sustainable tobacco farming system allowing a more efficient use of production factors (e.g., mineral N fertilizer) thanks to larger commercial yields, albeit diversified (smoke products, bioactive compounds for nutraceutical and cosmeceutical uses, energy), per unit of land area.

Our investigation started by the following considerations: (i) previous studies showed that tobacco contents of phenolic acids and flavonoids were sufficiently high to consider the wastes of tobacco as economic and convenient natural sources of these salutistic compounds [6]. Interestingly, regardless of tobacco types, the most abundant components in phenolic profile were quinic and chlorogenic acids, rutin, and luteolin rutinoside [6,54–56]; (ii) due to safety concerns, natural antioxidants, obtained both from edible materials (or edible by-products) and from waste biomass of non-food crops, are receiving increasing interest by industries since consumers prefer natural to synthetic phenolic antioxidants. In this view, good new opportunities should be opened up also for tobacco crops; (iii) very few studies have been conducted up to now on the use of tobacco waste biomass as an energy source [49,50]; (iv) it is well known that the richness of polyphenols as well as polyphenolic profiles of plants are differently influenced by soil types [57,58] due to the combination of numerous factors [47,59–61]. Among them, different rates of N fertilizer generally applied in different kinds of soils can play an important role [62].

2. Materials and Methods

2.1. Plant Materials, Experimental Treatments, Crop Management and Samplings

A field experiment was conducted in 2021 at the Experimental Station of the Department of Agricultural Sciences (Portici, Naples, Italy; 40°48′ N; 14°20′ E; 70 m a.s.l.). Three tobacco types (dark air-cured Badischer Geuderhetimer, hereafter referred to as IBG, cv. PMBG; light air-cured Burley, hereafter referred to as Bu, cv. PMs; dark fire-cured Kentucky, hereafter referred to as Ky, local ecotype Riccio Beneventano) were grown in three different soils (sandy clay loam, SCL; sandy loam, SL; clay loam, CL) whose physical and chemical characteristics are reported in Table 1.

Table 1. Physical and chemical characteristics of soils. SCL, sandy clay loam; SL, sandy loam; CL, clay loam; OM, organic matter; OC, organic carbon; EC, electrical conductivity; FC, field capacity; WP, wilting point.

	SCL	**SL**	**CL**
Sand	49.5	70.5	36.0
Clay	25.5	6.5	39.5
Silt	25.0	23.0	24.5
N (Kjeldhal, %)	0.106	0.074	0.080
NO_3-N (mg kg^{-1})	14.81	62.37	17.42
NH_4-N (mg kg^{-1})	11.84	13.37	8.17
OM (%)	1.71	1.61	0.95
OC (%)	0.99	0.94	0.55
C/N	9.4	12.7	6.9
$EC_{1:5}$ (dS m^{-1})	0.153	0.106	0.148
pH	7.56	7.83	7.67
FC (%)	31	18	38
WP (%)	18	9	24

Seedlings were transplanted on 5 May 2021, at 0.8 × 0.5 m distance for IBG, 1.0 × 1.0 m distance for Ky and 0.8 × 0.4 m distance for Bu (25,000, 10,000 and 30,000 plants per ha, respectively).

Plants were fertilized with nitrogen (N) at doses calculated by a specific N fertilization plan [41], and both gains (mineralization of the organic matter, basic soil fertility, residues from the previous year's crop, atmospheric depositions like precipitation, etc.) and losses (crop requirements, fixed assets, and deep dispersion, leaching) were considered. The following specific N doses were applied as ammonium sulphate (26% N): 158 (IBG), 181 (Bu), 112 (Ky) kg ha^{-1} in SCL soil; 171 (IBG), 194 (Bu), 125 (Ky) kg ha^{-1} in SL soil; and 162 (IBG), 185 (Bu), 116 (Ky) kg ha^{-1} in CL soil. N fertilizer doses within each soil also varied with different kinds of tobacco, depending on the expected yield. N doses of each treatment's combination were applied at side-dressing, split in two applications, the first at 10 (rosette phase) and the second at 40 (beginning of stalk elongation phase) days after transplanting (DAT).

According to standard practice of the cultivation area, Ky tobacco plants were topped at a height of 14–16 leaves per plant, and suckering was then controlled by applying a contact chemical (n-decanol). Plants were regularly irrigated up to the last commercial harvest (30 August). Pest and disease control were carried out according to standards on site.

Waste biomasses, which were (i) early stripping basal leaves and residual stalks at the end of cropping from IBG, Bu and Ky tobaccos and (ii) residual, not commercial, leaves and final suckers' material (leaves and stalks) from Ky tobacco only, were collected on 29 June and 4 October (early stripping basal leaves and residual stalks, respectively), and on 4 August and 3 September (residual, not commercial, leaves and final suckers' material, respectively). They were separately weighted, after being oven-dried at 60 °C up to constant weight, to determine dry matter content.

Early stripping basal leaves (from IBG, Ky and Bu), residual, not commercial, leaves and final suckers' material (from Ky) were then prepared to determine total polyphenols content (TP, mg kg^{-1} dry weight, d.w.). TP was calculated both separately for each type of waste biomass (TP_1) or for Ky as the average of those of all kinds of waste biomass (TP_2). On residual stalks (IBG, Ky and Bu), calorific value (CV, MJ kg^{-1} d.w.), volatile matter (VM, %) and ash (%) contents were determined. Biomass energy yield (BEY, GJ ha^{-1} yr^{-1}) was calculated.

The total waste biomass (WB, kg d.w. ha^{-1}, as the sum of early stripping basal leaves and residual stalks for IBG, Ky and Bu, and residual, not commercial, leaves and suckers' material for Ky) and yield of polyphenols (PY, kg ha^{-1}) were also calculated.

Commercial leaves of Bu and IBG tobaccos were harvested on 14 and 26 July, 4 and 30 August, while those of the Ky type on 4 August only. The commercial leaves yield (CLY, kg d.w. ha^{-1}) was finally calculated.

2.2. *Polyphenols Determination, Orbitrap High-Resolution Mass Spectrometry Analysis and Antioxidant Activity (ABTS, DPPH and FRAP Assays)*

The extraction protocol of polyphenolic compounds and their quali-quantitative profile was determined using ultrahigh-performance liquid chromatography–quadrupole-Orbitrap high-resolution mass spectrometry (UHPLC-Q-Orbitrap HRMS; Thermo Fisher Scientific, Waltham, MA, USA) according to the protocol previously described [6], and the results are presented as mg kg^{-1} DW.

A total of three different assays were carried out for antioxidant activity determination: the ABTS scavenging capacity based on the method described by Re et al. [63], the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity using the procedure proposed by Brand-Williams et al. [64] and the ferric reducing antioxidant power (FRAP) as reported by Benzie and Strain [65]. Methodological details on the antioxidant activity measurements are reported in our previous work [6].

2.3. N Agronomic Efficiency Calculation and Calorific Measurements

Nitrogen agronomic efficiency (NAE, kg d.w. kg^{-1} N) [21,23,24,64,66,67] was calculated as follows:

$$NAE_1 = CLY/N \text{ applied by fertilizer}$$
$$NAE_2 = (CLY + \text{total WB})/N \text{ applied by fertilizer}$$

The relative changes of NAE_2 vs. NAE_1 were then calculated to measure the effect of multiple commercial products' combination (CLY and total WB) on the efficiency of N use.

For calorific measurements, residual stalks, collected as previously reported, were transported to the Mechanics Laboratory in sealed containers and protected from light. The moisture content (MC, %) was determined in accordance with EN ISO 18134-2: 2015 regulations [68]. In brief, a 100 g sample by each treatment was weighed and subjected to drying in a Binder FD-155 oven (BINDER, Tuttlingen, Germany) at a temperature of 103 ± 2 °C until a constant mass was reached between two consecutive measurements, with an interval of 8 h. Measurements were replicated three times.

The higher heating value (HHV) was measured according to UNI EN ISO 1716: 2010 regulations [69]. In brief, three subsamples by each treatment were ground using a rotary blade mill and then compressed into 1 g pellets. The obtained pellets were then burned in an adiabatic bomb calorimeter (S.D.M. Apparecchi Scientifici© Mahler, Turin, Italy). A Pt100 thermoresistance connected to the cDAQ-9171 data logger (National Instruments Corporation, TX, USA) was used to record the temperature increase. All temperature data were processed using LabVIEW 2019 software (National Instruments Corporation, TX, USA). The test was considered complete when the final temperature remained constant for approximately five minutes [70]. To ensure accuracy, the calorimeter was tested before the experiment with six individual calibrations using pre-weighed 1 g pellets of benzoic acid. Measurements were made in triplicate. The biomass energy yield (BEY, GJ ha^{-1} yr^{-1}) was calculated.

The obtained tobacco stalk powder was used for further analysis. Specifically, ash content (%) was determined in accordance with EN ISO 18122: 2016 regulations [71]: three subsamples of 10 g each were taken by each treatment and placed in ceramic crucibles in a muffler (Carbolite CB ELF1123A, Verder Scientific, Haan, Germany) at a temperature of 550 ± 10 °C for 3 h [72]. Afterwards, the crucibles were cooled in a glass dryer in the presence of silicon granules to prevent reabsorption of moisture and weight variations. VM (%) was then calculated.

2.4. Experimental Design and Statistical Analyses

The experiment was a factorial combination of three different types of tobacco (IBG, Ky and Bu) and soils (SCL, SL and CL), with three replications. Results were subjected to a two-way analysis of variance (ANOVA) [73] using a split-plot design, with soils as the main factor and tobacco types as the subfactor. For Ky tobacco only, there were (i) a one-way ANOVA using a completely randomized design with three replications for residual, not commercial, leaves and (ii) a two-way ANOVA using a split-plot design with soils as the main factor and leaves and stalks from suckers' material as the subfactor, with three replications. Means were separated by the least significance difference (LSD) test at $p \leq 0.05$ and $p \leq 0.01$.

3. Results

The main phenolic and flavonoid components in all kinds of waste biomass extracts were 3-O-CQA, rutin, luteolin 7 rutinoside and quinic acid (Figure 1). On average, they accounted for: (i) 88 ± 1% (mean ± standard error) of total polyphenols (TP_1) in early stripping basal leaves, (ii) 91 ± 0.5% of TP_1 in residual, not commercial, leaves, and (iii) 96 ± 1% of TP_1 in after-topping suckers' material (92% in leaves and 99% in stalks).

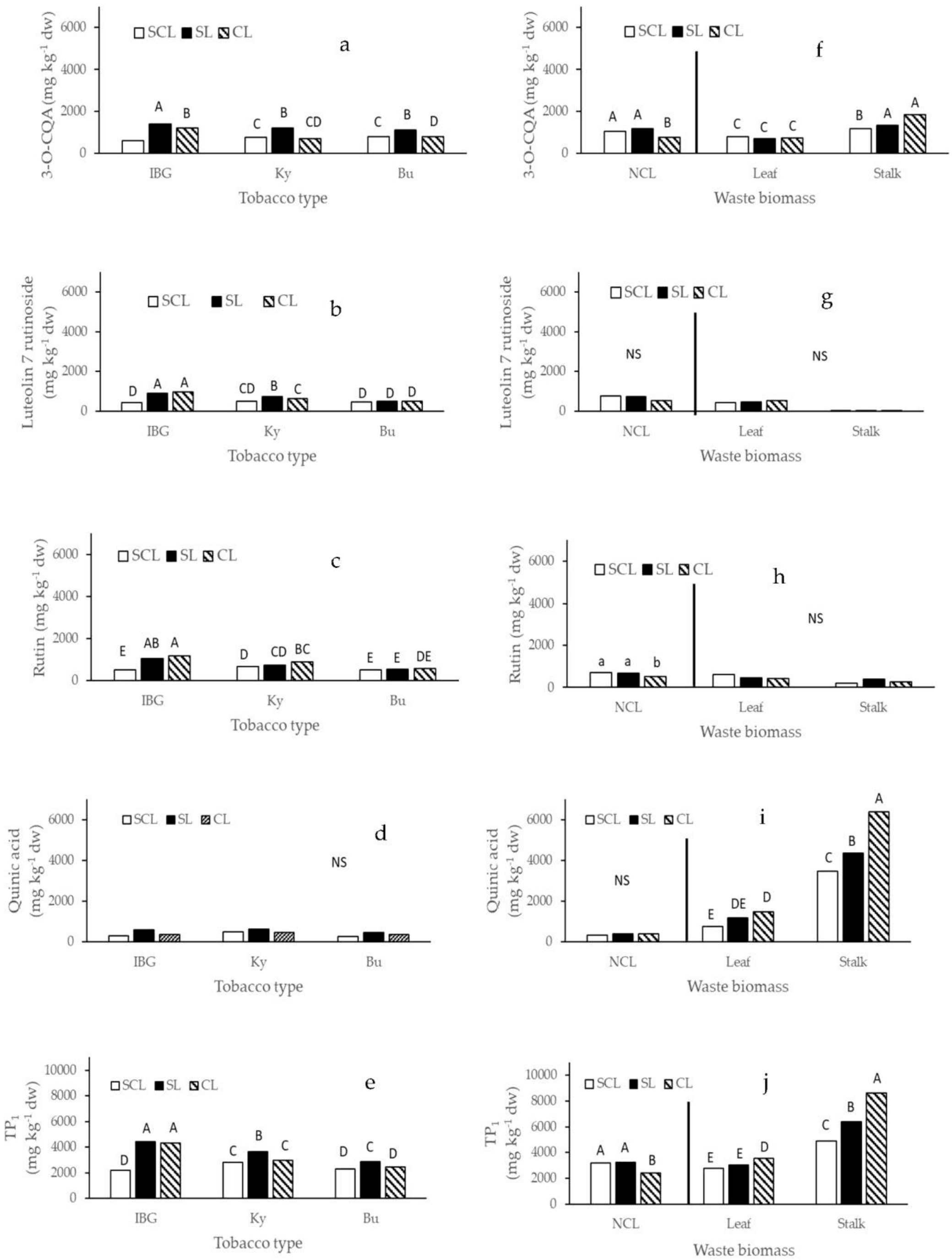

Figure 1. Main phenolic and flavonoid components and total polyphenol content (TP_1) of dark air−cured Badischer Geudertimer (IBG), dark fire−cured Kentucky (Ky) and light air−cured Burley (Bu) tobacco crops, grown in sandy clay loam (SCL), sandy loam (SL) and clay loam (CL) soils. All results were obtained by shredded leaves, picked up on 29 June 2021 (**a**–**e**), and residual, not commercial, leaves (NCL, residual, not commercial, leaves) or suckers' materials (leaves and stalks) picked up on 4 August and 3 September 2021, respectively (**f**–**j**). Different letters indicate least significant differences of interaction by soil × tobacco type at $p \leq 0.05$ and $p \leq 0.01$ (capital letters). NS, not significant; d.w., dry weight.

As for early stripping basal leaves, 3-O-CQA content was the highest in leaves from SL soil in all tobacco types (Figure 1a). Luteolin 7 rutinoside and rutin showed the significant highest values in IBG crops grown in both SL and CL soils (Figure 1b,c). There was no significant effect of treatments' combination (soil x tobacco type) on quinic acid content

(Figure 1d); however, the significant highest content was recorded in both SL and Ky tobacco (on average). TP_1 of early stripping basal leaves ranged between a minimum of 2215 (IBG in SCL soil) and a maximum of 4414 (IBG in SL soil) mg kg^{-1} d.w. (Figure 1e). Generally, Ky and Bu tobaccos reached the highest TP_1 in SL soil (Figure 1e).

The content 3-O-CQA and rutin of residual, not commercial, leaves of Ky tobacco did not vary significantly in SCL and SL soils, but it decreased in CL (Figure 1f,h). No significant effect of soils was recorded for luteolin 7 rutinoside and quinic acid on the same waste biomass (Figure 1g,i).

As for final suckers' material (leaves and stalks) of Ky (Figure 1), there was no effect of soil type on leaf content of 3-O-CQA, luteolin 7 rutinoside and rutin (Figure 1f–h), while quinic acid increased significantly passing from SCL to CL soils with intermediate values in SL (Figure 1i). The stalk content of both luteolin 7 rutinoside and rutin did not change in different soils (Figure 1g,h), but both 3-O-CQA and quinic acid increased according to, respectively, the following order: SCL < SL $\leq$ CL and SCL < SL < CL (Figure 1f,i). TP_1 of Ky residual, not commercial, leaves ranged between 2435 (CL) and 3252 (SL) mg kg^{-1} d.w., but TP_1 of suckers' material (leaves and stalks) varied from 2687 (leaves of SCL soil) to 8591 (stalks of CL soil) mg kg^{-1} d.w. (Figure 1j). TP_1 of residual, not commercial, leaves of Ky tobacco grown in CL soil was significantly lower, as well as that of leaves from suckers' material significantly higher, than that of both SCL and SL (Figure 1j). As for stalks from suckers' material, TP_1 increased significantly according to the following order: SCL < SL < CL (Figure 1j).

The results of antioxidant activity measurements (both on early stripping basal leaves of IBG, Ky and Bu tobaccos, and on residual, not commercial, leaves or final suckers' material of Ky tobacco) are reported in Figure 2. Treatments' combination (soil x tobacco type) was significant in each kind of assay. As for early stripping basal leaves, in all tobacco types the greatest antioxidant activity was that recorded in SL soil (Figure 2a–c), sometimes not different from CL (i.e., DPPH in IBG; Figure 2a), and only in few cases significantly different from SCL (i.e., DPPH and ABTS in IBG and Ky; FRAP in IBG; Figure 2a–c).

Both DPPH and FRAP of residual, not commercial, leaves of Ky were significantly higher in SCL and SL than CL (Figure 2d,f), whereas the highest ABTS was recorded in SL soil (Figure 2e). Leaves and stalks of suckers' material of Ky showed the same response to different soils in DPPH, ABTS and FRAP assays (Figure 2d–f). The highest values were in each case those of materials (both leaves and stalks) coming from crops grown in CL soil (Figure 2d–f).

Ky tobacco gave significantly less commercial yield than IBG and Bu (1 vs. 4 harvests), and the highest value was obtained with SCL soil. IBG yielded more, as well as Bu tobacco less, in CL than both SCL and SL soils (Figure 3). The highest WB was produced by IBG and Bu tobaccos grown in SL soil but by Ky tobacco grown in CL soil (Figure 3). Regardless of soil types, the WB coming from Ky tobacco was found to be significantly lower than that from both IBG and Bu tobaccos (Figure 3).

TP_2 content of Ky tobacco increased significantly from SCL to SL and then to CL (Figure 3). As for the IBG type, a significantly lower value of TP_2 was recorded in SCL as compared to both SL and CL, while in the Bu type no difference emerged in TP_2 due to soils (Figure 3). The PY of Ky was significantly higher than that of both IBG and Bu tobaccos (Figure 3). Moreover, the PY of IBG tobacco obtained in SL soil was higher than those obtained in both SCL and CL conditions (Figure 3). Finally, according to the results of TP_2, the PY of Bu tobacco did not change significantly with soil type (Figure 3).

Both NAE_1 and NAE_2 of Ky tobacco were significantly lower than those of IBG and Bu (Figure 3). There was little or no difference due to different soil types in NAE_1 and NAE_2, respectively, when they were measured for Ky tobacco. However, both NAE_1 and NAE_2 of IBG and Bu were significantly higher (IBG), or significantly lower (Bu), in CL than SCL and SL soils (Figure 3).

Table 2 reports relative changes of NAE_2 vs. NAE_1 under each treatment combination (soil × tobacco type): they varied from 1.85 (CL) to 2.16 (SL) in IBG, from 2.11 (CL) to 2.33 (SL) in Bu and from 5.46 (SCL) to 8.30 (SL) in Ky (Table 2).

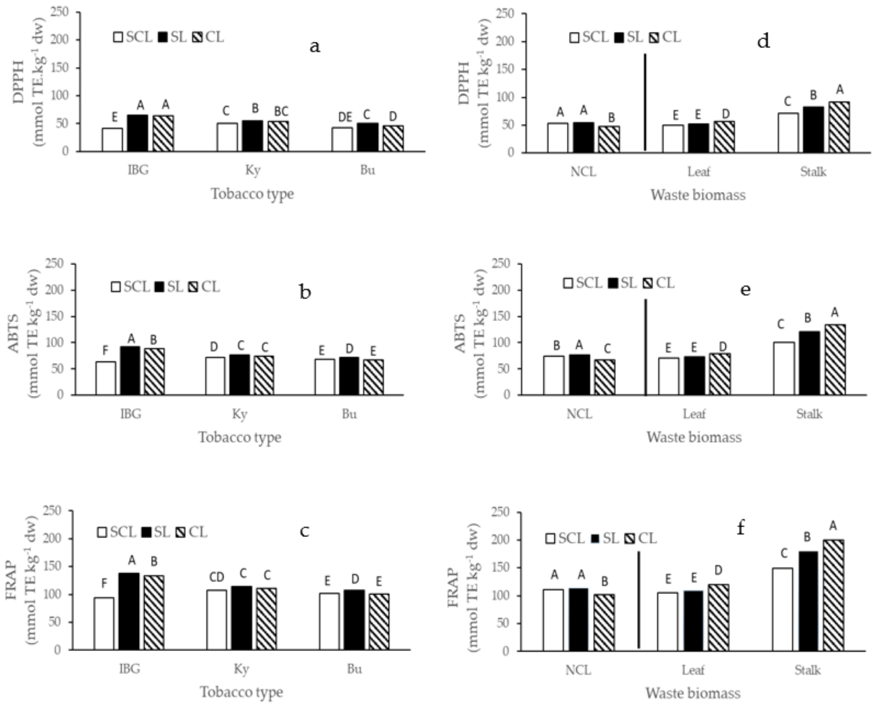

Figure 2. Antioxidant activity (ABTS, DPPH and FRAP Assays) of extracts from dark air−cured Badischer Geudertimer (IBG), dark fire−cured Kentucky (Ky) and light air−cured Burley (Bu) (**a**–**c**) and dark fire-cured Kentucky (Ky) (**d**–**f**) tobacco crops, grown in sandy clay loam (SCL), sandy loam (SL) and clay loam (CL) soils. All results were obtained by the waste biomass (shredded leaves), picked up by growers on 29 June 2021 (**a**–**c**) and by the waste biomass picked up by growers on 4 August 4(NCL, residual, not commercial, leaves) and 3 September 2021 (leaves and stalks of suckers) (**d**–**f**). Different letters indicate least significant differences of interaction by soil × tobacco type at $p \leq 0.01$. d.w., dry weight.

Results of calorific measurements are reported in Table 3. There were significant interactions soil × tobacco type for both SB and BEY (Table 3, Figure 4). On average, SB and BEY of Ky type were significantly lower than those of both IBG and Bu tobaccos (Figure 4). The SB and BEY of IBG and Bu tobaccos were negatively affected by SCL (IBG) and Cl (Bu) soil (Figure 4), but there was no effect of soils on the SB and BEY of Ky tobacco (Figure 4). Waste materials (stalks collected after the last commercial harvest) of Ky tobacco showed a significantly lower moisture content than those of both IBG and Bu tobacco types (Table 3). There was no effect of both soils and tobacco types on CV (Table 3). The lowest VM was that measured in SCL soil (Table 3), while, by contrast, the highest VM was that obtained by Ky tobacco (Table 3). Ky tobacco showed the lowest ash content, also (Table 3).

Table 2. Relative changes of NAE_2 vs. NAE_1 at each treatment combination. IBG, dark air−cured Badischer Geudertimer; Ky, dark fire−cured Kentucky; Bu, light air−cured Burley; SCL, sandy clay loam, SL, sandy loam and CL, clay loam soils. Different letters indicate least significant differences at $p \leq 0.01$. For an explanation of NAE_1 and NAE_2, see the Materials and Methods section.

		SCL			**SL**			**CL**	
Variables	**IBG**	**Ky**	**Bu**	**IBG**	**Ky**	**Bu**	**IBG**	**Ky**	**Bu**
NAE_1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
NAE_2	1.89	5.46	2.17	2.16	8.30	2.33	1.85	7.06	2.11

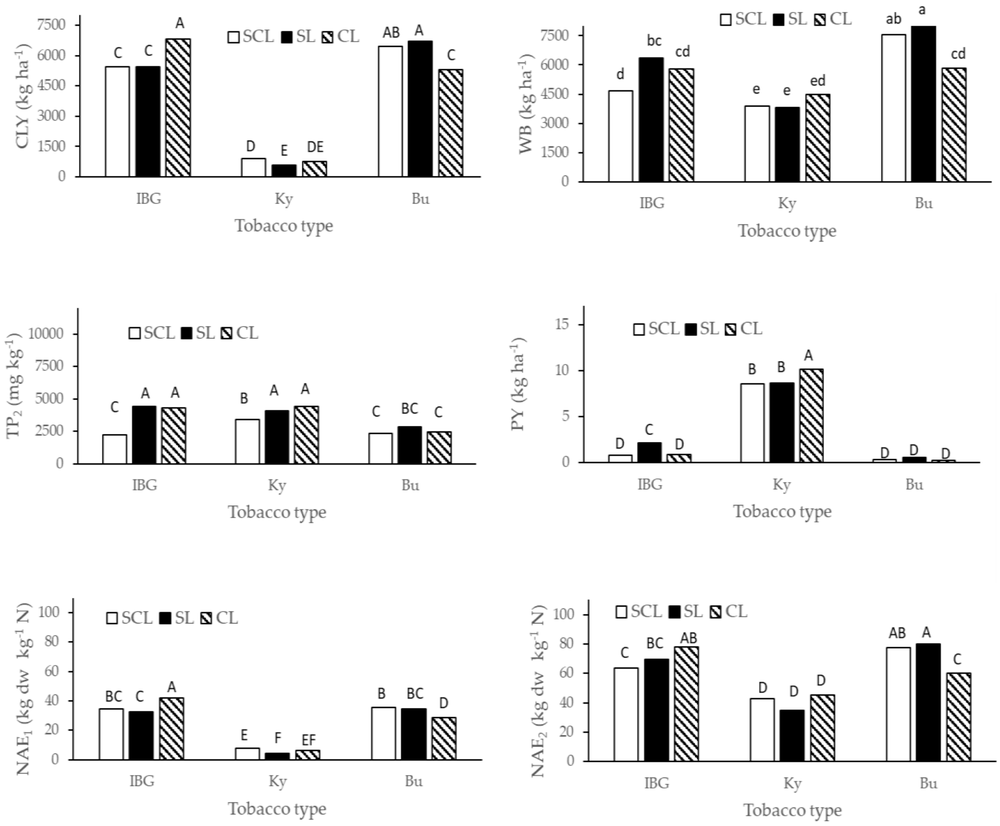

Figure 3. Yield of commercial leaves (CLY), total waste biomass (WB), total polyphenols (TP_2) and polyphenols yield (PY) and nitrogen use efficiency (NUE_1 and NUE_2) of dark air−cured Badischer Geudertimer (IBG), dark fire−cured Kentucky (Ky) and light air−cured Burley (Bu) tobacco crops, grown in sandy clay loam (SCL), sandy loam (SL) and clay loam (CL) soils. For an explanation of NAE_1 and NAE_2, see the Materials and Methods section. Different letters indicate least significant differences of interaction by soil × tobacco type at $p \leq 0.05$ and $p \leq 0.01$ (capital letters). d.w., dry weight.

Table 3. Stalk biomass (SB, kg ha^{-1}), moisture content (MC, %), calorific value (CV, MJ kg^{-1} d.w.), biomass energy yield (BEY, GJ ha^{-1} yr^{-1}), volatile matter (VM, %) and ash (%) contents of dark air−cured Badischer Geudertimer (IBG), dark fire−cured Kentucky (Ky) and light air−cured Burley (Bu) tobacco crops, grown in sandy clay loam (SCL), sandy loam (SL) and clay loam (CL) soils. All results were obtained by the waste biomass (residual stalks after final harvest), picked up by growers on 4 October 2021. Different letters indicate least significant differences at $p \leq 0.05$ and $p \leq 0.01$ (capital letters). The ANOVA table reports the significance of treatments and their interaction. NS, not significant; *, significant at $p \leq 0.05$; **, significant at $p \leq 0.01$.

Source of Variation		SB	MC	CV	BEY	VM	Ash
Soil (S)							
	SCL	4325.2 b	84.2	17.1	74.2	94.6 b	5.4 a
	SL	5315.1 a	83.5	17.4	90.6	96.0 a	4.0 b
	CL	4397.0 b	82.3	16.2	72.0	96.3 a	3.7 b
Tobacco type (T)							
	IBG	5251.8 B	84.7 A	16.6	86.6 B	94.9 b	5.1 a
	Ky	1551.1 C	81.6 B	17.3	29.9 C	96.6 a	3.4 b
	Bu	7234.2 A	83.6 A	16.9	122.3 A	95.3 b	4.7 a
ANOVA							
S		*	NS	NS	NS	*	*
T		**	**	NS	**	*	*
S × T		*	NS	NS	*	NS	NS

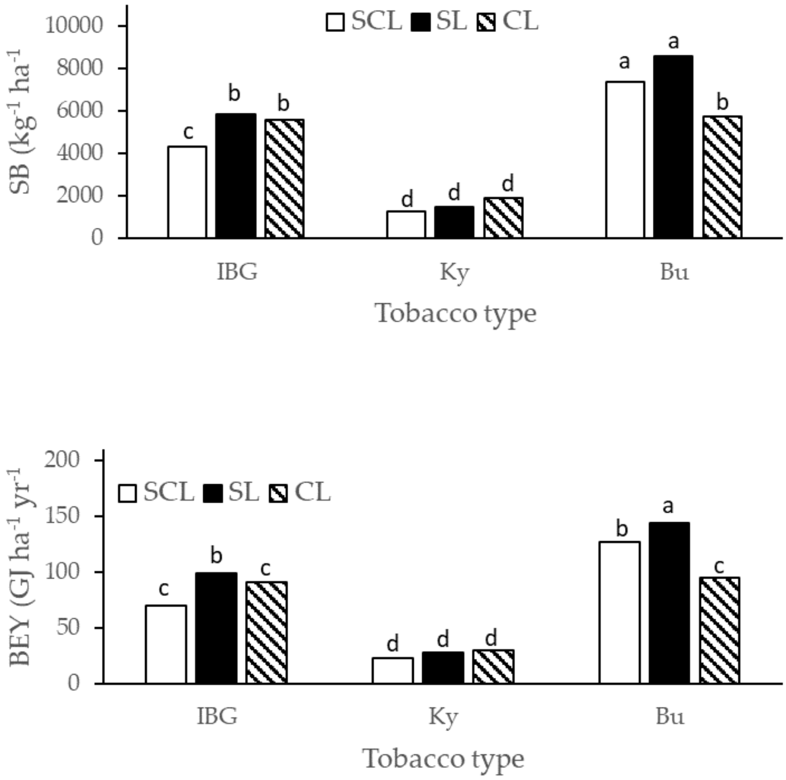

Figure 4. Stalk biomass (SB, kg ha^{-1}) and biomass energy yield (BEY, GJ ha^{-1} yr^{-1}) of dark air−cured Badischer Geudertimer (IBG), dark fire−cured Kentucky (Ky) and light air−cured Burley (Bu) tobacco crops, grown in sandy clay loam (SCL), sandy loam (SL) and clay loam (CL) soils. Different letters indicate least significant differences of interaction by soil × tobacco type at $p \leq 0.05$.

4. Discussion

The effects of soil × tobacco type interaction were significant on almost all investigated parameters, but there was no clear trend due to soils for all tobacco types. This latter result was expected since soil effects are generally due to a wide combination of factors like water, nutrients, pH, EC, rhizosphere microbiome, etc. [74–79], that may influence, both directly and indirectly, growth, development, and plant metabolism, including the secondary one [47,57–61]. For this reason, the influence of soils is generally reported to be more attributable to fertility overall (i.e., OM and OC content, mineral nitrogen richness, etc.) than to a single specific soil property [47,57,58,61].

Based on physical (texture) and chemical (pH, EC, OM, mineral N content, etc.) characteristics, soils used in the present experiment resulted all suitable for tobacco cultivation. As for the CL type, it is specifically recommended for dark fire-cured Kentucky tobacco [36]. On the contrary, SL is the most recommended type for IBG [37] and Bu [38] since both these tobacco types are reported to suffer the water excess typical of clayey soils. In the present experiment, together, to texture the soils differed in OM, which was higher in SCL and SL soils than CL (in a range of low, medium and very low content, respectively), and in mineral N content (both NO_3-N and NH_4-N quotas), which was higher in SL than both SCL and CL (in any case, in a range of good supply).

As expected, marked differences among soil types in water retention capacity (FC and WP, Table 1) were also evident. This last aspect may play an important role.

Our study confirmed the richness in important phenolic compounds of all kinds of tobacco pre-harvest waste [6]. According to what was previously reported by Sifola et al. [6], four main components were found in the tobacco polyphenols profile, namely 3-O-CQA, luteolin 7 rutinoside, rutin and quinic acid. In the present experiment, they always accounted for more than 80% of TP (i.e., reaching 99% in final suckers' material of Ky). Interestingly, the quinic acid content found in stalks of Ky final suckers' material was higher than that in both residual, not commercial, leaves of Ky or in early stripping basal leaves of IBG, Ky and Bu. This result was consistent with that already reported by Sifola et al. [6]. Quinic acid is one of the most important components in polyphenol profile of tobacco, contributing to both cured leaves taste and smoke aroma [80].

The relative weight of each phenolic component significantly changed with type of soil. Soil effect was more evident when polyphenols were extracted by early stripping basal leaves (IBG, Ky and Bu) than by residual, not commercial, leaves and final suckers' material (Ky). As previously reported, soil type, together with several other environmental factors like sun exposure, rainfall, etc., may have a major effect on polyphenol content [48,57,58]. Oney-Montalvo et al. [57] reported that polyphenols were affected by soil concentrations of minerals and specifically by N content in soils. In particular, the OM and N content, together with K and P, showed to have an important role in the synthesis of some enzymes involved in polyphenol biosynthesis [57]. In our experiment, regardless of tobacco type the highest content of nitric N of SL soil, associated to OM that was in a medium range for that kind of texture, could explain the good results of TP_1 content of early stripping basal leaves of plants grown in SL condition.

The antioxidant capacity was, on average, greater than that reported in Sifola et al. [6] and appeared influenced by both soil, tobacco and waste biomass types. According to what was already discussed for TP_1, an improvement in OM and mineral N could also explain the improvement of antioxidant capacity due to soil type [81,82]. TP_1 and antioxidant capacity were both higher in stalks of Ky tobacco suckers' material than other waste biomasses by IBG, Ky and Bu, confirming what was already found by Sifola et al. [6].

The total amount of biomass produced by different kinds of tobaccos varied between 10,102 (SCL) and 12,600 (CL) kg ha^{-1} for IBG, 4370 (SL) and 5249 (CL) kg ha^{-1} for Ky and 11,139 (CL) and 15,500 (SL) kg ha^{-1} for Bu. CL soil influenced positively the biomass accumulation of both IBG and Ky tobaccos but not the Bu type, confirming, for the latter, recommendations previously reported [36–38]. Interestingly, the following very high percentages of total biomass were pre-harvest waste, which markedly changed according to both different soils and tobacco types: 46 (CL) to 54 (SL) % for IBG, 81 (SCL) to 87 (SL) % for Ky, 52 (CL) to 57 (SL) % for Bu (minimum and maximum values, respectively). These amounts were consistent with what was already found in the same cultivation area by previous experiences in Ky [41] and Bu tobacco [28,39,40] and with that reported by Berbec and Matyka [49]. Thus, these results confirmed the great chance for the tobacco farming system to find profitable uses for such waste biomass other than burial, usually practiced by tobacco farmers [36–38].

Ky tobacco showed a significantly higher PY than other tobacco types since polyphenols were extracted by residual leaves and final suckers' material also. The PY of Ky reached the maximum value of 10 kg ha^{-1} when Ky tobacco was grown in CL soil. In this case, considering what was reported by Sifola et al. [6], the extra gross income from polyphenols could vary from EUR 100 to EUR 1000 per hectare. The polyphenols content of tobacco biomasses appeared competitive with that of several species [83,84] and, considering the quality of each main component, could represent a good opportunity for the food industry to find new natural ingredients [6]. Nevertheless, as far as we know at present, no study is available on the yield of polyphenols on area basis confirming that a cropping system like that proposed herein still needs to be applied to an agronomic context.

As expected, NAE_2 was always greater than NAE_1. It was almost 2- (IBG in SCL and CL soils) to more than 8-fold (Ky in SL soil) greater than NAE_1 thanks to a greater potential commercial biomass produced (CLY and WB) with the same amount of N fertilizer used. Considering that NAE is an indicator of sustainability [22–24], a potential threshold appears greatly overcome with these values [13]. The increase in N fertilization efficiency when using pre-harvest waste biomass (NAE_2 vs. NAE_1) was certainly the greatest in Ky tobacco. This result was also expected since, thanks to the high economic value of commercial yield by smoke products (sell price of smoking products from 2022 growing season was, on average, EUR 7.25/kg vs. EUR 2.26 and 2.79/kg, respectively, for IBG and

Bu; personal communication Organizzazione Nazionale Tabacchicoltori), fewer commercial leaves are often harvested (a single harvest is generally practiced by growers of Ky tobacco), and, consequently, like already reported, great amounts of pre-harvest waste biomass are often produced.

As for calorific measurements, in the present experiment, stalks collected at the end of the growing period represented about 89–96, 32–46 and 97–99% of total pre-harvest waste biomass in IBG, Ky and Bu tobacco types, respectively. The results showed that there was no change due to soils or tobacco types on CV of stalk biomass. The measured values (16.9 MJ kg^{-1}, on average) were in line with those reported for tobacco by Berbec and Matyka [49] (17.6 MJ kg^{-1}, on average) and consistent with those of other herbaceous crops like wheat, barley and rice (straw), cotton (coirs), soybean, etc. [4,85]. As for different tobacco types, Berbec and Matyka [49] measured the CV of 17.8 and 17 MJ kg^{-1} for Virginia and Burley types, respectively. In addition, our results showed that there was a greatly different BEY among the investigated tobacco types, with the highest values in Bu (122.3 GJ ha^{-1} yr^{-1}, on average) and the lowest in Ky (29.9 GJ ha^{-1} yr^{-1}, on average), the latter due to the low amount of stalk biomass produced per unit land area (less seedlings transplanted per hectare). A greater BEY was found by Berbec and Matyka [49] in Virginia and Burley tobacco (227.6 GJ ha^{-1} yr^{-1}, on average), but they processed both stalk and leaf materials.

In the present experiment, a negative characteristic of stalks was the high moisture content that could limit their use for energy production [4,85]. Biomasses for these purposes are, in fact, properly selected based on this aspect since, whatever being the transformation (gasification, pyrolysis or combustion), moisture influences conversion processes through biologically mediated reactions [85]. Moreover, moisture may also have a negative impact on the transport and storage of biomass materials. Nevertheless, a low ash content was always found, which means that all tested materials have a good potential to be used as fuel [4]. Overall, Ky tobacco had the lowest ash content coupled to the highest content of volatile matter, and, for this reason, it showed the greatest potential among tobacco types to be used as fuel [4].

5. Conclusions

Here, we show for the first time that a multipurpose use of tobacco crop is possible since there are a lot of biomasses, other than those for smoke products, that might be profitably used for polyphenols or energy production. Lower usable biomasses were found in Ky than in IBG and Bu types, due to the usual lower planting density of this kind of tobacco as compared with others (10,000 vs. 25,000 and 30,000 plants per hectare, respectively), but differences among tobacco types may be reduced if economic values of biomasses are considered (smoke products of Ky tobacco generate higher income as compared with others).

Polyphenolic profiles with the four well-known and widely utilized components (rutin for cosmeceutical uses, chlorogenic acids for blood pressure control, etc.) were found in all types of tobacco under investigation. Both polyphenols yield and energy potential per unit land area and/or per growing season appeared competitive with those from other herbaceous crops.

Ky tobacco showed clearer behavior than the other tobacco types to the different kinds of soil, responding well to the CL soil (i.e., the highest biomass accumulation, TP content, PY, etc.) that is properly recommended for it. Among tobacco types, it showed the greatest potential to be used as fuel. As expected, the multipurpose use of tobacco increased the agronomic efficiency of N applied as fertilizer with particularly evident benefits for Ky tobacco (particularly great thresholds of such indicator).

In the proposed multipurpose sustainable tobacco farming system, the conversion of waste into revalued and diversified products would allow for a production circuit characterized by a virtuous flow of resources, in line with the European Commission's new Circular Economy Action Plan (CEAP), one of the main building blocks of the European Green Deal. Overall, this system for tobacco crops should be quite easy to carry out. In fact, new products might be obtained with an involvement equal to or slightly higher than that necessary for traditional smoke products (cured leaves), considering that in most cases tobacco farms/companies are already equipped with spaces and systems suitable for the needed operations of collection, drying, cutting and packaging of residual biomass deriving from cultivation. Therefore, a new multipurpose tobacco farming system might be easily applicable to all farms and, overall, does not envisage lasting investments or additional labor also (it is already a labor-intensive crop). Further study will need both to measure additional labor, if any, and to investigate economic aspects (potential incomes and costs) of this multipurpose tobacco system.

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Abbreviations

Abbreviation	Definition
IBG	Ibrid Badischer Geuderhetimer
Bu	Burley tobacco
Ky	Kentucky tobacco
SCL	Sandy Clay Loam soil
SL	Sandy Loam soil
CL	Clay Loam soil
WB	Waste Biomass
CLY	Commercial Leaves Yield
NAE	Nitrogen Agronomic Efficiency
TP	Total Polyphenols
PY	Polyphenols Yield
CV	Calorific Value
VM	Volatile Matter
BEY	Biomass Energy Yield

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