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Effects of Fertilization on Morphological and Physiological Characteristics and Environmental Cost of Maize (*Zea mays* L.)

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Abstract: Maize (*Zea mays* L.) is one of the most important crops in the world and fertilization is the most important management practice which contributes to high yield. The objective of this study was to determine the effect of different fertilizers on maize crop and their contribution to the carbon footprint. The experiments were conducted in a commercial field in the area of Thessaloniki during the growing seasons of 2019 and 2020. During the experiment a number of physiological and morphological characteristics, and the energy output/input ratio, energy efficiency, and carbon footprint were determined. The results of the experiment showed that the inorganic fertilizers and manure improved the morphological and physiological characteristics that were studied compared to the green manure treatment and the control. In addition, it appeared most of the energy input of maize cultivation is from fertilizers (52%), followed by diesel (25%) and the use of machinery (14%). The treatments with the slow release fertilizers and the manure gave satisfactory results with an average of 42.1 Mg ha^{-1} in 2019 and 43.6 Mg ha^{-1} in 2020 for both fertilization treatments. Therefore, it is necessary to use the appropriate fertilizers in order to maintain the productivity of the crop and reduce the environmental costs.

Keywords: nitrogen fertilizer; green manure; manure; energy equivalent; carbon footprint

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1. Introduction

Climate change is an important issue for the 21st century and agriculture contributes to the release of 10–14% of greenhouse gas and 95% of ammonia emissions [1]. One of the most important factors that contribute to greenhouse gas emissions is the use of inputs, such as nitrogen (N) fertilizers. Additionally, excessive use of fertilizers can lead to pollution of surface and ground water [2]. Moreover, there is a significant cost due to the pollution from nitrogen fertilizers that are used in modern agriculture, which can reach up to 320 billion euros. Therefore, it is important to reduce the use of fertilizers and at the same time to use the inputs more sustainably [3].

Nitrogen is one of the most important nutrients for crop production, especially for maize production. It affects leaf area development, maintenance, and dry matter production [4]. In addition, N fertilization affects leaf chlorophyll content and is used to determine the N status of the plant [5]. Moreover, maize dry matter (DM) production increases linearly with N application and also maize silage quality up to 200 kg N ha^{-1} [6]. However, higher N rates can lead to a significant increase in residual NO_3-N concentrations and to underground water contamination. Therefore, there is a need to minimize NO_3-N leaching and at the same time to maintain or improve crop yield. Timing of N application and form of N can be an adequate strategy to ensure N availability when crops need it and minimize N losses.

In addition, the increase in fossil fuel prices led to the increase in fertilizer cost and also the inputs that are used in crop production have risen as one of the most important issues.

There is also a great interest in identifying sustainable management combinations of N forms, type of fertilizers, time of application, and N amendments that could result in more efficient use of N. Alternative forms of fertilizers such as manures (liquid manure, farmyard manures, composts, and green manures) can be used as sources of plant nutrients and at the same time increase N use efficiency and crop yield [7–10]. Manures can increase the seasonal soil N mineralization available to the crops [11–13]. However, manures also have disadvantages, such as odor, high fly breeding potential, possible transfer of pathogens and weeds, NO_3-N leaching, methane emission that contribute to the greenhouse gases, increase of soil salinity, etc. [11–13].

Modern agriculture depends on utilizing large amounts of energy from fossil fuels and there is a need to reduce the inputs and maintain the productivity in high levels and the sustainable management in order to reduce the negative effect of the excessive inputs on climate change. According to others [14–18], fertilizers have the highest energy equivalent in maize production, up to 51% of the energy that is required, while electricity accounts for 20%, and fossil fuels for 23% of the total energy. In addition, the ratio of outputs/inputs is 0.76 in maize production and this ratio indicates that the inputs are not used efficiently.

Furthermore, the agricultural sector is the major contributor of greenhouse gas (GHG) emissions, which are related to direct losses of soil organic carbon (SOC) and nitrogen in forms of CH_4, CO_2, and N_2O globally [15,16]. Improper farming operations will release substantial amounts of GHGs, thereby increasing the carbon footprint that ultimately leads to climate change [17]. Considering that the inputs with high carbon footprint are the fertilizers, fuel, and machinery, producers should implement practices to reduce their effects [17]. According to other researchers [15–18], the GHGs emissions released from maize production increased from 3633.7 kg CO_2-eq ha^{-1} in 2004 to 4043.3 kg CO_2-eq ha^{-1}. Fertilizer application, soil N_2O emission, and irrigation contributed more than 85% of total GHGs emissions. The reduction of GHGs emissions from agricultural practices is a quite complex and multifaceted challenge. In addition, the actions to reduce GHG emissions are limited and most of them are strongly connected to management practices. The effect of agriculture on GHGs emissions can be reduced by using sustainable practices such as crop rotation, reduced or no tillage, use of renewable energy sources, organic cultivation and integrated crop management, reduction of nitrogen fertilizers, and use of alternative organic N fertilization [19–21].

Nitrogen fertilization of maize is one of the most important management practices that affects the growth and the yield of the crop [13,22–25]. However, there is limited published work about the effects of application of cattle manure and green manure compared with the inorganic fertilizer and the effect of the different forms of N application on maize growth and development. In addition, any crop practice such as the application of cattle manure and green manure should be evaluated for its suitability in the cropping system before its adoption by the farmers. The objective of the present study was to evaluate the different types of fertilizers on the growth and yield of maize and also on the energy and carbon footprint of the crop.

2. Materials and Methods

2.1. Experimental Site

The experiment was conducted in a commercial field in the area of Thessaloniki, (40°34′11.4″ N 22°59′16.0″ E, 30 m), in North Greece for two years 2019 (Y1) and 2020 (Y2). The soil type was clay loam with pH 7.8 (1:2 water), EC_{se} 0.67 dSm^{-1}, and contained organic matter of 23 $g \cdot kg^{-1}$, N-NO_3 23.8 $mg \cdot kg^{-1}$, P (Olsen) 29.6 $mg \cdot kg^{-1}$, and exchangeable K 800 $mg \cdot kg^{-1}$. The weather conditions were recorded daily with an automated weather station, which was located on site, and they are referred to as monthly mean value data for both years (Table 1).

Table 1. The main weather parameters (average temperature and rainfall) in a commercial field crop in the area of Thessaloniki during the growing seasons 2019 and 2020. The weather data were recorded by a weather station on site.

Month	Rainfall (mm)		Temperature (°C)	
	2019	**2020**	**2019**	**2020**
April	71.4	97.8	14.5	12.8
May	23	36.6	19.5	19.3
June	52	25.6	26.6	24.2
July	41	13.0	27.3	27.4
August	1.8	74.8	28.4	26.6
September	13.2	15.4	23.6	24.6

2.2. Experimental Design

The experimental design that was used was a completely randomized block design with four replications. The following treatments were used: (1) no fertilization (control), (2) green manure with common vetch (GM), (3) conventional fertilizer (CF) with application of NP (12-20-0) fertilizer and urea (46-0-0) in an amount of N 310 kg Nha^{-1} and 40 kg P_2O_5 ha^{-1}, (4) slow-release fertilizer (SRF) with application of NP (16-20-0) and SRF Stabil N-M 40-0-0+14SO_3 (with a urease inhibitor) in an amount of 310 kg N ha^{-1} and 40 kg P_2O_5 ha^{-1} respectively, and (5) application of cattle manure (CM) in an amount equal to that of the conventional fertilizing in N and quantity of 20 Mg ha^{-1} (Table A1, Appendix A). The chemical properties of manure were as follows: pH 7.8, organic matter 383.8 g·kg^{-1}, N 16.9 g·kg^{-1}, P 680 mg·kg^{-1}, and K 2500 mg·kg^{-1}. The manure was incorporated with a tandem harrow disc to a depth of 12–15 cm within 15 days before sowing.

Two maize hybrids which are widely used in Greece, Pioneer 1291 (H1) (FAO 700) and Dekalb 6777 (H2) (FAO 700), were used in the study. Common vetch (*Vicia sativa* L.) was sown and used as a green manure crop in both growing seasons. The common vetch cultivar that was used was "Marianna" at a seeding rate of 150 kg·ha^{-1} and it was incorporated into the soil 15 days before the sowing of maize. The sowing of maize was conducted on 4 April 2019 and on 8 May 2020 with a four-row pneumatic seeding machine, at a seeding rate of 80,000 plants/ha. The experimental area that was used was 2.300 m^2, and each plot was 5.6 m × 20 m, covering an area of 112 m^2. The emergence of the maize plants was reported on the 17 April 2019 during the first year and on the 26 May 2020 during the second year, while harvest took place on the 10 August 2019 and on the 14 September 2020 for 2019 and 2020, respectively. All agricultural activities were recorded including the working time of the agricultural tractor and the fuel consumption.

2.3. Crop Management

The experimental area was irrigated with overhead sprinklers with a 400 mm total amount of water. First irrigation took place within the first week after maize sowing for all years. Weed control was achieved with Terbuthylazine 594 g ai ha^{-1}, Mesotrione 126 g ai ha^{-1}, and Nicosulfuron 116 g ai ha^{-1}. Additional mechanical weeding was performed to control escaped weeds in both years. No other pesticides were used.

There were eight rows in each plot and representative plants were used from the two center rows of each plot and were measured for the physiological and morphological characteristics and the silage yield. Representative plants are considered plants in full growth, with healthy and uninfected leaves, with full exposure to sunlight and plants in the same growth stage. Furthermore, the energy equivalent and the carbon footprint were calculated. The measurements of the morphological and physiological characteristics occurred during the months June–August, for both years, the first one at the stage of anthesis (GS1) and the second one 20 days later (GS2). More specifically, the following measurements were conducted:

2.4. Morphological Characteristics

2.4.1. Plant Height

Plant height was determined with a measuring tape in five plants, which were in the central rows of each plot for both growth stages.

2.4.2. Leaf Area Index

The LAI was determined by using the AccuPAR, LP–80 (Decagon Devices, Inc., NE Hopkins Ct, Pullman, DC, USA). The device comprises an external sensor, a microprocessor, and a data recorder. The sensors record the photosynthetically active radiation, in the 400 to 700 nm waveband, in units of micromoles per meter squared per second ($\mu mol \cdot m^{-2} \cdot s^{-1}$). The measurements took place during the hours between 11 a.m.–1 p.m. During this time three measurements were conducted within the canopy. The mean value of these measurements was used as the value of LAI.

2.5. Physiological Characteristics

Leaf Greenness Index (SPAD Index)

The leaf greenness index was measured using the portable device SPAD-502 (Minolta Co Ltd., Osaka, Japan) [26]. This meter determines the intensity of the green color on the leaves on a plant, according to the light absorbance in two wavelengths (650 and 940 nm). The measurements were taken from 16 plants of the main rows of each plot. The measurements were taken in the middle from the leaf of the main cob [27].

2.6. Energy Equivalent

Agricultural practices should take into consideration energy efficiency, so that a low input management can be implemented and the environmental effects can be reduced [28]. The energy approach is based on the conversion of all production factors, and even of every cultivation product, into energy units. Table 2 shows the energy equivalents that are used in agricultural production. The amount of input in this study was calculated per hectare and these data were multiplied by the coefficient of the energy equivalent. The energy equivalents were conveyed in megajoules (MJ). To determine the output/input ratio [1] and the efficiency of the energy used [2] while producing maize, the following formulas were used [14].

$$\text{Output/input ratio} = \frac{\text{The amount of energy (Output)(MJ/ha)}}{\text{The amount of energy (Input)(MJ/ha)}} \quad (1)$$

$$\text{Energy efficiency} = \frac{\text{Maize Production (kg/ha)}}{\text{The amount of energy (Input)(MJ/ha)}} \quad (2)$$

Table 2. Energy equivalents of inputs and outputs in agricultural production.

Inputs	Unit	Energy Equivalent Coefficient (MJ/unit)	Reference
Pesticides, Fungicides	kg	120	[29]
Labor	h	1.96	[29]
Machinery	h	64.8	[14]
Nitrogen (N)	kg	66.14	[30]
Phosphorus (P)	kg	12.44	[30]
Potassium (K)	kg	11.15	[30]
Manure	ton	303.1	[14]
Diesel	L	56.31	[31]
Electricity	kWh	3.6	[32]
Irrigation water	m^3	0.63	[32]
Seed for vetch	kg	10	[33]
Seed for maize	kg	14.7	[29]

2.7. Carbon Footprint

In the present study carbon (C) emissions were calculated by taking into account the C emissions that are derived directly from crop management practices, materials, and machinery inputs. The total sum of the maize C footprint of the growing season was calculated by using the following formula [34]:

$$\text{Carbon footprint} = \text{SUM (IR} \times \text{CE)} \quad (3)$$

where IR is the input ratio and CE is the coefficient of greenhouse gas emissions for each input (kg CO_2-eq kg^{-1}) (Table 3).

Table 3. Emission coefficient for each input that was used in the present study.

Inputs	Emission Factor	Reference
Nitrogen (N)	8.30 kg CO_2-eq kg^{-1} N	[35]
Phosphorus (P)	0.61 kg CO_2-eq kg^{-1} P_2O_5	[36]
Potassium (K)	0.44 kg CO_2-eq kg^{-1} K_2O	[36]
Seeds	3.85 kg CO_2-eq kg^{-1}	[36]
Electricity	0.80 kg CO_2-eq kW h^{-1}	[37]
Pesticides, Fungicides	18 kg CO_2-eq kg^{-1}	[36]
Diesel	2.63 kg CO_2-eq L^{-1}	[38]

2.8. Statistical Analysis

Data for height, LAI, and SPAD were analyzed according to a 2 × 5 × 2 × 2 experiment based on the Randomized Complete Block Design. The experiment involved four factors, in a split-split-split plot arrangement [39,40], with four replications (blocks) per combined treatment: the "year", the "fertilizer treatment", the "maize hybrid", and the "growth stage". The two years were considered as the main plots, the five fertilizer treatments were the sub-plots, the two maize hybrids were the sub-sub plots, and, finally, the two growth stages were considered as the sub-sub-sub plots. Data for energy output/input ratio, energy efficiency, and silage yield were analyzed according to a 2 × 5 × 2 experiment based on the Randomized Complete Block Design. The experiment involved three factors, in a split-split plot arrangement [39,40], with four replications (blocks) per combined treatment: the "year", the "fertilizer treatment", and the "maize hybrid". The two years were considered as the main plots, the five fertilizer treatments were the sub-plots, the two maize hybrids were the sub-sub plots. In all cases, data were analyzed within the methodological frame of Mixed Linear Models with the ANOVA method [39,40]. The ANOVA method was used mainly for computing the correct standard errors of the differences between mean values of treatments' combinations. Mean values were compared using the "protected" Least Significant Difference (LSD) criterion. The combined analysis over the two growing seasons facilitated the calculation of a common LSD value for conducting all interesting comparisons between treatments' combinations means. In all hypothesis testing procedures, the significance level was predetermined at $a = 0.05$ ($p \leq 0.05$). Statistical analyses were accomplished with the IBM SPSS v.26.0 statistical software.

3. Results

The weather conditions were quite different in the two growing seasons (Table 1). The first growing season was characterized by a warm and dry summer and the second growing season was characterized by a mild spring with significant rainfall during the spring and also during the summer. Additionally, the nonhomogeneous variation of the data across years reflected climatic fluctuations and prevented a combined analysis.

3.1. Morphological Characteristics

3.1.1. Plant Height

The plant height was affected by the main effects of "year" (Y, $p < 0.001$), "fertilizer" (F, $p < 0.001$), "hybrid" (H, $p = 0.035$), and "growth stage" (S, $p < 0.001$) and also by the two-way interaction "fertilizer × year" ($p = 0.007$). The fertilizer treatments showed the tallest plants was the conventional fertilizer with a total mean of 2.66 m for both years, while the slow-release fertilizer and application of cattle manure treatments also showed satisfactory results (with a total mean of 2.59 and 2.57 m for both years, respectively) (Table 4). Additionally, in the control treatment were found the shortest plants in both years, while the tallest plants were observed in the conventional fertilizer treatment. In addition, in the second year of the experimentation (2020) the plants were higher in all the fertilizer treatments compared to the first year (2019). Higher plants were found on hybrid Dekalb 6777 compared to hybrid Pioneer 1291 (2.46 and 2.43 m, respectively). Additionally, difference was observed between the two growth stages, with the second growth stage showing taller plants.

Table 4. Plant height (m) for the years 2019 and 2020, for both hybrids, Pioneer 1291 and Dekalb 6777, for two growth stages. Data presented are mean values, where $LSD_{0,05}$ is the least significant difference at the 0.05 significance level.

Fertilizer Treatments	**Year 2019 ***	**Year 2020 ***	**Total Mean ***
GM	2.04 e	2.52 c	2.28 c
Control	1.92 f	2.34 d	2.13 d
CF	2.35 d	2.97 a	2.66 a
SRF	2.33 d	2.85 b	2.59 b
CM	2.33 d	2.81 b	2.57 b
Total mean	2.19	2.69	
$LSD_{0.05}$ for interaction F × Y	0.08		
$LSD_{0.05}$ for main effect of F			0.06
Significance of main effect of Y (p-value)		<0.001	
Hybrids	Year 2019	Year 2020	Total mean
H1	2.18	2.68	2.43
H2	2.21	2.72	2.46
Significance of main effect of H (p-value)			0.035
Growth Stages	Year 2019	Year 2020	Total mean
GS1	2.16	2.66	2.41
GS2	2.23	2.74	2.48
Significance of main effect of GS (p-value)			<0.001

Notes: F: fertilizer; Y: year; H: hybrid; GS: growth stage; Control: no fertilization; GM: green manure; CF: conventional fertilizer; SRF: slow-release fertilizer; CM: application of cattle manure; H1: Pioneer 1291; H2: Dekalb 6777; GS1: growth stage at the stage of anthesis and GS2: growth stage 20 days later from the stage of anthesis. * Means followed by the same letter are not statistically significantly different, at significance level 0.05, according to the LSD criterion.

3.1.2. Leaf Area Index (LAI)

Leaf area index (LAI) was affected by the main effects of "year" (Y, $p < 0.001$), "fertilizer" (F, $p < 0.001$), "hybrid" (H, $p = 0.048$), and "growth stage" (S, $p < 0.001$) and also by two-way interaction "fertilizer × growth stage" ($p < 0.001$). Comparing the LAI values among the different fertilizer treatments of the experiment, it was observed that the higher values were found in the plants with the conventional fertilizer, cattle manure, and slow-release fertilizer treatments (2.82, 2.77, and 2.76, respectively) (Table 5). For both growth stages in which the measurements were conducted, the lowest values of LAI index were found in the control and green manure treatment, while in the conventional fertilizer treatment LAI had the highest values. Additionally, the hybrid Pioneer 1291 showed the lowest LAI values with an average of 3.45 compared with the hybrid Dekalb 6777 which showed the highest LAI values with an average of 3.56. Leaf area index had the highest value during the 2020 year with an average of 4.07, while LAI value was lower in 2019 (2.94). Additionally, a significant difference was observed between the two growth stages, with the first growth stage showing a higher leaf area index than the second, with an average of 3.55 and 2.85, respectively.

Table 5. Leaf area index (LAI) for the two years 2019 and 2020, for both hybrids Pioneer 1291 and Dekalb 6777, for two growth stages (GS). Data presented are mean values, where $LSD_{0.05}$ is the least significant difference at the 0.05 significance level.

Fertilizer Treatments	**GS1 ***		**GS2 ***	**Total Mean ***
GM	3.30 b		3.21 bc	3.25 b
Control	2.96 cd		2.86 d	2.91 c
CF	3.87 a		3.78 a	3.82 a
SRF	3.81 a		3.71 a	3.76 a
CM	3.82 a		3.72 a	3.77 a
Total mean	3.55		2.85	
$LSD_{0.05}$ for interaction F × GS		0.35		
$LSD_{0.05}$ for main effect of F				0.24
Significance of main effect of GS (*p*-value)		<0.001		
Hybrids	GS1		GS2	Total mean
H1	3.49		3.40	3.45
H2	3.61		3.51	3.56
Significance of main effect of H (*p*-value)				0.048
Years	GS1		GS2	Total mean
Year 2019	2.98		2.90	2.94
Year 2020	4.13		4.01	4.07
Significance of main effect of Y (*p*-value)				<0.001

Notes: F: fertilizer; Y: year; H: hybrid; GS: growth stage; Control: no fertilization; GM: green manure; CF: conventional fertilizer; SRF: slow-release fertilizer; CM: application of cattle manure; H1: Pioneer 1291; H2: Dekalb 6777; GS1: growth stage at the stage of anthesis and GS2: growth stage 20 days later from the stage of anthesis. * Means followed by the same letter are not statistically significantly different, at significance level 0.05, according to the LSD criterion.

3.2. *Physiological Characteristics*

Leaf Greenness Index (SPAD Index)

The leaf greenness index (SPAD) was affected by the main effects of "year" (Y, $p < 0.001$), "fertilizer" (F, $p < 0.001$), and "growth stage" (S, $p < 0.001$) and also by the two-way interaction "fertilizer × year" ($p = 0.004$). The SPAD index values, irrespective of the year, were highest in the conventional fertilizer treatment with an average of 58.87, followed by the slow-release fertilizer treatment with an average of 57.57 and cattle manure with an average of 57.56 (Figure 1). For both years, 2019 and 2020, the lowest values of SPAD index were found in the control (45.05 and 44.79, respectively) and in the green manure treatments (51.05 and 48.86, respectively). In contrast, the highest SPAD values were observed for both years in the conventional fertilizer treatment with an average of 59.75 in year 2019 and 58.00 in year 2020. The slow-release fertilizer and manure treatments showed high values similar to the conventional fertilizer in both years (58.37 and 58.36 in year 2019 and 56.78 and 56.76 in year 2020, respectively). The plants displayed greater leaf greenness index during 2019 with a value of 54.52, while during 2020 the value had an average of 53.04. Furthermore, in both years (2019 and 2020) SPAD decreased from the first growth stage to the second. There were no statistically significant differences between the two hybrids that were tested, Pioneer 1291 and Dekalb 6777.

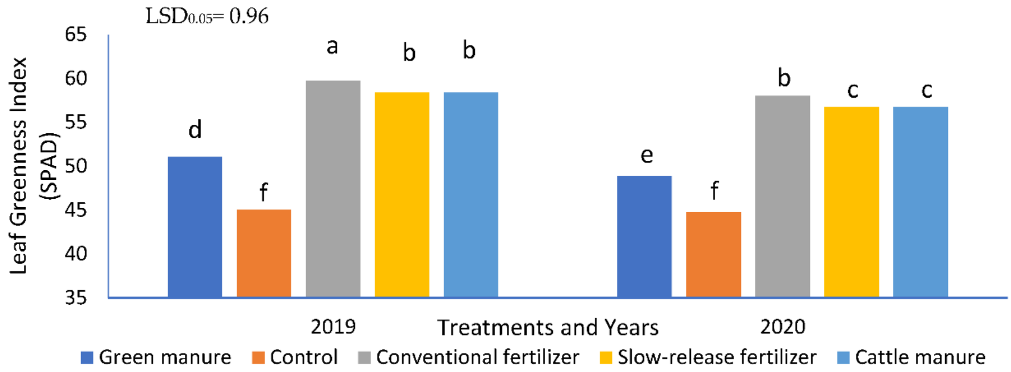

Figure 1. Leaf Greenness Index (SPAD) for the two years 2019 and 2020. Notes: Control: no fertilization; GM: green manure; CF: conventional fertilizer; SRF: slow-release fertilizer; CM: application of cattle manure. Within each year, means (bars) denoted by the same letter are not statistically significantly different, at significance level 0.05, according to the LSD criterion.

3.3. Energy Equivalent

The total energy input equivalents were calculated by multiplying the amount of input by unit with the corresponding coefficient of the energy equivalent (Table 6). The total energy input amounts to 477.49 and 555.28 MJ ha^{-1} in 2019 and 2020, respectively. The percentages of the energy input equivalents used in maize cultivation for the 2019 year were determined as follows: plant protection products 0.28%, labor 0.21%, machinery 13.5%, fertilizers 56.6%, diesel 20.4%, electricity 1.5%, irrigation 3.9%, and seeds 3.7%. Similar results were recorded in 2020 with the following percentages: plant protection products 0.24%, labor 0.26%, machinery 15.1%, fertilizers 48.7%, diesel 29.6%, electricity 1.9%, irrigation 3.7%, and seeds 3.2%. The experimental results showed that the largest part of energy input is due to the use of fertilizers (56.6% in 2019 and 48.7% in 2020), followed by the use of diesel (20.4% in 2019 and 29.6% in 2020) and machinery (13.5 in 2019 and 15.1% in 2020).

Table 6. Energy input and output equivalents in maize cultivation for the years 2019 and 2020.

Inputs	Unit	The Amount of Input/Unit		Energy Equivalent Coefficient (Mj/Unit)	Energy Equivalent (Mj/Ha)	
		2019	2020		2019	2020
Pesticides, Fungicides	kg	0.11	0.11	120	1.32	1.32
Labor	h	5	7.5	1.96	0.98	1.47
Machinery	h	10	13	64.8	64.80	84.24
Nitrogen (N)	kg	31	31	66.14	205.03	205.03
Phosphorus (P)	kg	4	4	12.44	4.97	4.976
Manure	Mg	2	2	303.1	60.62	60.62
Diesel	L	17	26.3	56.31	95.72	148.09
Electricity	kWh	20	30	3.6	7.2	10.8
Irrigation water	m^3	300	330	0.63	18.9	20.79
Seed for vetch	kg	15	15	10	15	15
Seed for maize	kg	2	2	14.7	2.94	2.94
Total input energy (Mj)					477.49	555.28

The ratio output/input was affected by the main effects of "fertilizer" (F, $p < 0.001$) and "hybrid" (H, $p < 0.001$), while the energy efficiency input was affected by the main effects of "fertilizer" (F, $p < 0.001$) and "hybrid" (H, $p < 0.001$), and also by the two-way interactions "fertilizer × year" ($p = 0.015$). The ratio output/input showed similar tendencies during both years of the experiments, with the highest values being found in the green manure treatment and cattle manure treatment for both hybrids, while the lowest values were found in the slow release fertilizer and the conventional fertilizer treatment, with the hybrid Pioneer 1291 having the lowest values during both years when compared with the hybrid Dekalb 6777. Differences were also observed among the fertilizer treatments that applied. The highest values were observed in the green manure treatment for both years (0.89 and 0.78 for the year 2019 and 2020, respectively) while the conventional fertilizer and the slow-release fertilizer treatments showed the lowest values in the energy efficiency (0.58 and 0.56 for the year 2019, 0.56 and 0.50 for the year 2020, respectively) (Table 7). Significant differences were also observed in the energy efficiency between the two hybrids. More specifically, the hybrid Dekalb 6777 showed higher values with an average of 0.70, in contrast to the hybrid Pioneer 1291 with an average of 0.66. It seems that the ratio energy output/input fluctuates from 0.94 to 1.73 in the different fertilizer treatments, indicating that the ratio is low, a fact that shows that the inputs are not used efficiently.

Table 7. Energy efficiency in maize cultivation the two years 2019 and 2020. Data presented are mean values, where $LSD_{0,05}$ is the least significant difference at the 0.05 significance level.

Fertilizer Treatments	**Year 2019**		**Year 2020**	**Total Mean**
GM	0.89 a *		0.78 b	0.83 a
Control	0.73 c		0.70 c	0.72 c
CF	0.58 d		0.53 de	0.55 d
SRF	0.56 d		0.50 e	0.53 d
CM	0.86 a		0.70 c	0.78 b
Total mean	0.72		0.64	0.68
$LSD_{0.05}$ for interaction F × Y		0.06		
$LSD_{0.05}$ for main effect of F				0.04
Significance of main effect of Y (p-value)		0.062		
Hybrids	Year 2019		Year 2020	Total mean
H1	0.70		0.62	0.66
H2	0.74		0.66	0.70
Significance of main effect of H (p-value)				<0.001

Notes: F: fertilizer; Y: year; H: hybrid; Control: no fertilization; GM: green manure; CF: conventional fertilizer; SRF: slow-release fertilizer; CM: application of cattle manure; H1: Pioneer 1291; H2: Dekalb 6777. * Means followed by the same letter are not statistically significantly different, at significance level 0.05, according to the LSD criterion.

3.4. Carbon Footprint

In Table 8 is presented the amount of inputs used for maize production, the CO_2 emission factors, and the sum of CO_2 emissions. The results for 2019 showed that the inputs with the highest values of CO_2 emissions are nitrogen with a value of 2.573 kg CO_2-eq ha^{-1}, diesel with 447.1 kg CO_2-eq ha^{-1}, electricity with 160 kg CO_2-eq ha^{-1}, maize seeds 77 kg CO_2-eq ha^{-1}, phosphorus 24.4 kg CO_2-eq ha^{-1}, and the plant protection products with 19.8 kg CO_2-eq ha^{-1}. Similarly, in 2020 the results showed the same tendency, with differences being found in diesel with a value of 691.69 kg CO_2 eq ha^{-1} and in electricity with 240 kg CO_2-eq ha^{-1}. The results indicated that, of the different inputs in maize cultivation, the ones that contribute the most in the CO_2 emissions are the N fertilizers, the use of diesel, and electricity.

Table 8. Emission factors for each input during the two years.

Inputs	**Emission Factor**	**The Amount of Input**		**CO_2 Emissions**	
		2019	**2020**	**2019**	**2020**
Nitrogen (N)	8.30 kg CO_2-eq kg^{-1} N	310 kg ha^{-1}	310 kg ha^{-1}	2.573 kg CO_2-eq ha^{-1}	2.573 kg CO_2-eq ha^{-1}
Phosphorus (P_2O_5)	0.61 kg CO_2-eq kg^{-1} P_2O_5	40 kg ha^{-1}	40 kg ha^{-1}	24.4 kg CO_2-eq ha^{-1}	24.4 kg CO_2-eq ha^{-1}
Electricity	0.80 kg CO_2-eq kWh^{-1}	200 kWh ha^{-1}	300 kWh ha^{-1}	160 kg CO_2-eq ha^{-1}	240 kg CO_2-eq ha^{-1}
Seeds	3.85 kg CO_2-eq kg^{-1}	20 kg ha^{-1}	20 kg ha^{-1}	77 kg CO_2-eq ha^{-1}	77 kg CO_2-eq ha^{-1}
Pesticides, Fungicides	18 kg CO_2-eq kg^{-1}	1.1 kg ha^{-1}	1.1 kg ha^{-1}	19.8 kg CO_2-eq ha^{-1}	19.8 kg CO_2-eq ha^{-1}
Diesel	2.63 kg CO_2-eq L^{-1}	170 L ha^{-1}	263 L ha^{-1}	447.1 kg CO_2-eq ha^{-1}	691.69 kg CO_2-eq ha^{-1}
Total emissions CO_2	8.30 kg CO_2-eq kg^{-1} N	310 kg ha^{-1}	310 kg ha^{-1}	3.301 kg CO_2-eq ha^{-1}	3.625.89 kg CO_2-eq ha^{-1}

3.5. Silage Yield

The silage yield was influenced only by the factor "fertilizer" (F, $p < 0.001$). The lowest yield was found in the control and green manure treatments for both hybrids during both years (Table 9). On the contrary, higher yields were observed in the conventional and the slow release fertilization (4.42 Mg ha^{-1} and 4.31 Mg ha^{-1}, respectively), while equally high yields were found in the cattle manure application, with an average of 4.25 Mg ha^{-1}.

Table 9. Silage yield during the two years 2019 and 2020. Data presented are mean values, where $LSD_{0,05}$ is the least significant difference at the 0.05 significance level.

Fertilizer Treatment	Total Mean (Mg ha^{-1}) *
GM	4.04 b
Control	3.65 c
CF	4.42 a
SRF	4.31 a
CM	4.25 ab
$LSD_{0.05}$ for main effect of Fertilizer	0.23

Notes: Control: no fertilization; GM: green manure; CF: conventional fertilizer; SRF: slow-release fertilizer; CM: application of cattle manure. * Means followed by the same letter are not statistically significantly different, at significance level 0.05, according to the LSD criterion.

4. Discussion

4.1. Morphological Characteristics

4.1.1. Plant Height

It was found that in the control the plants were shorter, while in the slow release fertilizer, the cattle manure, and the conventional fertilizer treatments the plant height was increased compared with the green manure treatment. The plant height increase that was found may be due to the positive effect of nitrogen on plant growth which leads to the increase of internode length, as well as to the cell division and consequently the increase of the plant height [41–43]. These results are in agreement with other studies where it was found that the conventional fertilizers, the slow release fertilizers, and the cattle manure increased the plant height compared with the control [44–46].

4.1.2. Leaf Area Index (LAI)

The smallest increase of the leaf area index was recorded at the green manure treatment compared with the fertilizer treatments. The results of the present study are similar to those reported from other studies [45,47]. The slow release fertilizer treatment together with the cattle manure treatment caused a greater increase of LAI compared with the control and the green manure treatment. Similar results were reported by other scientists [48–50]. Additionally, the conventional fertilization caused an increase of leaf area index that is in agreement with other studies [50–53]. In conclusion, the results show that the leaf area index was increased more with the mineral fertilizers and the use of cattle manure compared with the green manure. According to other studies [51,53,54], this increase is caused by the immediate effect of nitrogen on the leaf size, which leads to an increase in leaf area and to interception of solar radiation [52].

4.2. Physiological Characteristics

Leaf Greenness Index (SPAD)

Chlorophyll content measured with SPAD meters is directly related to nitrogen uptake from the plants and can be used as an index for the immediate and precise detection of the nutrient conditions [55]. In the present study significant differences were found between control and fertilizer treatments (cattle manure, conventional fertilizer, slow release fertilizer) where the level of chlorophyll was increased compared with the control and the green manure treatments. The lower amount of N available in control treatment remobilized from the leaves towards the grains caused a decrease of the leaf chlorophyll content and a premature leaf ageing [9,56]. This means that when there is an adequate amount of N in the soil, the leaf ageing is slower and the plant supplies the grain with N for a longer period of time, resulting in higher performance [5,57].

4.3. Environmental Costs

4.3.1. Energy Equivalent

This study shows that most of the energy input is caused by fertilizers, diesel, and machinery. Similar data were reported in other studies where the fertilization, diesel, and machinery had the highest energy inputs in maize cultivation [14,58–60]. Therefore, for improving energy efficiency of maize it is important to use machinery and fertilizers more efficiently. In addition, the ratio energy output/input ranged from 0.94 to 1.73 among the different fertilizers, revealing that the inputs are not being used efficiently [14,31,61]. The ratio of the current research is recorded as lower than that presented in other studies [29,62] due to the high energy consumption by the fertilizers. Hence, farmers should be trained on how to achieve an efficient usage of inputs in maize production while maintaining a high performance.

4.3.2. Carbon Footprint

The present study shows that from the different inputs in maize cultivation the ones that contribute the most in CO_2 emissions are the chemical fertilizers, diesel, and electricity. In addition, other also studies reported that fertilization, diesel, and electricity were the main inputs in maize cultivation which contribute to the C footprint [34,63,64]. Fertilizers can contribute up to 60% of CO_2 emissions [38] and are the highest source of CO_2 emissions. Despite the fact that the use of conventional fertilizers has the greatest effect on the C footprint, diesel and electricity contribute equally greatly and, thus, these inputs should also be reduced to improve the machinery efficiency, irrigation as the implementation of electricity, and diesel usage during cultivation and fertilization performance so that their contributions to the C footprint can be reduced.

4.4. Silage Yield

From the present study it was found that the slow release fertilizer and cattle manure treatments increased silage yield compared with the control and were comparable with the conventional fertilizers. This effect may be because of the nutrients that are available in cattle manure and also the slow release of N from the fertilizer [9,10,65–68].

5. Conclusions

Maize is a crop species that requires high amount of nutrients due to the high production of dry matter and grain yield. One of the most important factors that contribute to an increase in the dry matter production is fertilization. In the present study, which was conducted in a commercial field in the area of Thessaloniki, it was found that fertilization affects the morphological and physiological characteristics of maize plants. The mineral fertilizers and cattle manure increased the plant height, LAI, and SPAD index compared with the use of green manure and the control treatment. It was found that the highest percentage of energy input in maize cultivation is because of the use of fertilizers, diesels, and machinery. Consequently, application of slow release fertilizers and cattle manure can improve all the characteristics that were studied and perform similarly to conventional fertilizers, which means that they can be used as alternatives for fertilization. This study provides new information regarding the effect of N application on maize phenology, growth, and development, which affects the productivity of the crop. Therefore, they can be used by the farmers in Mediterranean areas as they maintain or improve crop yield. It is important that the production rate can maintained and that the environmental cost is decreased with the use of appropriate fertilizers.

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Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to privacy restrictions. Data are presented in the article and Appendix A.

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Appendix A

Table A1. The experimental design that was used in the study with the five fertilizer treatments (control, green manure, conventional fertilizer, slow-release fertilizer, and cattle manure) and the two hybrids (Dekalb 6777 and Pioneer 1291) for the first growing season, with different randomization for the second growing season.

Block 1				**Block 2**				**Block 3**				**Block 4**		
Dekalb 6777	Pioneer 1291	control		Pioneer 1291	Dekalb 6777	conventional fertilizer		Pioneer 1291	Dekalb 6777	green manure		Dekalb 6777	Pioneer 1291	slow-release fertilizer
Pioneer 1291	Dekalb 6777	green manure		Dekalb 6777	Pioneer 1291	cattle manure		Dekalb 6777	Pioneer 1291	conventional fertilizer		Dekalb 6777	Pioneer 1291	control
Dekalb 6777	Pioneer 1291	conventional fertilizer	corridor	Dekalb 6777	Pioneer 1291	control	corridor	Pioneer 1291	Dekalb 6777	slow-release fertilizer	corridor	Dekalb 6777	Pioneer 1291	green manure
Dekalb 6777	Pioneer 1291	slow-release fertilizer		Pioneer 1291	Dekalb 6777	slow-release fertilizer		Pioneer 1291	Dekalb 6777	control		Pioneer 1291	Dekalb 6777	cattle manure
Pioneer 1291	Dekalb 6777	cattle manure		Dekalb 6777	Pioneer 1291	green manure		Dekalb 6777	Pioneer 1291	cattle manure		Pioneer 1291	Dekalb 6777	conventional fertilizer

Table A2. Plant height for the years 2019 and 2020, for both hybrids, Pioneer 1291 and Dekalb 6777, for two growth stages. Data presented are mean values and CV stands for coefficient of variation.

Treatments	Height							
	2019				2020			
	1° Growth Stage		2° Growth Stage		1° Growth Stage		2° Growth Stage	
	Pioneer 1291	Dekalb 6777	Pioneer 1291	Dekalb 6777	Pioneer 1291	Dekalb 6777	Pioneer 1291	Dekalb 6777
GM	2.01	1.99	2.10	2.07	2.48	2.47	2.57	2.57
Control	1.86	1.91	1.93	2.00	2.27	2.34	2.35	2.41
CF	2.29	2.33	2.37	2.40	2.88	2.96	2.98	3.06
SRF	2.28	2.30	2.36	2.37	2.78	2.82	2.88	2.91
CM	2.29	2.31	2.36	2.38	2.77	2.78	2.84	2.84
CV%	0.71							

Table A3. Leaf area index (LAI) for the years 2019 and 2020, for both hybrids Pioneer 1291 and Dekalb 6777, for two growth stages. Data presented are mean values and CV stands for coefficient of variation.

Treatments	LAI							
	2019				2020			
	1° Growth Stage		2° Growth Stage		1° Growth Stage		2° Growth Stage	
	Pioneer 1291	Dekalb 6777	Pioneer 1291	Dekalb 6777	Pioneer 1291	Dekalb 6777	Pioneer 1291	Dekalb 6777
GM	2.69	2.74	2.64	2.66	3.85	3.90	3.76	3.77
Control	2.29	2.35	2.22	2.27	3.57	3.62	3.45	3.51
CF	3.27	3.30	3.18	3.21	4.32	4.62	4.22	4.51
SRF	3.26	3.28	3.18	3.20	4.17	4.54	4.07	4.41
CM	3.28	3.30	3.20	3.23	4.23	4.47	4.12	4.32
CV%	0.77							

Table A4. Leaf Greenness Index (SPAD) for the years 2019 and 2020, for both hybrids Pioneer 1291 and Dekalb 6777, for two growth stages. Data presented are mean values and CV stands for coefficient of variation.

Treatments	SPAD							
	2019				2020			
	1° Growth Stage		2° Growth Stage		1° Growth Stage		2° Growth Stage	
	Pioneer 1291	Dekalb 6777	Pioneer 1291	Dekalb 6777	Pioneer 1291	Dekalb 6777	Pioneer 1291	Dekalb 6777
GM	51.42	51.25	50.87	50.65	49.62	48.77	48.87	48.20
Control	45.60	45.07	45.10	44.45	45.37	44.72	44.87	44.20
CF	60.07	60.12	59.32	59.47	58.47	58.10	57.85	57.57
SRF	58.75	58.57	58.05	58.12	56.95	57.20	56.42	56.55
CM	58.72	58.60	58.25	57.90	56.97	57.15	56.47	56.47
CV%	0.67							

Table A5. Energy output/input ratio and energy efficiency in maize cultivation for the years 2019 and 2020. Data presented are mean values and CV stands for coefficient of variation.

Treatments	Output/Input Ratio				Energy Efficiency			
	2019		2020		2019		2020	
	Pioneer 1291	Dekalb 6777	Pioneer 1291	Dekalb 6777	Pioneer 1291	Dekalb 6777	Pioneer 1291	Dekalb 6777
GM	1.68	1.73	1.45	1.53	0.88	0.90	0.76	0.80
Control	1.33	1.41	1.31	1.36	0.71	0.75	0.69	0.72
CF	1.08	1.12	0.97	1.04	0.57	0.59	0.51	0.55
SRF	1.01	1.08	0.94	0.96	0.54	0.57	0.49	0.50
CM	1.58	1.70	1.29	1.37	0.83	0.89	0.68	0.72
CV%	5.4				4.6			

Table A6. Silage yield during the years 2019 and 2020. Data presented are mean values and CV stands for coefficient of variation.

Treatments	Silage Yield (Mg ha^{-1})			
	2019		2020	
	Pioneer 1291	Dekalb 6777	Pioneer 1291	Dekalb 6777
GM	38.20	38.80	41.82	42.90
Control	33.50	34.30	38.77	39.45
CF	42.70	43.70	44.47	46.27
SRF	41.50	43.10	43.70	44.48
CM	41.20	42.60	42.62	43.92
CV%		8.11		

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