Life Cycle Assessment of Biomass Production from Lignocellulosic Perennial Grasses under Changing Soil Nitrogen and Water Content in the Mediterranean Area

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Abstract: Low iLUC risk feedstocks, such as lignocellulosic no-food crops, have been indicated as sustainable crops for the transition to a bio-based economy. Given the high output to input ratio and the environmental benefits that can be obtained from renewable heat production replacing fossil fuels, the present study addressed the biomass yield, CO$_2$-sequestration, and life cycle assessment of giant reed (Arundo donax L.) and miscanthus (Miscanthus × giganteus Greef et Deuter) growing under different soil water availability and nitrogen fertilization for three consecutive growing seasons in a semiarid Mediterranean environment. Giant reed outperformed miscanthus, showed a higher CO$_2$-sequestration and a lower overall environmental impact. In case of both crops, the irrigation effect was significant, while the one of nitrogen fertilization was not apparent. While giant reed responded positively to reduced irrigation, compared to its highest level, as the plantation became older, miscanthus needed high water volume to get most out its potential yield. Nonetheless, the growing season had also a significant effect on both crops, mainly when low yields were achieved following the establishment year. Unlike the environmental benefits in the impact categories “non-renewable energy use” and “global warming potential”, environmental burdens concerning ozone depletion, acidification, and eutrophication were observed, indicating that further improvements of the evaluation of impact assessment associated with bioenergy production might be necessary.

Keywords: miscanthus; giant reed; LCA; CO$_2$-sequestration; environmental impact

1. Introduction

Lignocellulosic biomass is the most abundant raw material on earth and bioenergy from lignocellulosic crops can address indirect land use changes (iLUC) emissions and avoid conflict with food markets. In the European Union, high iLUC risk feedstock (first generation crops) has been judged to be unsustainable; nowadays, emphasis is given to bioenergy and biofuels from low iLUC risk feedstock, either by promoting a biomass productivity increase per unit of land used or by growing low input requiring lignocellulosic crops on unused, abandoned, idle lands [1,2].

In the last 40 years, perennial energy grasses have been deeply investigated under countless environmental conditions and agronomic practices [3]. The wish list for a sustainable transition from a fossil to a biobased economy includes feedstock that has high and stable yield and composition, needs low agrochemical inputs, has a low water requirement and a low moisture content at harvest [4]. Whereas perennial energy grasses display most of those traits, their improper agronomic management can cause environmental burdens, either directly to the surrounding agroecosystem or indirectly elsewhere [5]. Agricultural practices thus need to be optimized according to the site-specific environmental conditions and crop requirements to maximize the net environmental benefit.
The life cycle assessment (LCA) is a methodology widely used for the evaluation of the environmental burdens associated with bioenergy/biofuel production, by identifying energy and materials used as well as waste and emissions released to the environment [6]. Several LCAs studies have found a significant net reduction in greenhouse gas (GHG) emissions and fossil energy consumption with bioenergy crops replacing fossil fuels [7,8]. However, some also ascertained other impact categories, such as local air pollution, acidification, eutrophication, ozone depletion, and land use, among others [9,10], often concluding that many bioenergy crops might cause contamination of water and soil resources, mainly when irrigation, fertilizers, and pesticides are intensively used. Nonetheless, these environmental burdens are even more affected by site-specific assumptions than the GHG and energy balances, suggesting that it is not easy to draw simplified conclusions [11].

Further uncertainties might arise in geographical areas where agriculture systems are subjected to increasing pressure due to global warming, such as the Mediterranean. Indeed, the Mediterranean region has been identified as a climate change hot spot, with most countries already experiencing a rise in temperatures, freshwater scarcity, and high risk of prolonged drought [12,13]. In addition, semiarid climates in the Mediterranean are typically characterized by nutrient deficiency [14] and, as a result, substantial areas of agricultural land are already becoming marginal and often abandoned due to increasing dryness and fertility loss leading to bio-physical and socio-economic limitations [2,15].

On the other hand, cropping these lower grade lands with bioenergy crops might help to meet many sustainability requirements and ecosystem services, such as no direct competition with food/feed, limited iLUC, benefits to biodiversity, and reduction of erosion risks, among others [16]. However, tailored crops, input amount, and timely application, primarily of water and nitrogen, can in part enhance cropping system outcomes and restore rhizospheric microbial community functions for optimum productivity [14].

Previous studies have addressed input reduction to improve water and nutrient use efficiency in many bioenergy crops in the semiarid Mediterranean climate [17,18]. The present study focused on a three-year field trial, comparing two perennial grasses—namely, giant reed (Arundo donax L.) and miscanthus (Miscanthus × giganteus Greef et Deuter)—under different soil water availability and nitrogen fertilization in a semiarid Mediterranean environment. The aim was to ascertain the biomass yield, the CO\textsubscript{2}-sequestration, and the life cycle assessment of biomass use for renewable heat production replacing fossil fuels.

2. Materials and Methods

2.1. Agronomic Data

Giant reed (Arundo donax L.) and miscanthus (Miscanthus × giganteus Greef et Deuter) were grown in Enna, Italy (37°23′ lat. N, 14°21′ long. E, 450 m a.s.l.), in a deep, loam soil for three consecutive growing seasons. Field and bed preparation followed an autumn ploughing at 30 cm depth and a spring disc-harrowing at 20 cm depth before transplant. Rhizomes were mechanically transplanted in spring 2002, at a depth of 0.15 m and at a density of 20,000 rhizomes ha\textsuperscript{-1} for both species.

Throughout the three-year experimental period, two levels of nitrogen (N) fertilization—namely, 50 kg N ha\textsuperscript{-1} (N\textsubscript{50}) and 100 kg N ha\textsuperscript{-1} (N\textsubscript{100})—and two levels of soil water availability—namely, 25% and 75% of maximum evapotranspiration restoration (I\textsubscript{25} and I\textsubscript{75}, respectively)—were differentiated. Crops were grown in a split–split plot experimental design with three replications, where the species was the main plot (12 m × 4 m), the irrigation the subplot (6 m × 4 m), and the N fertilization the sub-sub plot (3 m × 4 m). The N fertilization was applied at the beginning of each growing season as ammonium nitrate (27%), while a basal dressing with 120 kg P\textsubscript{2}O\textsubscript{5} ha\textsuperscript{-1} as superphosphate (18%) was applied in the establishment year. Irrigation water was supplied by means of a drip system during summer months according to the water balance method [17,18]. The irrigation volume in I\textsubscript{25} was 115 mm, 146 mm, and 150 mm in both species in the first, second and third growing seasons, while I\textsubscript{75} received 441 mm, 438 mm, and 450 mm, respectively. Aboveground biomass was harvested in wintertime in representative subplots of 6 m\textsuperscript{2} for
all species, treatments, and replications. Biomass subsamples were placed in a ventilated oven at 65 °C, up to a constant weight, for dry matter determination.

The CO$_2$-sequestration (Mg ha$^{-1}$) was calculated according to the aboveground biomass yield ($Y$), the biomass carbon content (C%), and the conversion of C to CO$_2$-equivalent:

$$\text{CO}_2\text{-sequestration} = [(Y \times C) \times (44/12)]$$

where: $Y = \text{dry biomass yield (Mg ha}^{-1})$; $C = \text{carbon content of miscanthus and giant reed biomass of 45.7% and 45.3%, respectively [19]}$; 44 = CO$_2$-equivalent atomic weight; 12 = carbon atomic weight; and 16 = the oxygen atomic weight.

The maximum and minimum air temperature and rainfall were measured by a weather station connected to a data logger (CR 10, Campbell Scientific, Logan, UT, USA). The reference crop evapotranspiration ($ET_{0}$) was calculated from the class A evaporation pan (mm) by the pan coefficient of 0.80 [20]. Equipment were set-up nearby the experimental field and daily data were aggregated on ten-day basis.

### 2.2. Life Cycle Assessment

The LCA followed the ISO standards 14040 and 14044 guidelines [21,22]. The impact on the environment of the solid biomass from giant reed and miscanthus in a combustion boiler to produce heat for domestic use was compared to the impact of a fossil fuel for heat production and use. The life cycle of the biogenic system included soil tillage and bed preparation, nursery activities for rhizome fragmentation and establishment, cropping system differentiation (i.e., nitrogen fertilization rates, and irrigation levels), biomass harvest, logistics, conversion, use phase, and end of life (residual ash). The life cycle of the conventional reference system included crude oil extraction, processing, transportation, use phase, and end of life.

The reference unit was the thermal energy in MJ of energy produced. The primary energy consumption (MJ h$^{-1}$) and emissions (g h$^{-1}$) for each specific operation were obtained by multiplying the time of resource use by the amount of input, according to the database of the Institute for Energy and Environmental Research [23].

Although the lifespan of perennial grasses is fifteen years or more [24], the LCA was conducted for the establishment, second, and third growing season separately. For each crop and treatment, farming energy input (both direct and indirect) were acquired by Mantino et al. [25] because the current data came from the same field experiment.

The reference cropping system considered in this work is a grass-fallow in which the agronomic management was defined by a first soil tillage (ploughing and harrowing) and two mowing operations per year.

Field emissions from the use of fertilizers included nitrous oxide (N$_2$O), nitric oxide (NO), and ammonia (NH$_3$) in the atmosphere, and nitric ions (NO$_3^-$) and orthophosphoric acid (H$_3$PO$_4$) in surface and subsurface waters.

The multivariate empirical models developed by Bouwman et al. [26] were used for the assessment of direct NO$_2$ and NO:

$$\text{NO}_2 = e^{\text{Constant} + (\sum \text{factor class})}$$

where the constant is $-0.4136$ and the coefficients for factor classes are the fertilizer type (ammonium nitrate, 0.0061) by N rate, the crop type (grass, $-1.268$), the soil texture (medium, $-0.472$), the soil drainage (good, $-0.420$), the soil pH (>7.3, $-0.352$), the climate (temperate, 0) and the length of experiment (>300 days, 0.825).

$$\text{NO} = e^{\text{Constant} + (\sum \text{factor class})}$$

where the constant is $-1.527$ and the coefficients for factor classes are the fertilizer type (ammonium nitrate, 0.0040) by N rate, and the soil drainage (good, 0.946).
The NH$_3$ emissions were estimated according to Bouwman et al. [27]:

$$\text{NH}_3 = e^{\sum \text{factor class}}$$  \hspace{1cm} (4)

where the factor classes are the crop type (grass, $-0.158$), the fertilizer type and amount (ammonium nitrate $\leq 50$ kg, $0.134$; $\leq 100$ kg, $1.936$), the application mode (broadcast, $-1.305$), and the soil drainage (good, $0.946$).

The amount of indirect emissions can be converted to NO$_2$ emission by multiplying NO and NH$_3$ emissions by the default value 0.01 [28,29].

NO$_3$-leaching was calculated according to the semi-empirical model of Di and Cameron [30] considering volatilization and denitrification:

$$\text{NO}_3 = 0.00143(N_{PL})^2 - 0.0229N_{PL}$$  \hspace{1cm} (5)

where $0.00143$ and $-0.0229$ are regression coefficients and $N_{PL}$ is the nitrogen (predominantly NO$_3$) potentially leached (kg N ha$^{-1}$ 100 mm$^{-1}$ drainage).

The emission factor of 0.33% per kg P applied has been assumed for phosphates leached to surface and subsurface waters [31].

Heavy metals emissions (cadmium, chromium, copper, iron, lead, manganese, mercury, nickel, selenium, and zinc) were calculated according to the share in fertilizers, in vegetable biomass, for the heavy metals naturally occurring in the soil and those from atmospheric depositions [23].

In the industrial phase, the hourly unit emissions of the bioconversion for the production of thermal energy—which includes the transport, storage, combustion, and the treatment of residual ashes—were considered. The carbon dioxide (CO$_2$) emissions at the biomass combustion stage were set to zero due to the CO$_2$ uptake from the atmosphere by the crop photosynthetic process.

The relative emissions (g h$^{-1}$) were determined for each sub-phase, taking into account the hourly use of each equipment and the production means of the heating system. The emission values, referred to one hectare (MJ ha$^{-1}$; g ha$^{-1}$), were converted to the unit of energy produced (MJ MJ$^{-1}$; g MJ$^{-1}$). The biomass moisture content and the lower heating value were set at 15% and 15.6 MJ kg$^{-1}$ for both crops [20] and the thermal bioconversion efficiency at 90%.

The conventional reference system was based on fossil fuel oil for the production of thermal energy resulting from the refining of oil, which is imported into Europe from the OPEC countries.

The impact assessment followed the classification that grouped emissions into specific impact categories represented by the consumption of abiotic resources and linked them to the emissions of chemical substances.

The consumption of abiotic resources category included the set of primary energy resources used for each production process. Each production means used was characterized by a total energy cost ($E_1$), given by the sum of an operating energy cost and a depreciation cost, expressed in MJ h$^{-1}$, which derived from the sum of primary energy resources (crude oil, coal, lignite, natural gas, uranium) and abiotic resources (water), which entered its production process or during its use, as:

$$E_1 = \frac{RE}{HU} \times LHV$$  \hspace{1cm} (6)

where $RE = \text{sum of the energy resources used for the production or use of the means (kg)}$; $HU = \text{technical life of the machine (hours)}$; $LHV = \text{lower heating value (MJ kg}^{-1}$): 45.6 for crude oil, 48.8 for natural gas, 19.0 for hard coal, 9.5 for lignite, and 451,000 for uranium [32].

The greenhouse effect category included carbon dioxide and monoxide (CO$_2$ and CO), methane (CH$_4$), nitrous oxide (N$_2$O), and volatile organic compounds (VOC). According to the Intergovernmental Panel on Climate Change (IPCC), the equivalence factor is expressed
as g CO₂ kg⁻¹ [33,34]. The time horizon to which the greenhouse effect was referred was 100 years.

The potential depletion of the stratospheric ozone layer considered only the nitrous oxide (N₂O) emissions for this impact category.

The gases that contribute to the acidification effect of the atmosphere are ammonia (NH₃ in the gaseous form in the air and NH₄⁺ in the ionic form in water), hydrogen chloride (HCl), nitrogen oxides (NOₓ), and sulphur dioxide (SO₂), all expressed in g SO₂-equivalent [35].

For the eutrophication of water bodies, the effects of ammonia (NH₃ and NH₄⁺), nitrogen oxides (NOₓ), nitrates (NO₃⁻), and phosphates (PO₄³⁻) were aggregated and expressed in g NO₃⁻ per g of substance [36].

We normalised all results by relating them to the environmental situation in Europe; i.e., by dividing them by the annual average resource demand and the average emissions of various substances per capita in Europe [6]. The results were thus expressed in so-called inhabitant equivalents per 100 TJ of energy consumed in one year. This way, the relative magnitude of the results could be better interpreted. Due to the uncertainty related to future emissions of various substances, inhabitant equivalents were calculated based on the year 2000 values. Despite the representativeness of the collected agronomic data in terms of temporal and geographical correlation (data collected within 3 years from the study and from the same area) [37], our assumptions about the post-harvest and technological uses might have caused uncertainties. An assessment of uncertainty was not performed; nonetheless, in order to avoid biased comparisons, mature technologies were taken as a basis for the assessment, and discussions about the main source of uncertainties took expert judgments into account. According to the goal of the present study and the system boundaries, the highest priority was given to the greenhouse gas emission savings resulting from better agronomic practices to maximize yield, which in turn determined the amount of conventional products that could be substituted within the used area.

2.3. Statistical Analysis

Data were subjected to the two-way analysis of variance (ANOVA) using repeated measurements in time (SPSS, PASW Statistics 18). Measurements were performed throughout growing seasons within-subjects, while species, irrigation, and nitrogen fertilization as between-subjects. When data failed Mauchly’s sphericity test, the univariate results were adjusted by using the Greenhouse–Geisser Epsilon and the Huynh–Feldt Epsilon correction factors. When univariate results satisfied sphericity tests for within-subjects effects, the F-values and associated p-values for between-subjects effects were tested. The Duncan’s post-hoc test was used for mean separation at a 95% confidence level.

3. Results

3.1. Meteorological Conditions

The crop establishment was accomplished in mid-March, when mean air temperature was consistently higher than 10 °C (Figure 1). After the establishment, an even rainfall distribution was observed up to May, thereafter a long dry period with high reference evapotranspiration (ET₀) was registered in summertime. The first growing season was untypically dry with only 334 mm of rainfall throughout the whole growing season. The first biomass harvest was conducted in March 2003, then stem re-sprouting for the second growing season coincided with the increase in air temperatures in spring.
Figure 1. Meteorological trends, maximum (Tmax) and minimum (Tmin) air temperature (°C), rainfall and reference evapotranspiration (ET0, mm) at the experimental site (37°23′ lat. N, 14°21′ long. E, 450 m a.s.l.) during 2002/03, 2003/04, and 2004/05 growing seasons.

The second growing season was particularly hot and dry in the summertime, with very high ET0 (nearly 7.5 mm per day) and air temperature close to 38 °C without significant rain events, except in late September and throughout the autumn. The rainfall in the whole growing season was 605 mm, of which 43% was registered from October to November. The second season biomass harvest was carried out in March 2004; thereafter, the third growing season started with stem re-sprouting in spring.

The third growing season had a quite even rainfall distribution (28% in spring, 32% in autumn, and 32% in winter), except in the summertime when only 8% out of 650 mm of the whole growing season was registered. The air temperature, both maximum and minimum, was cooler than the previous growing season, notably the minimum air temperature from the end of December up to the biomass harvest time was the lowest of the overall experimental period.

The whole season ET0 was 1152 mm in 2002/03, 1245 mm in 2003/04, and 1174 mm in 2004/05, and the mean air temperature was 16.8 °C, 17.4 °C, and 16.2 °C, respectively. The irrigation, at the planned amount (I25 and I75), was scheduled in summertime, approximately from the end of May to the beginning of September, when the highest crop water demand coincided with the dry period in this environment.

3.2. Biomass Yield and Aboveground CO2-Sequestration

The ANOVA showed that the species and irrigation main effects on biomass yield and CO2-sequestration were significant. The harvest, which represented the within-subject effect, was also significant, while neither the fertilization nor the interactions were (Table 1).
Table 1. Repeated measures ANOVA for main effects and interactions on aboveground dry matter yield (DMY) and aboveground CO₂-sequestration of Arundo donax and Miscanthus × giganteus. Degrees of freedom (DF) adjusted mean square (Adj MS) and significance: p ≤ 0.001 (***) or not significant (ns).

| Source         | DF | DMY Adj MS | CO₂-Sequestration Adj MS |
|----------------|----|------------|--------------------------|
| Harvest        | 2  | 5427.01 *** | 15,079.6 ***             |
| Species (S)    | 1  | 2259.60 *** | 6088.6 ***               |
| Irrigation (I) | 1  | 530.46 ***  | 1476.2 ***               |
| Fertilization (F) | 1  | 26.49 ns    | 73.1 ns                  |
| S × I          | 1  | 0.20 ns     | 0.30 ns                  |
| S × F          | 1  | 21.51 ns    | 59.4 ns                  |
| I × F          | 1  | 3.53 ns     | 9.8 ns                   |
| S × I × F      | 1  | 1.49 ns     | 4.1 ns                   |
| Error          | 62 | 33.99 ns    | 93.5 ns                  |

Mean separation of main effects indicated that giant reed outperformed miscanthus across fertilization and irrigation levels (26.1 and 14.9 Mg ha⁻¹, respectively). The irrigation I₂₅ was significantly lower than I₇₅ across species and fertilization (17.8 and 23.2 Mg ha⁻¹, respectively), while fertilization, although higher in N₁₀₀ than N₅₀ across the average of the other factors (18.9 and 21.1 Mg ha⁻¹, respectively), was not different (Figure 2).

Biomass yield increased from the first to the third growing season, and the increase was much more apparent in miscanthus, which followed a linear trend, irrespective of nitrogen and irrigation levels. Nonetheless, yield response seemed more affected by the irrigation than by the nitrogen. The highest yields in miscanthus were observed in the third year (I₇₅N₁₀₀) and I₇₅N₅₀ (30.6 and 29.3 Mg ha⁻¹, respectively), while reduction of the irrigation decreased the biomass yield to 23.5 and 24.1 Mg ha⁻¹ (I₂₅N₁₀₀ and I₂₅N₅₀, respectively). Giant reed increased the biomass yield almost linearly in the lowest input combination (I₂₅N₅₀), while increasing input levels, particularly irrigation, boosted yield response from the second growing season to the third one, where biomass yield levelled off irrespective of the input supplied. The highest input combination in giant reed (I₇₅N₁₀₀) boosted biomass yield from 9 Mg ha⁻¹ at the first to 41 Mg ha⁻¹ at the second harvest, levelling off thereafter.
The aboveground CO₂-sequestration (CO₂-equivalent) was very low in miscanthus but also in giant reed under the lowest irrigation level. From the second growing season onward, the CO₂-sequestration increased in both species and it was proportional to the input supplied in giant reed in the second season ($I_{75}N_{100} > I_{75}N_{50} > I_{25}N_{100} > I_{25}N_{50}$), while no differences were observed in the third year, where the CO₂-sequestration averaged 68 Mg ha⁻¹ (Figure 3).

In miscanthus, the CO₂-sequestration was different between irrigation levels both in the second and third growing seasons, with the $I_{75}$ always higher than $I_{25}$, but the fertilization did not improve it. Across the average of experimental factors, giant reed had a significantly higher CO₂-sequestration than miscanthus (43.4 and 24.9 Mg ha⁻¹), and $I_{75}$ was higher than $I_{25}$ (38.7 and 26.6 Mg ha⁻¹). Fertilization $N_{100}$ enhanced of only 1.9 Mg ha⁻¹ the CO₂-equivalent as compared with the $N_{50}$.

3.3. Life Cycle Assessment

The ANOVA showed a significant effect of the species on all impact categories. The irrigation was significant for all categories, except for the non-renewable energy use (NREU). The fertilization was significant only for the ozone depletion (OD) and the eutrophication (EU). The harvest, which represented the within-subject effect, was also significant in all impact categories, while no significant interactions of main effects occurred (Table 2).

### Table 2. Repeated measures ANOVA for main effects and interactions on impact categories of *Arundo donax* and *Miscanthus × giganteus*. Degree of freedom (DF), adjusted mean square (Adj MS), and significance: $p \leq 0.001 (***)$, $p \leq 0.01 (**)$, $p \leq 0.05 (*)$, not significant (**).
The three experimental years showed environmental benefits of the biogenic system for the impact categories non-renewable energy use (NREU) and global warming potential (GWP). On the contrary, environmental burdens were observed for the ozone depletion (OD), acidification (AC), and eutrophication (EU). Burdens were also registered in miscanthus I₂₅N₁₀₀ and I₂₅N₅₀ in the first growing season for the GWP impact category (Figure 4).

![Figure 4](image_url)

**Figure 4.** Impact categories (NREU—non-renewable energy use; GWP—global warming potential; OD—ozone depletion; AC—acidification; EU—eutrophication), expressed in European inhabitant equivalent per 100 TJ of energy consumed in one year, of *Arundo donax* and *Miscanthus × giganteus* throughout the experimental period and investigated main effects (green arrow—environmental benefits; red arrow—environmental burdens).

Environmental burdens were higher in the first than in the second and third years in both species. Environmental benefits were also more contained in the first than in the second and the third growing seasons.

In the first growing season, giant reed I₇₅N₅₀ showed the best combination for both benefits and burdens, while the worst was in I₂₅N₁₀₀. Miscanthus I₂₅N₅₀ and I₂₅N₁₀₀ showed the worst combination for all impact categories in the first growing season, while the I₇₅N₅₀ was the best.
Within the same species, combination of experimental factors showed very similar results for all impact categories in the second growing season. In the third, the benefits were also similar between species and input combinations, while within species, the burdens were higher in the case of giant reed I$_{25}$N$_{100}$ for the OD and EU and in the case of miscanthus I$_{25}$N$_{100}$ for OD.

Across the average of experimental factors, the mean separation showed a greater performance of giant reed than miscanthus, with higher benefits and lower burdens of impact categories investigated (Table 3). Increasing irrigation, across species and fertilization, worsened the GWP, the OD, the AC, and the EU. Increasing the fertilization, across species and irrigation, worsened both the OD and the EU, while it was ineffective for the other impact categories.

### Table 3. Mean separation of main effects on impact categories, expressed in European inhabitant equivalent per 100 TJ of energy consumed in one year of Arundo donax and Miscanthus × giganteus grown under different irrigation levels (I$_{25}$ and I$_{75}$) and nitrogen rates (N$_{50}$ and N$_{100}$). Different letters within main effect indicate statistical different mean ($p \leq 0.05$).

|                | NREU     | GWP      | OD       | AC       | EU       |
|----------------|----------|----------|----------|----------|----------|
| **Species**    |          |          |          |          |          |
| Arundo         | −870.26a | −691.44a | 820.45b  | 339.44b  | 429.16b  |
| Miscanthus     | −776.78b | −547.85b | 1075.95a | 517.06a  | 632.42a  |
| **Irrigation** |          |          |          |          |          |
| I$_{25}$       | −799.97a | −568.40b | 1081.12a | 498.91a  | 610.98a  |
| I$_{75}$       | −847.07a | −670.89a | 815.29b  | 357.59a  | 450.61b  |
| **Nitrogen**   |          |          |          |          |          |
| N$_{50}$       | −833.12a | −651.38a | 826.37b  | 409.57a  | 507.96b  |
| N$_{100}$      | −813.92a | −587.91a | 1070.03a | 446.92a  | 553.62a  |

### 4. Discussion

The three-year experimental period was characterized by interannual variability of air temperature, rainfall, and evapotranspiration. Beside the air temperature increase from spring to reach maximum values in summertime, which sustained stem sprouting and rapid elongation of the perennial grasses, the evapotranspiration (ET$_0$) increased as well, while rainfall followed an opposite trend, decreasing strongly in summertime when crop water demand was the highest. Early/middle autumn is usually wet with suitable air temperatures for growth; however, the photoperiod triggers flowering in perennial grasses and, consequently, the crops suspend soil water uptake and start senescence to overwinter [36].

With regards to the climatic parameters measured, the first experimental year was the driest, the second season had the highest ET$_0$ and air summer temperature, while the third was the most favourable, although the coldest in winter. The irrigation provided in summertime was calculated to cope with either 75% or 25% of the maximum crop ET in order to reduce this high energy input. According to previous results, the biomass yield followed an asymptotic pattern in giant reed as a function of crop water use; thus, it becomes clear that the yield response flattens at high soil water availability [17]. The nitrogen (N) was supplied according to previous observations, where the N removed with the aboveground biomass ranged from 50 to 120 kg N ha$^{-1}$ (unpublished data). This agrees with Monti and Zegada-Lizarazu [38], who calculated a N removal of 117 Kg N ha$^{-1}$ yr$^{-1}$ in giant reed fertilized at 160 kg N ha$^{-1}$ yr$^{-1}$. Giant reed half N fertilized (80 kg N ha$^{-1}$ yr$^{-1}$) removed 90 kg N ha$^{-1}$ yr$^{-1}$, while the N removal was 75 kg N ha$^{-1}$ yr$^{-1}$ in unfertilized plots. Thus, the present experiment was designed according to the low-input cultivation system.

Crops that were established by rhizome fragments grew well and followed the typical yielding phase, namely increasing from the first to the second and further to the third year [24]. However, the best combination of irrigation and fertilization on biomass yield in giant reed was the I$_{75}$N$_{100}$, which boosted biomass yield to the ceiling already in the
second year. The other treatments that were somehow limited in N or irrigation took an additional year to reach the maximum biomass yield achieved in this study. In miscanthus, the input level did not change the yield response trend, and the biomass yield followed a linear increase, reaching the maximum only in the third year. No interaction of main effects was observed in both species; thus, the yield change was explained only by the irrigation rather than by the fertilization. This is particularly true for miscanthus because irrigation, irrespective of nitrogen fertilization, improved biomass production either at first, second, or third growing season. In the case of giant reed, the irrigation seemed more effective in the first two years, while its effect disappeared in the third. In the second year, the N effect was somehow noteworthy in the case of giant reed; however, N was ineffective in the third year as well. Similar results had been already observed, where the nitrogen use efficiency became not significant in the third growing season of giant reed [17].

In accordance with other studies in the Mediterranean environment, giant reed outperformed miscanthus across the range of growing seasons, irrigation, and fertilization levels [24,25].

The low productivity of perennial grasses in the establishment year is a well-known drawback [39,40]. This led to a very low CO$_2$-sequestration, particularly in miscanthus in all input combinations, but also in giant reed I$_{25}$N$_{100}$ and I$_{25}$N$_{50}$. However, from the second growing season, the CO$_2$-sequestration was quite high and ranged from 37 to 68 Mg ha$^{-1}$ in giant reed and from 18 to 32 Mg ha$^{-1}$ in miscanthus. The CO$_2$-sequestration was proportional to the input supplied in giant reed in the second year, while no input effect was observed in the third one. On the contrary, the CO$_2$-sequestration was mostly affected by the irrigation rather than by the fertilization in miscanthus, in both the second and the third year. Other studies with miscanthus showed a maximum CO$_2$-sequestration up to 30.6 Mg CO$_2$ ha$^{-1}$ yr$^{-1}$ in good soil conditions in Central Europe, while 19.2 Mg CO$_2$ ha$^{-1}$ yr$^{-1}$ and 24.0 Mg CO$_2$ ha$^{-1}$ yr$^{-1}$ were achieved on marginal sites limited by cold or drought [41]. In this study, a positive linear relationship was found between CO$_2$-sequestration and biomass yield (y = 1.66x + 0.1028, $R^2$ = 0.99, data not shown), meaning that each ton of lignocellulosic biomass could save up to 1.66 Mg CO$_2$-equivalent ha$^{-1}$.

The life cycle assessment showed large environmental benefits of the renewable source in two impact categories—namely, NREU, and GWP—after normalisation on the average European inhabitant equivalent. In this case, the amount of fossil fuels saved was equal to the amount that nearly 870 (giant reed) or 776 (miscanthus) European citizens would consume on average per 100 TJ of heat in one year. The GWP avoided would be nearly 690 inhabitants equivalent for giant reed and 547 for miscanthus. Benefits were a function of biomass yield, as indicated by the growing season effect in both species. The low biomass yield at the first harvest reduced NREU and GWP benefits in both species, and even environmental burdens were associated with miscanthus under reduced irrigation and high fertilization dose (I$_{25}$N$_{100}$) for the GWP impact category. The irrigation supply had a significant effect on GWP across species and fertilization treatments, so increasing the irrigation volume would increase the g CO$_2$-equivalent saved leading to GWP. Although the nitrogen had no significant effect, decreasing the N dose (N$_{50}$) would increase by 20 and 62 inhabitant equivalents the NREU and GWP, respectively.

Environmental burdens of the renewable source, compared to fossil fuels, were registered for the OD, AC, and EU. Even in this case, burdens were greater in the first year due to lower biomass yield and higher input used (e.g., soil tillage and bed preparation, nursery activities for rhizome fragmentation and establishment) than the second and third years. However, increasing the irrigation tended to reduce OD, AC, and EU burdens, while the N fertilization at the highest dose exacerbated OD and EU. Considering all impact categories, giant reed resulted as the winner crop simply because of the clearly highest productivity under the same growing conditions, input supply, and bioconversion technology. However, as emphasized by many studies, open questions still remain about benefits and burdens deriving from the use of energy crops, because positive or negative effects strongly depend on different methodological assumptions and technical means [5,6,11,42]. The use
of agricultural input like irrigation and fertilization to increase biomass yield, which is the key trait in environmental studies, is unacceptable for bioenergy crops, and it might add further uncertainty to the result interpretation. As long as LCA is not able to adequately address water use, a careful site-specific analysis of water availability is necessary before recommending this agronomic practice, because irrigation of non-food crops may reduce water availability for other crops, causing water-induced iLUC. Excessive fertilization should be avoided in light of the present results because it can cause further environmental burdens. This is particularly true under these favourable climate conditions and soil type (deep loam) with good macronutrient availability and organic matter content. On the contrary, in areas with a lower fertility or other factors limiting crop growth, fertilization, also through organic fertilizers, is fundamental to maintain or improve soil fertility for the whole plant lifespan.

Other sources of uncertainty might be related to the post-harvest and technological processes. Data for the transportation, storage and bioconversion were assumed for feedstock at 15% moisture content for both crops. While miscanthus matched these values at the harvest time, giant reed biomass was usually wetter, which could add further environmental burdens associated with the downstream phases. In a sensitivity analysis, Schmidt et al. [6] found out that drying giant reed using light fuel oil instead of natural gas worsened the GHG balance significantly due to the higher CO$_2$ emissions during combustion. The use of biomass instead of natural gas for drying largely decreased GHG emissions but at the expenses of biomass quantity to produce renewable heat and power and so less GHG savings.

Although a quantitative assessment of the uncertainty related to the use of unrepresentative data may be preferable, and despite the efforts made in the past years to decrease the uncertainty in LCA outcomes, other studies showed that even when using robust statistical methods for the uncertainty analysis, such as the Monte Carlo simulation, the uncertainty sometimes remains too high to identify a statistically significant alternative [43].

5. Conclusions

Main findings suggested giant reed had a greater performance than miscanthus in terms of biomass yield, aboveground CO$_2$-sequestration, and environmental impact of biomass use for heat production. In the present environmental and soil conditions, giant reed responded positively to reduced irrigation (I$_{25}$), but the ceiling yield was achieved one year earlier when irrigation was increased (I$_{75}$). On the contrary, miscanthus needed high water volume to get most out its potential yield. The nitrogen effect was not obvious in both crops; therefore, it is recommended to reduce this input to further improve not only the non-renewable energy use and global warming potential but also the overall net environmental benefit.

In general, large environmental benefits were achieved for NREU and GWP impact categories after normalisation on average European inhabitant equivalent and following crop establishment (from second growing season onward). However, unlike the positive impacts on GHG-emissions and fossil energy consumption saved, other activities affecting the environment (i.e., land use change emission, soil carbon pools, soil nutrient content, and ecosystem services) should be accounted for when the evaluation of environmental burdens associated with bioenergy production is conducted because they may carry environmental risks in other areas.

It is worth mentioning that an uncertainty analysis was not considered in the present study, and the result interpretation derived from specific site conditions, crop management, bioconversion, system boundaries, and reference system; hence, it is highly recommended to limit these findings to the cultivation area and post-harvest technologies used to compare the bioenergy and the fossil fuel chain assumed in the present study.

Author Contributions: All authors contributed equally to this work. All authors have read and agreed to the published version of the manuscript.
**Funding:** The research was partly supported by the Italian Ministry of Agriculture in the framework of the project “Sustainable innovative techniques for energy and non-food crops (TISEN)”.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Data is not applicable.

**Acknowledgments:** Authors gratefully acknowledge Carmelo Maugeri, Matteo Maugeri, and Dario Maugeri of the University of Catania for field trial set-up and maintenance.

**Conflicts of Interest:** The authors declare no conflict of interest.

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