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# Leaf Area Duration and Crop Radiation Use Efficiency Determine Biomass Yield of Lignocellulosic Perennial Grasses under Different Soil Water Content

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**Abstract:** The aim of the present work was to assess the leaf area duration (LAD) and the radiation use efficiency (RUE) of six warm-season perennial biomass grasses (PBGs) in a two-year field trial in the semiarid Mediterranean climate under different soil water availability. Two ecotypes of giant reed (*Arundo donax* L., ARCT and ARMO), one ecotype of African fodder cane (*Saccharum spontaneum* L. subsp. *aegyptiacum* (Willd.) Hack., SAC) and three hybrids of *Miscanthus* (the commercial *M.* × *giganteus* J.M. Greef, Deuter ex Hodk., Renvoize, M × G, and two new seed-based hybrids, GNT9 and GNT10) were compared under three levels of soil water availability: rainfed, 50% and 100% of maximum crop evapotranspiration (ETm) restoration. The determination of RUE of perennial plants is controversial and has led to contrasting results in past studies. In the present work, LAD and RUE differed among crops and irrigation regimes, being positively affected by supplemental water inputs. SAC, ARCT and ARMO showed both high LAD and RUE, which determined the high biomass yield than both the commercial M × G and the improved *Miscanthus* hybrids GNT9 and GNT10. RUE was particularly high and less affected by soil water availability during the mid-season, while the effect of irrigation and the differences among the genotypes were larger during the late season. Adequate biomass yield can be achieved by sub-optimal soil water availability, thus reducing the water footprint and increasing the sustainability of these biomass perennial grasses selected for the Mediterranean climate.

**Keywords:** perennial energy crops; lignocellulosic; RUE; LAD; marginal land; Mediterranean

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## 1. Introduction

The European Renewable Energy Directive (RED II) has set several targets to promote renewable energy and to shrink greenhouse gas (GHG) emission by 2030. It also set criteria for the phase out of energy and biofuels based on high-iLUC (iLUC: indirect Land Use Change) risk food and feed crops, in favour of low-iLUC risk feedstocks [1].

Among the low-iLUC risk feedstocks, biomass perennial grasses (BPGs) combine high lignocellulosic biomass yield, high radiation use efficiency (RUE) and high water use efficiency (WUE) [2–5], along with a low agronomic input request and traits of resistance to multiple biotic and abiotic stresses [6]. Several studies have demonstrated their adaptability to low grade agricultural land, including anthropogenically contaminated sites, making BPGs the "best bet" to avoid land use competition with food production [7–9].

RUE is defined as the amount of dry matter produced by the plant per unit light intercepted [10,11]. As a crop-specific parameter, has been extensively used in crop growth models that simulate photosynthesis and consequently biomass accumulation [12].

The determination of the RUE of perennial plants is controversial and has led to contrasting results [2–5,13]. This is due in part to translocation of photosynthates from rhizomes to the aerial organs during shoot emission and from the aerial organs to the rhizomes during leaf senescence, leading to the shifting of plant resources between subsequent growing seasons [14–16]. Assessing how RUE changes during the growing seasons could be useful to clarify this controversy.

Crop photosynthesis and therefore the amount of dry matter produced by the crop are determined by the photosynthetically active surface, which is measured as the leaf area index (LAI), i.e., the one-sided green leaf area per unit ground surface area, and leaf area duration (LAD), i.e., the LAI over a period of time.

The potential yield of BPGs in the Mediterranean area is limited by the low soil water availability and high vapour pressure deficit during summer months, which induce stomata closure, reducing plant $CO_2$ assimilation, and leaf senescence [17–19]. Soil moisture declines also lead to modifications in plant morphological features as LAI and LAD [20], leading to a reduction of the rate and the time of the intercepted photosynthetically active radiation (IPAR) and consequently in the amount of dry matter produced trough photosynthesis.

As a consequence, in water-limited Mediterranean climates, supplementary water input during summer dry periods has been proven to enhance LAI, LAD and RUE for BPGs [3,5,21]. In order to achieve high productive performances at a low water input in these climates, the selection of the BPGs should focus on traits that enhance the accumulation of dry matter in periods with a higher soil water availability, such as the early emergence in spring or the delayed senescence in autumn [22], or by increasing RUE at low levels of soil water availability.

In Europe, the hybrid *Miscanthus* × *giganteus* J.M. Greef, Deuter ex Hodk., Renvoize has been recognized as the most suited species for continental and oceanic environments [23], while giant reed (*Arundo donax* L.) and African fodder cane (*Saccharum spontaneum* L. spp. *aegyptiacum* (Willd.) Hack.) are more adapted to the warm and hot Mediterranean climates [7,24].

Giant reed has a C3-pathway metabolism; nonetheless, it is able to activate a C4 photosynthetic cycle under stress conditions [25]. African fodder cane is a C4 with an efficient stomata regulation under drought conditions [24].

Although *M.* × *giganteus* has a C4 cycle, it is too sensitive to hot and dry summers typical of the Mediterranean area [26,27]. New *Miscanthus* seed-based hybrids, *M. sinensis* × *M. sinensis* and *M. sacchariflorus* × *M. sinensis*, have been developed to cope with changing environmental conditions in Europe [26,27]. Field trials employing *M. sacchariflorus* × *M. sinensis* hybrids have demonstrated higher yields compared with *M.* × *giganteus*, both under northern and southern European environments [26,27]. Multi-location trials highlighted a better adaptability of *M. sinensis* × *M. sinensis* hybrids to northern and *M. sacchariflorus* × *M. sinensis* hybrids to southern Europe [28].

The present work investigated the effect of three different levels of soil water availability on giant reed, African fodder cane and *Miscanthus* hybrids under a semiarid Mediterranean environment in terms of LAI, LAD, IPAR and RUE over the whole growing season and during both the periods from shooting until the end of the stem elongation phase and from the end of the stem elongation phase until flowering, and their implications on aboveground biomass productivity.

## 2. Materials and Methods

### *2.1. Field Trial*

A two-year field trial was carried out at the Experimental Farm of the University of Catania (10 m a.s.l., 37°24′ N, 15°03′ E) in a typical Xerofluvent soil (USDA, 1999) in the second and third growing seasons in 2019 and 2020,respectively. Soil conditions, experimental design and treatments, and agronomic practices were extensively reported in [27]. Briefly, six BPGs were compared in a split-plot experimental design with three replications, where irrigation, at three levels, represented the main plot, and genotypes, in six levels, the sub-plot.

The irrigation was provided during the summer months (June–August) to restore the 100% (I100) or 50% (I50) of maximum crop evapotranspiration or rainfed conditions (I0). The crop water use from plant re-growth up to the onset of senescence of genotypes (water supplied by irrigation + precipitation ± difference between soil water content at plant re-growth and soil water content at the onset of senescence) ranged between 263.13 ± 11.2 mm in I0 in 2019 and 1062.11 ± 21.1 mm in I100 in the 2020 growing season [27].

The BPG genotypes were two giant reed (*A. donax*) ecotypes named ARCT and ARMO (collected in Italy and Morocco, respectively), the commercial *Miscanthus* × *giganteus* named M × G (provided by Energene sp. z o.o, Wroclaw, Poland), two seed-based *M. sacchariflorus* × *M. sinensis* hybrids named GNT9 and GNT10 (provided by Terravesta Ltd., Lincoln, UK) and one ecotype African fodder (*S. spontaneum* spp. *aegyptiacum*) cane named SAC (collected in Italy).

Transplanting was carried out by hand in May 2018 at a density of 1 plant $m^{-2}$ in unfertilized plots. The first-year harvest was executed in February 2019. In the second and third growing seasons, mean air temperature (18.3 °C in 2019 and 18.0 °C in 2020) and the reference evapotranspiration (1291 mm in 2019 and 1257 mm in 2020) were quite similar. On the contrary, rainfall was lower in 2019 than the 2020 growing season (473 mm and 779 mm, respectively).

### *2.2. Measurements*

Incident solar radiation and PAR were measured hourly by a weather station connected to a data logger (CR10, Campbell Scientific, Logan, UT, USA), located 150 m from the experimental field.

The ratio of solar radiation interception was measured at periodic intervals between shooting and harvesting using an AccuPAR model LP-80 PAR/LAI Ceptometer (Decagon Devices, Inc., Pullman, WA, USA). The instrument measures the ratio between the amount of PAR transmitted through the canopy plus the PAR scattered by leaves within the canopy and the above canopy PAR. Leaf area index (LAI) was determined according to the following equation:

$$\mathrm{LAI} = \frac{-\ln(1-\mathrm{R})}{\mathrm{k_e}} \quad (1)$$

where R is the ratio of intercepted PAR and $\mathrm{k_e}$ is the extinction coefficient of the crop reported for the local environmental conditions (0.56 for *Miscanthus* hybrids, 0.405 for giant reed and 0.87 for African fodder cane) [3,5,21].

To estimate the amount of solar radiation actively intercepted by the crop canopy, the fraction of the intercepted PAR (FPAR) was calculated on the basis of green LAI (gLAI). Green LAI (gLAI) was calculated by multiplying the LAI and the ratio between green and total LAI measured by using the Easy leaf area software [29] on three-stem samples collected monthly. Daily gLAI were calculated by performing a linear interpolation between the measured points of gLAI.

The leaf area duration (LAD) was calculated according the following equation:

$$\mathrm{LAD} = \sum_{i=1}^{n} \mathrm{gLAI}_i \quad (2)$$

where $n$ is the duration of the growing season from shooting until harvesting in days and $\mathrm{gLAI}_i$ is the gLAI value at day $i$.

The FPAR at day $i$ was calculated according to:

$$\mathrm{FPAR}_i = \mathrm{e}^{-\mathrm{k_e} \cdot \mathrm{gLAI}_i} \quad (3)$$

where $\mathrm{gLAI}_i$ is the gLAI at day $i$ and $\mathrm{k_e}$ is the extinction coefficient of the crop.

The amount of PAR intercepted by the crop (IPAR) was calculated as follows:

$$\mathrm{IPAR} = \sum_{i=1}^{n} \mathrm{FPAR}_i \cdot \mathrm{PAR}_i \quad (4)$$

where $\mathrm{PAR_i}$ is PAR at day *i* and the FPAR*i* is the fraction of the intercepted PAR at day *i*.

Aboveground biomass was collected monthly throughout the growing season from random subplots of 0.5 × 0.5 m (0.25 $m^2$) given the even stem density achieved in the whole plots. Three stems per plot were sorted randomly among the collected stems to measure the fresh and the dry weight after drying the samples at 65 °C until the weight was constant.

Total aboveground biomass at harvest was collected in January 2020 and 2021, when *Miscanthus* hybrids were in the senescence phase and giant reed and African fodder cane reached the minimum extent of green leaf area. The whole aboveground fresh biomass was collected and weighted from a 4 $m^2$ subplot. During harvest, five stems per plot were weighted and then dried to a constant weight at 65 °C.

The radiation use efficiency (RUE) was calculated as the slope of the linear regression between the aboveground dry biomass produced per unit of intercepted PAR (g $MJ^{-1}$). Three values of RUE were calculated: (i) for the whole growing season (i.e., from shooting until harvesting, $RUE_{tot}$); (ii) at mid-season (i.e., from shooting until the end of the stem elongation phase, $RUE_I$) and (iii) at late season (i.e., from the end of the stem elongation phase until flowering, $RUE_{II}$).

### 2.3. Statistical Analysis

The data were subjected to an analysis of variance (two-way ANOVA) to assess the effect of genotype, irrigation (as between factors) and their interaction on LAD and RUE.

Differences between means were evaluated for significance using the Fisher's LSD test at a 95% confidence level. All analysis were performed using the R CRAN software [30]. Linear regressions to estimate $RUE_{tot}$ and $RUE_I$ were forced through the origin. The Shapiro–Wilk test was developed to test residuals for normality, and the goodness of fit was assessed by calculating $R^2$.

## 3. Results

The experimental factors (genotype and irrigation) had a significant effect on green LAI (gLAI), leaf area duration (LAD), aboveground biomass (AGB) and on radiation use efficiency (RUE) for the whole growing season (RUE tot), at mid-season (RUE I) and at late season (RUE II) (Table 1). The interaction of genotype x irrigation was significant for gLAI, LAD and AGB.

**Table 1.** Three-way ANOVA for main effects and interaction on green Leaf Area Index (gLAI), Leaf Area Duration (LAD), Aboveground Biomass (AGB), intercepted Photosynthetically Active Radiation (iPAR) and Radiation Use Efficiency (RUE). The *p*-value is reported. Irrigation and genotypes are the between-factor effects.

| Source of Variation | gLAI | LAD | AGB | iPAR | $RUE_{tot}$ | $RUE_I$ | $RUE_{II}$ |
|---|---|---|---|---|---|---|---|
| Genotype | $<10^{-16}$ | $<10^{-16}$ | $<10^{-16}$ | $<10^{-16}$ | $<10^{-16}$ | $<10^{-16}$ | $<10^{-16}$ |
| Irrigation | $<10^{-16}$ | $1.09 \times 10^{-15}$ | $<10^{-16}$ | $<10^{-16}$ | $<10^{-16}$ | $<10^{-16}$ | $<10^{-16}$ |
| Whole-Plot Error | $4.93 \times 10^{-12}$ | 0.42 | 0.95 | 0.34 | 0.69 | 0.95 | 0.93 |
| Genotype × Irrigation | $<10^{-16}$ | 0.02 | $2.95 \times 10^{-7}$ | 0.43 | 0.09 | 0.21 | 0.11 |

### 3.1. Green Leaf Area Index (gLAI)

Giant reed (ARCT and ARMO) reached the highest maximum gLAI, both in the 2019 and 2020 growing season, followed by African fodder cane (SAC) and the *Miscanthus* GNT9 (Figure 1). The gLAI in the hybrid *Miscanthus* M × G was lower than 3 $m^2$ $m^{-2}$ in both years. Irrigation water significantly improved gLAI development during summer months and postponed the leaf senescence in autumn. During the 2019 growing season, leaf development started earlier than the 2020, due to the higher maximum temperature registered in March. In 2020, green LAI was higher for giant reed ecotypes and African fodder cane due to the stem density higher than 2019 growing season. Contrarily, all *Miscanthus* hybrids reached a similar maximum gLAI in both experimental years, except for the GNT9 which showed a lower gLAI in I0 in 2019 as compared with the 2020 growing season. In both years, giant reed and African fodder cane maintained a fraction of the green leaf area during winter, while *Miscanthus* hybrids reached leaf senescence in autumn (early November in M × G and late November in GNT9 and GNT10), and their gLAI approached zero during winter.

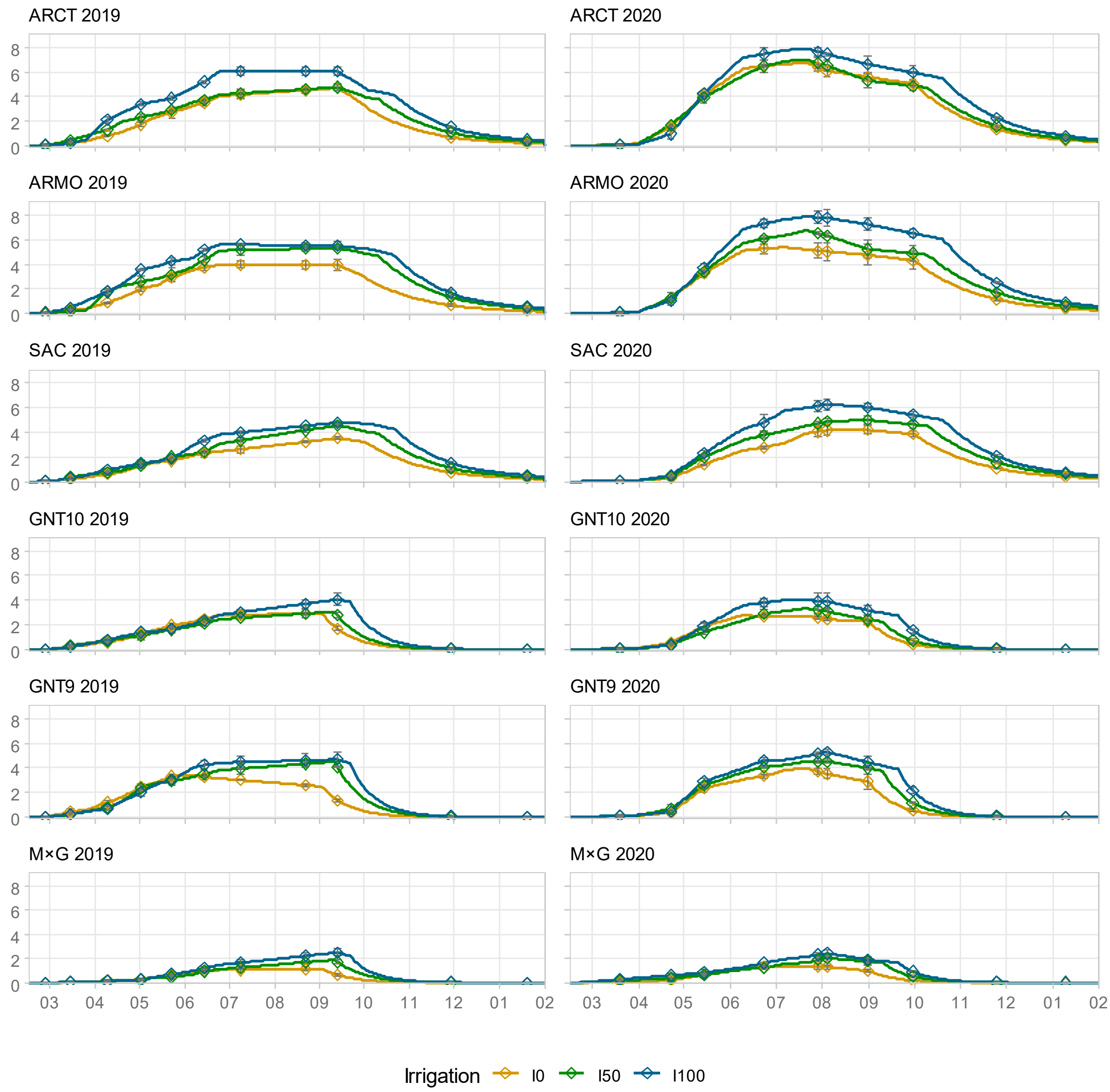


**Figure 1.** Green leaf area index (gLAI, $m^2\ m^{-2}$) trend over two growing seasons (February 2019 to February 2021) for 3 water restoration levels (I0, I50, I100) and for the 6 genotypes exanimated in this study—ARCT: *A. donax* ecotype Catania, ARMO: *A. donax* ecotype Morocco, SAC: *S. spontaneum*, GNT10: *Miscanthus* hybrid 10, GNT9: *Miscanthus* hybrid 9, M × G: *Miscanthus* × *giganteus*. The month is reported as a number (1–12) on the abscissa.

### 3.2. Leaf Area Duration (LAD)

LAD followed the same pattern as LAI, and it was the longest for both giant reed ecotypes in both years. In the 2020, LAD was between 15 and 18% higher than those in 2019 for giant reed and African fodder cane, respectively, while the *Miscanthus* hybrids M × G and GNT10 had a similar LAD in the two years. *Miscanthus* M × G showed the lowest LAD overall, while GNT9 had the highest value among the *Miscanthus* hybrids (Figure 2). The full irrigation regime (I100) significantly improved the LAD, and almost proportionally extended the LAD in all perennial grasses. Mid-level irrigation (I50) was similar to rainfed conditions (I0) in ARCT and GNT10 in 2019 and 2020, while I50 and I100 were similar in GNT9 in 2019.

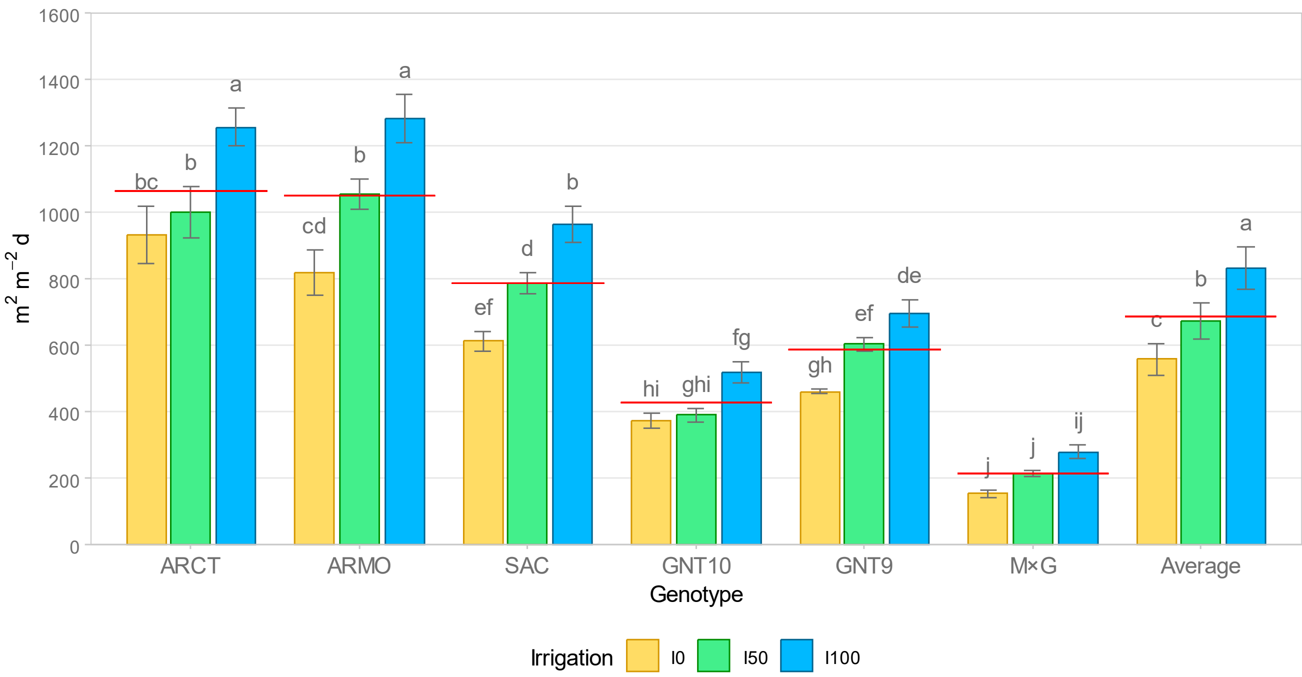


**Figure 2.** Leaf area duration (LAD, $m^2\ m^{-2}$ d) during two growing seasons for 3 water restoration levels (I0, I50, I100) and for the 6 genotypes examined in this study—ARCT: *A. donax* ecotype Catania, ARMO: *A. donax* ecotype Morocco, SAC: *S. spontaneum*, GNT10: *Miscanthus* hybrid 10, GNT9: *Miscanthus* hybrid 9, M × G: *M.* × *giganteus*. The red segments represent the average of 3 water restoration levels for each genotype. LSD = 130 $m^2\ m^{-2}$ d. Letters indicate significant differences between irrigation levels (for average bars) and for the interaction of genotype and irrigation using Fisher's LSD post hoc test.

### *3.3. Fraction of the Intercepted Photosynthetically Active Radiation (fPARi)*

The fPARi showed smaller differences among genotypes and among irrigation levels than LAI and LAD, although the ANOVA was highly significant for the fixed effects (Figure 3). African fodder cane (SAC) showed the highest fPARi in both years, approaching the 100% of the PAR interception at I50 and I100. Lower fPARi values were observed in giant reed ARMO and ARCT, and *Miscanthus* GNT9 and GNT10 (between 80% and 95%), which varied in accordance with the irrigation effects. The *Miscanthus* M × G showed the lowest fPARi, lower than the 80% at the full irrigation (I100) and lower than 55% in the rainfed condition. Giant reed and African fodder cane extended the fPARi during winter and up to the final season harvest (between 10% and 35%, varying with the irrigation input), while leaf senescence already reduced the fPARi in all *Miscanthus* hybrids and all irrigation levels in the mid-autumn.

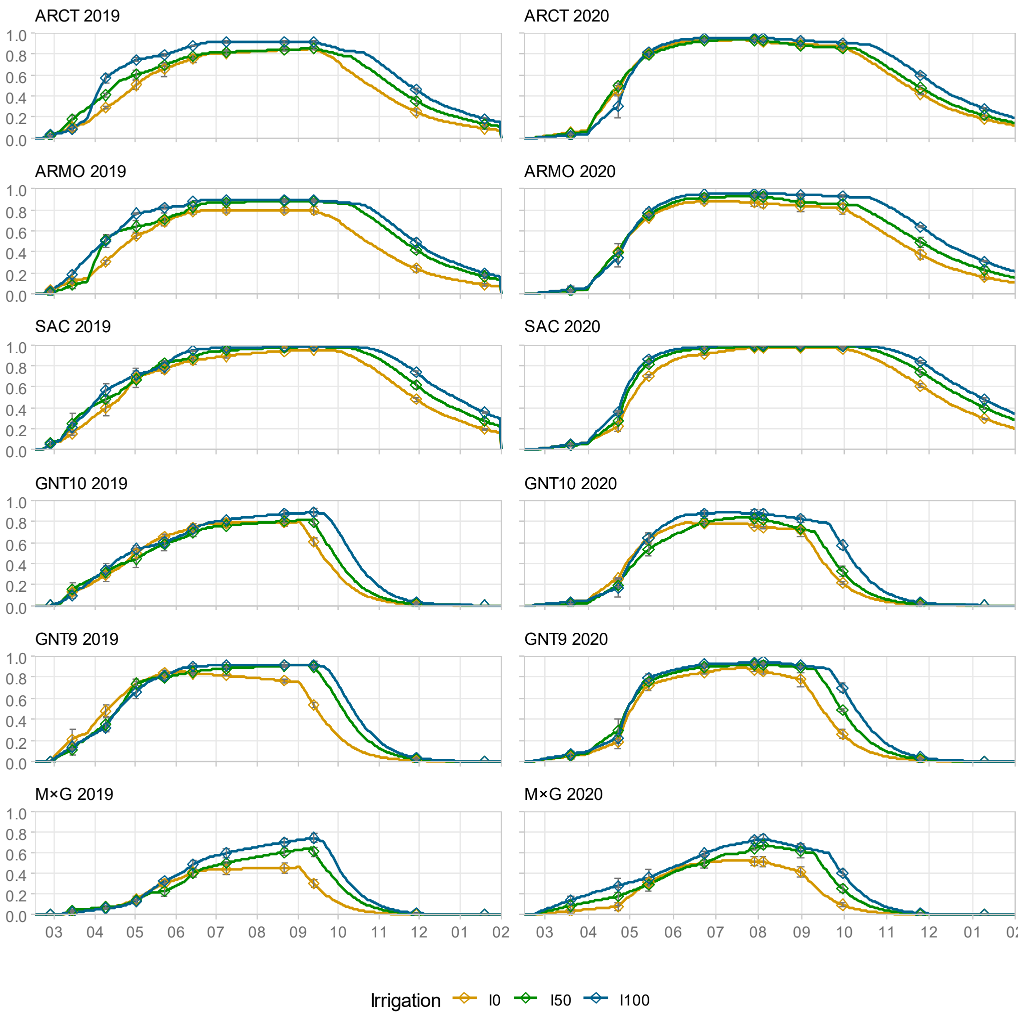


**Figure 3.** The fraction of PAR intercepted (fPARi) trend over the crop cycle (February 2019 to February 2021) for 3 water restoration levels (I0, I50, I100) and for 6 genotypes exanimated in this study—ARCT: *A. donax* ecotype Catania, ARMO: *A. donax* ecotype Morocco, SAC: *S. spontaneum*, GNT10: *Miscanthus* hybrid 10, GNT9: *Miscanthus* hybrid 9, M × G: *Miscanthus* × *giganteus*. The month is reported as a number (1–12) on the abscissa.

### 3.4. Aboveground Dry Biomass Accumulation (ABG)

At harvest, giant reed and African fodder cane were the most productive in terms of AGB yield, both in the first and second year of the trial (Figure 4). ARMO reached the highest AGB yield with the I100 irrigation input: 3510 g $m^{-2}$ and 3680 g $m^{-2}$ in 2019 and 2020, respectively. *Miscanthus* hybrids had lower AGB yields, ranging from 1950 g $m^{-2}$ for GNT10 to 1170 g $m^{-2}$ for M × G in 2020 with the I100 irrigation input. A lower irrigation input led to a significant reduction in the AGB yields in comparison with the full irrigation, an average of 47.8% and 20.3% for the rainfed and the I50 irrigation input, respectively.

African fodder cane reached the highest amount of PAR intercepted in both growing seasons and with all irrigation levels. The values of intercepted PAR were 1743 MJ $m^{-2}$, 1880 MJ $m^{-2}$ and 1961 MJ $m^{-2}$ with the I0, I50 and I100 treatments, respectively. *Miscanthus* hybrids intercepted a lower amount of PAR, ranging from 1560 MJ $m^{-2}$ for GNT9 with I100 in 2019 to 607 MJ $m^{-2}$ for M × G in the rainfed condition in 2019. Decreasing the irrigation input led to a reduction in the iPAR in comparison with the full irrigation, an average of 16.8% and 8.7% for the rainfed and the I50 conditions, respectively.

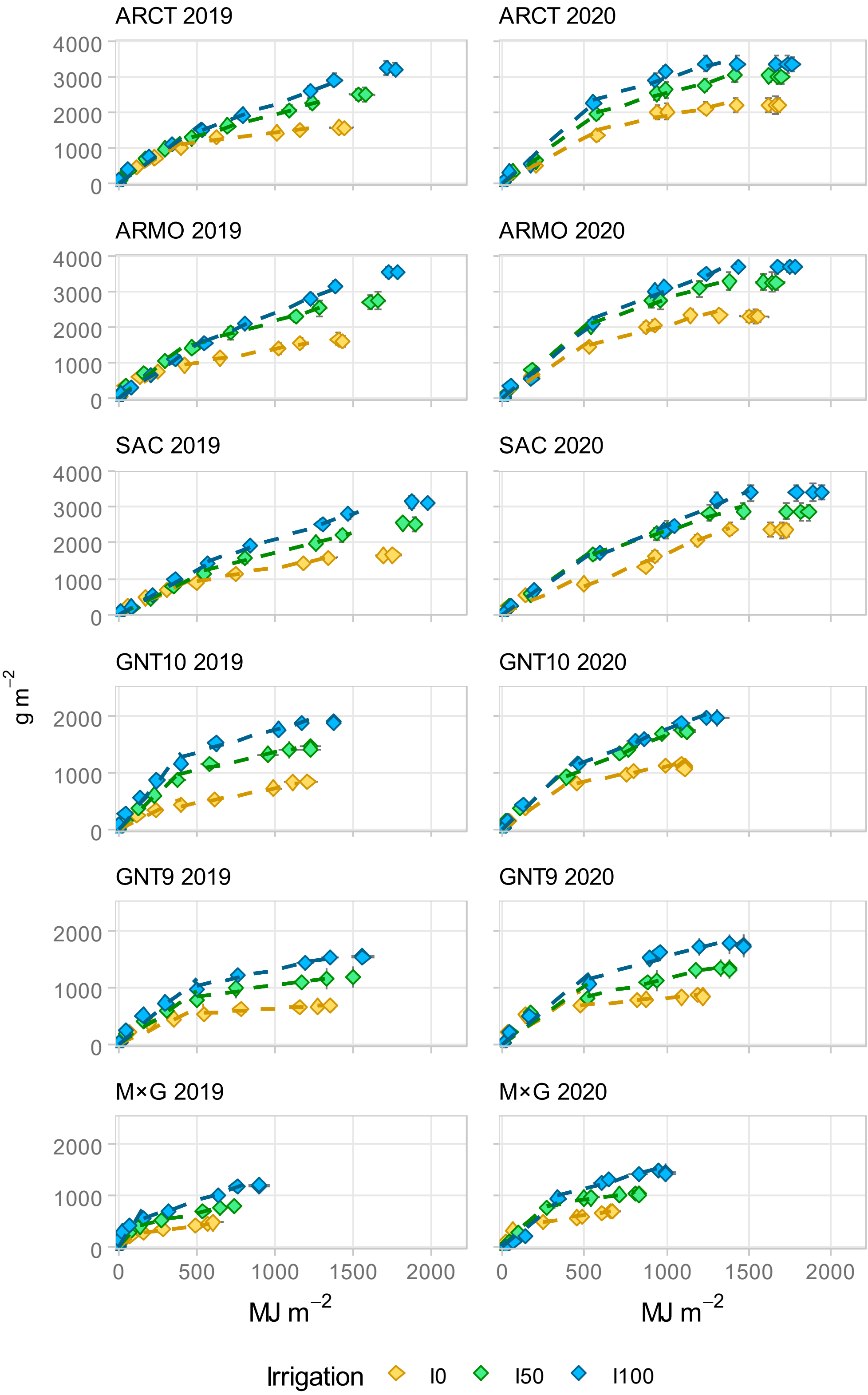


**Figure 4.** Relationships between AGB (g m$^{-2}$) and intercepted PAR (MJ m$^{-2}$) during two growing seasons for 3 water restoration levels (I0, I50, I100) and for the 6 genotypes exanimated in this study—ARCT: *A. donax* ecotype Catania, ARMO: *A. donax* ecotype Morocco, SAC: *S. spontaneum*, GNT10: *Miscanthus* hybrid 10, GNT9: *Miscanthus* hybrid 9, M × G: *Miscanthus* × *giganteus*. The dashed lines represent the fitted linear model for mid-season (from shooting until the end of the stem elongation phase) and at late season (from the end of the stem elongation phase until flowering). Biomass LSD = 204 g m$^{-2}$.

### 3.5. Radiation Use Efficiency (RUE)

Giant reed ecotypes had the highest $RUE_{tot}$: ARMO reached 2.31 g $MJ^{-1}$ and ARCT 2.15 g $MJ^{-1}$, both with the I100 irrigation input (Table 2). The $RUE_{tot}$ of SAC was significantly lower and reached 1.92 g $MJ^{-1}$ under the I100 condition. $RUE_{tot}$ was significantly lower with reduced irrigation input: the reduction was 13% and 37% for the I50 and the rainfed conditions, respectively. $RUE_{I}$ represents the mid-season radiation use efficiency. Giant reed ecotypes had the highest $RUE_{I}$ at all the levels of irrigation input. $RUE_{I}$ was significantly affected by the level of irrigation input: compared with I100, I50 and I0 led to a reduction of 7% and 32%, respectively. Among *Miscanthus* hybrids, M × G had the highest $RUE_{I}$, reaching 3.22 g $MJ^{-1}$ with the I100 irrigation input (Table 3). $RUE_{II}$ represents the late-season radiation use efficiency. Giant reed ARMO still showed the highest $RUE_{II}$ in (Table 4). $RUE_{II}$ was significantly affected by the level of irrigation: compared with I100, $RUE_{II}$ was reduced by 23% and 45% in the I50 and I0 conditions, respectively. In African fodder cane, the reduction in $RUE_{II}$ by reducing the soil water availability was less consistent than with the other genotypes. African fodder cane and the giant reed clone ARMO had the highest $RUE_{II}$ values, with no significant difference between the values.

**Table 2.** Radiation Use Efficiency for the whole growing season ($RUE_{tot}$, g $MJ^{-1}$) for 3 water restoration levels (I0, I50, I100) and for the 6 genotypes exanimated in this study—ARCT: *A. donax* ecotype Catania, ARMO: *A. donax* ecotype Morocco, SAC: *S. spontaneum*, GNT10: *Miscanthus* hybrid 10, GNT9: *Miscanthus* hybrid 9, M × G: *Miscanthus* × *giganteus.* Letters indicate significant differences between irrigation or genotype levels (for average values) and for the interaction of genotype and irrigation using Fisher's LSD post hoc test. LSD = 0.22 g $MJ^{-1}$.

| Irrigation | ARCT | ARMO | GNT10 | GNT9 | M × G | SAC | Average |
|---|---|---|---|---|---|---|---|
| I0 | 1.41 b | 1.53 b | 0.92 b | 0.69 c | 0.98 c | 1.31 b | 1.14 c |
| I50 | 1.92 a | 2.09 a | 1.48 a | 0.98 b | 1.29 b | 1.66 a | 1.57 b |
| I100 | 2.15 a | 2.31 a | 1.61 a | 1.24 a | 1.54 a | 1.92 a | 1.79 a |
| Average | 1.82 b | 1.98 a | 1.33 d | 0.97 e | 1.27 d | 1.63 c | 1.5 |

**Table 3.** Radiation Use Efficiency at mid-season (i.e., from shooting until the end of the stem elongation phase, $RUE_{I}$, g $MJ^{-1}$) for 3 water restoration levels (I0, I50, I100) and for the 6 genotypes exanimated in this study—ARCT: *A. donax* ecotype Catania, ARMO: *A. donax* ecotype Morocco, SAC: *S. spontaneum*, GNT10: *Miscanthus* hybrid 10, GNT9: *Miscanthus* hybrid 9, M × G: *Miscanthus* × *giganteus*. Letters indicate significant differences between irrigation or genotype levels (for average values) and for the interaction of genotype and irrigation using Fisher's LSD post hoc test. LSD = 0.45 g $MJ^{-1}$.

| Irrigation | ARCT | ARMO | GNT10 | GNT9 | M × G | SAC | Average |
|---|---|---|---|---|---|---|---|
| I0 | 2.55 b | 2.76 b | 1.55 b | 1.42 c | 2.07 b | 1.91 b | 2.04 c |
| I50 | 3.18 a | 3.61 a | 2.43 a | 1.74 b | 3.02 a | 2.6 a | 2.76 b |
| I100 | 3.53 a | 3.37 a | 2.87 a | 2.15 a | 3.22 a | 2.77 a | 2.98 a |
| Average | 3.09 a | 3.24 a | 2.28 c | 1.77 d | 2.77 b | 2.42 c | 2.59 |

**Table 4.** Radiation Use Efficiency at late season (i.e., from the end of the stem elongation phase until flowering, $RUE_{II}$, g $MJ^{-1}$) for 3 water restoration levels (I0, I50, I100) and for the 6 genotypes exanimated in this study—ARCT: *A. donax* ecotype Catania, ARMO: *A. donax* ecotype Morocco, SAC: *S. spontaneum*, GNT10: *Miscanthus* × *giganteus* hybrid 10, GNT9: *Miscanthus* × *giganteus* hybrid 9, M × G: *Miscanthus* × *giganteus*. Letters indicate significant differences between irrigation or genotype levels (for average values) and for the interaction of genotype and irrigation using Fisher's LSD post hoc test. LSD = 0.3 g $MJ^{-1}$.

| Irrigation | ARCT | ARMO | GNT10 | GNT9 | M × G | SAC | Average |
|---|---|---|---|---|---|---|---|
| I0 | 0.92 c | 1.12 b | 0.56 b | 0.2 b | 0.46 b | 1.3 a | 0.76 c |
| I50 | 1.27 b | 1.47 b | 0.99 a | 0.58 a | 0.63 b | 1.39 a | 1.05 b |
| I100 | 1.65 a | 1.96 a | 1.01 a | 0.81 a | 0.99 a | 1.75 a | 1.36 a |
| Average | 1.28 b | 1.51 a | 0.85 c | 0.53 d | 0.69 cd | 1.48 a | 1.06 |

## 4. Discussion

Previous field trials demonstrated the ability of giant reed and African fodder cane to produce high amounts of biomass in the Mediterranean environment both in rainfed and irrigated conditions [3,5,7,24]. High yields were attributed to long green LAD, high $CO_2$ assimilation rate and high RUE and WUE. Another biomass-related trait that has been reported for giant reed is its low light extinction coefficient which has been related to an optimal canopy architecture that allows for a good light distribution among the layers of leaves [3].

Soil water availability significantly affected several traits linked to productivity (LAD, iPAR, AGB yield and RUE during the whole growing season and at mid-season and late season), confirming the results previously observed in similar environmental conditions affected by summer drought [3,5,21].

The present study further highlights the high biomass productivity and the biomass-related traits of giant reed, African fodder cane and *Miscanthus* hybrids. LAD and iPAR during the growing season, as well as RUE, were similar to other findings reported in the literature. In particular, African fodder cane showed a similar or higher maximum LAI compared to those reported by Scordia et al. [5] in rainfed and irrigated conditions (50% and 100% of ETm restoration during summer months), while the RUE values over the two growing seasons were higher, due to the higher biomass production and the lower iPAR. These RUE differences can be ascribed to the plantation age: second and third in the present study, ninth in Scordia et al. [5].

On the other hand, the amount of PAR intercepted by giant reed was lower compared to the study of Cosentino et al. [3], mainly due to the lower extinction coefficient and the lower plant density at the establishment: 1 plant $m^{-2}$ in the present study vs. 2.5 plant $m^{-2}$.

Giant reed reached lower values of maximum LAI than the values reported by Cosentino et al. [2] in the rainfed condition and 120 kg $ha^{-1}$ of nitrogen fertilization, and by Cosentino et al. [3] at three irrigation levels (ranging from rainfed to 100% of ETm restoration) and three nitrogen fertilization levels (from 0 to 120 kg $ha^{-1}$). Cosentino et al. [2] reported a higher RUE for giant reed due to the higher biomass yield and lower iPAR (50% of the iPAR reported in the present study). Cosentino et al. [3] found similar RUE values for rainfed giant reed in comparison to the present study. Irrigated giant reed (50% and 100% of ETm restoration) reached a lower RUE compared to the present study, due to a similar AGB production but a higher iPAR. Nitrogen fertilization showed a slight increase on AGB and on iPAR [3], leading to a slightly higher RUE, which was very similar to the RUE found for ARCT, but lower than that of ARMO.

*Miscanthus* showed a slower leaf area development and reached lower values of maximum LAI and iPAR both in rainfed and irrigated conditions compared with the results obtained by studies conducted in a similar environment [21] and environments at higher latitudes [4,25]. Low iPAR was the consequence of the modest LAI development and the early leaf senescence of the *Miscanthus* hybrids, particularly of M × G.

*Miscanthus* (M × G) showed similar RUE values of those observed by Cosentino et al. [21] in the same environment, who registered a higher AGB yield and higher iPAR at the same levels of nitrogen and water input. At higher levels of nitrogen supply, Cosentino et al. [21] observed an increase in RUE. Davey et al. [4] and Kiniry et al. [13] studied the productivity of *Miscanthus* at higher latitudes in unfertilized plots and non-limiting water availability provided by natural rainfall. They reported a higher RUE related to high AGB yields despite the low iPAR. These results are caused by the longer vegetative phase experienced by *Miscanthus* at high latitudes, where the onset of senescence or the flowering is not triggered by day length [31].

Kiniry et al. [13] studied the productivity of *Miscanthus* at two latitudes (39° N and 31° N). They found that in similar conditions of non-limiting water availability, both AGB and RUE were higher at the higher latitude. Davey et al. [4] and Kiniry et al. [13] reported similar AGB yield and RUE values with low nitrogen and irrigation input, while both AGB yield and RUE with high irrigation and nitrogen input were higher than those observed for *Miscanthus* in the present study.

The *Miscanthus* hybrids GNT9 and GNT10 achieved higher AGB yield and IPAR values than M × G although the RUE was lower in GNT9 likely due to the higher amount of intercepted PAR when compared to the other *Miscanthus* hybrids. Despite the lowest RUE in the GNT9, the higher LAD has been found to be one of the traits that lead to the suitability of these new hybrids for the Mediterranean climate when compared to the commercial *Miscanthus* × *giganteus*. In addition, Scordia et al. [27] found out that high water use efficiency, another trait that supports these new *Miscanthus* hybrids in coping with the Mediterranean climate, was a determinant in its ability to outperform the commercial *Miscanthus* × *giganteus*.

$RUE_I$, representing the mid-season radiation use efficiency, included the phase during which the nutrient release from the rhizome fosters AGB growth. The *Miscanthus* hybrid M × G had the highest $RUE_I$ in 2019 due to the slow leaf area development and the fast biomass growth induced by the nutrient release from the rhizome.

$RUE_{II}$, representing the late-season radiation use efficiency, included the phenological phases after the conclusion of the nutrient release from the rhizome and therefore the AGB growth is only sustained by net photosynthesis during this stage. This reason, together with the less suitable atmospheric conditions of the late season (higher maximum temperature and lower relative humidity), led to lower $RUE_{II}$ values than $RUE_I$, particularly in the M × G and *Miscanthus* hybrids than the more adapted African fodder cane and giant reed.

## 5. Conclusions

The present study demonstrated the good productivity and desirable traits of the naturalized BPGs and their suitability for the Mediterranean environments affected by low soil moisture availability during summer months. The most important traits for biomass productions are LAD and RUE, which are both positively affected by supplemental water inputs. African fodder cane and giant reed showed both high LAD and RUE, which determined the high biomass yield.

RUE was particularly high during the mid-season (i.e., from shooting until the end of the stem elongation phase) in all perennial grasses. In the same period, RUE was less affected by soil water availability. On the other hand, the effect of irrigation on RUE and the differences in RUE among the genotypes were larger during the late season.

Giant reed and African fodder cane, endemic species in the area of the study, performed better in terms of LAD and RUE than both the commercial *Miscanthus* × *giganteus* and the improved *Miscanthus* hybrids GNT9 and GNT10. However, GNT9 and GNT10 achieved a higher AGB yield than *Miscanthus* × *giganteus* due to the higher LAD, although RUE was similar (for GNT10) or lower (for GNT9).

Irrigation input has proved to be necessary to achieve the potential yield of all perennial grasses tested here by improving both LAD and RUE as soil water availability increased. The genotypes responded similarly to sub-optimal soil water availability by reducing the leaf area expansion, which reduced the LAD and the intercepted iPAR, and depressed the RUE. However, adequate biomass yield can be achieved by monitoring soil water availability, thus reducing the crop water and energy footprint and increasing the sustainability of these BPGs selected for the Mediterranean climate.

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