

SORGHUM AND MAIZE BIOMASS, PROTEIN AND BIOETHANOL YIELDS IN A SEMI ARID ENVIRONMENT OF CENTRAL GREECE

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ABSTRACT: We investigated biomass and protein productivity of Sudan sorghum and maize crops in relation to nitrogen fertilization (6, 15 and 35 g m⁻²) in central Greece. Sudan sorghum was cultivated under two management techniques: (a) as a row crop similar to maize cultivation (row-to-row distance: 75 cm; observed shoot density: 27 per m²) with a single harvest per year; and (b) as a very dense crop (row-to-row distance: 10 cm; observed shoot density: 155 per m²) with a three harvests per year, each when plants reached 1 m in height. Results indicated that Sudan sorghum grown at high plant density and harvested three times per season gave 67, 23 and 40% higher fresh, dry, and protein biomass yields, respectively, than maize. This superiority was explained by the fast increase in leaf area early in the season and by the high leaf/stem ratio at harvest. However, this cultivation strategy only yielded well at large amounts of nitrogen fertilizer ($P < 0.05$ among N-levels). Our results are promising and provide an alternative cultivation opportunity for Greek farmers, but future cost/benefit studies are needed to assess whether this cultivation strategy is also profitable.

Keywords: Maize, sorghum, biomass production, protein, forage feed

1 INTRODUCTION

In the EU maize occupies large cropping areas while sorghum is marginally cultivated. In view of increasing biofuel production, sweet and fibre sorghum received particularly attention over the last decade. However, there are many constraints to growing sorghum for bioenergy production, which seem far from being solved [1, 2].

Given its high potential in terms of biomass production [3, 4], sweet and fibre sorghum was tested as alternative to maize for forage production. They found similar protein yields for maize and sorghum, but lower production costs for sorghum. Cultivating a crop for forage production requires minimum industrial support, while mechanical equipment for such purposes is largely available and farmers are well experienced. Thus sorghum can be a good alternative to maize cultivation for cheap production of forage feed by the Greek farmers.

This paper builds on previous work by Danalatos et al. [3] and Archontoulis et al. [4], but instead of using a sweet or fibre sorghum cultivar, this paper tests the productivity of a Sudan sorghum grass in a comparative way with a maize hybrid in central Greece. Moreover Sudan sorghum productivity was investigated under two different management techniques: (a) as a row crop (like for maize) with a single harvest per season; and (b) as a very dense crop (like for alfalfa) with three cuttings per season. The effect of nitrogen fertilization (at three levels) was also investigated.

We hypothesized that the dense sorghum cultivation would produce higher biomass yields compared to other treatments (cf. maize and sorghum crops grown at 75 cm row distance), because very dense planting promotes early canopy development and light interception, increases crop growth rates and therefore results in higher yields compared to less dense planting.

2 MATERIALS AND METHODS

2.1 Experimental site and field management

A field experiment was carried out in central Greece (Velesino, 39°23' N, 22°44' E, alt. 87 m asl) in 2010. The soil was a silt-loam (28% sand, 68% silt, 4% clay) with no groundwater table (dry soil), which is classified as Typic Xerofluvent (USDA, [5]). A 3×3 split-plot experimental design was used in six blocks (54 units). Main factor comprised the crop type: M = maize hybrid Ambizioso, S_1 = sorghum planted at 75 cm row-to-row distance, and S_2 = sorghum planted at 10 cm row-to-row distance; hybrid Honey Graze BMR (Table I). Sub-factor comprised the N-fertilization application ($N_1 = 6$, $N_2 = 15$ and $N_3 = 35$ g N m⁻²).

The size of each plot was 42 m² (3.8 m × 11 m). The S_2 plants were cut when plants reached 1 m in height and then biomass was removed from the field. The following day the plots were irrigated and sorghum plants re-grew very fast. In total three cuttings were carried out during 2010. In between the main harvests, destructive samplings were implemented to monitor the crop growth. Sowing took place on April 24th 2010 for all crops. The dates and the amounts of nutrients applied are shown in Table II. Irrigation was applied (every week; Fig. 1) using drip irrigation to ensure high accuracy.

Weather data such as temperature, rainfall, radiation, relative humidity and wind speed were recorded by an automatic meteorological station which was installed at the borders of the experimental site.

Table I:
Cropping treatments and plant densities

Crop	Plant arrangement (cm × cm)	Observed shoots m ⁻²
M : Maize	75 × 16.3	8 ± 0.3
S_1 : Sorghum	75 × 16.3	27 ± 0.9
S_2 : Sorghum	10 × 5.0	155 ± 65.9

Table II:
Time and amount of nutrient application

Element	Type	Date	Application rate (g m ⁻²)		
			N ₁	N ₂	N ₃
P	0-46-0	16/4	6	6	6
K	0-0-50	16/4	4.8	4.8	4.8
N	34-0-0	19/5	6	6	6
N	46-0-0	9/6	0	4.5	14.5
N	46-0-0	27/7	0	4.5	14.5
N-total			6	15	35

2.2 Measurements

Plant height, leaf area, fresh and dry biomass productivity per plant component (stem, leaves and storage organs) were measured during growth. Nine destructive harvests were conducted every 2-3 weeks. In each manual harvest (sampling area of 1 m² per plot) the plant sample was weighed fresh in the field. Then, the average plant height and the total number of tillers present in a sample were assessed as well. Subsequently, the samples (or subsamples at advanced growth stages) were divided into different plant components and oven-dried at 70°C until constant weight and weighed again to determine dry weights. Before drying, leaf area was measured using an area meter (LI-COR, LI-3000A). After drying, all samples were analysed for their total nitrogen concentration on dry basis (g N per 100 g dry matter) using the Kjeldahl method.

2.3 Calculations

Specific leaf area (SLA, m² kg⁻¹) was calculated as green leaf area over leaf dry weight. Leaf area index (LAI, m² green leaf m⁻² ground) was derived directly from leaf area measurements (note 1 m² harvest area) during initial growth stages. LAI was calculated indirectly during advanced growth stages as the product of green leaf dry weight (kg m⁻² ground) times the specific leaf area (SLA in m² leaf kg⁻¹).

The biomass increase over time was analyzed using the beta growth function [6]:

$$W = W_{max} \left(1 + \frac{t_e - t}{t_e - t_m} \right) \left(\frac{t}{t_e} \right)^{\frac{t_e}{t_e - t_m}} \quad [1]$$

with $0 \leq t_m \leq t_e$

where W_{max} is the maximum biomass weight (W), t is time in days after emergence, t_m is the day when $W = 0.5 W_{max}$, and t_e is the day when $W = W_{max}$. Eq. (1) obeys the constraints that $W=0$ at growth initiation (i.e. $t=0$), and $W=W_{max}$ when growth has ceased (i.e. $t = t_e$). From Eq. (1) the maximum crop growth rate (C_m , kg ha⁻¹ d⁻¹) was calculated as [6]:

$$C_m = \frac{2t_e - t_m}{t_e(t_e - t_m)} \left(\frac{t_m}{t_e} \right)^{\frac{t_m}{t_e - t_m}} W_{max} \quad [2]$$

Crude protein concentration for each plant component was calculated as $6.25 \times$ nitrogen concentration. Nitrogen and protein contents per ground area were calculated by multiplying concentration with the corresponding dry weights for each plant component (stem, leaves, grains and cobs). Total sugar content (only for sorghum) was approximated based on its correlation to Brix degree values using the following equation [7]:

$$\text{Total sugar content (\%)} = [0.8111 \times \text{Brix (\%)}] - 0.3728$$

Then, the theoretical ethanol yield for all crops was calculated according to Vasilakoglou et al. [8]:

$$\text{Total ethanol yield (L ha}^{-1}\text{)} = \text{total sugar content (\%)} \times \text{fresh biomass (Mg ha}^{-1}\text{)} \times 6.5 \text{ (conversion factor of ethanol from sugar)} \times 0.85 \text{ (process efficiency of ethanol from sugar)} \times (1.00/0.79) \text{ (specific gravity of ethanol; g mL}^{-1}\text{)}.$$

2.4 Statistics

Measured and calculated data for M and S_1 crops were subjected to analysis of variance following a 2×3 split-plot design in Genstat (13th version). Significant differences were assessed at $P=0.05$ and the $LSD_{0.05}$ criterion was used to separate differences among mean values. Measured data for the S_2 crop were analysed separately following a one way ANOVA because of the different management techniques used during its cultivation.

The final (M and S_1) or cumulative (S_2) biomass yields were analysed together in Genstat following the full experimental design (3×3). The parameters of the non-linear equation used to describe growth over time were derived from iterative non-linear least-square regression using the PROC_NLIN procedure in SAS software.

3 RESULTS

3.1 Whether conditions

The climate of the area is typical Mediterranean, with hot, dry summers and cool, humid winters. Figure 1 illustrates air temperatures, precipitation, and irrigation applied during the experimental period (~650 and 750 mm for maize and sorghum crops, respectively).

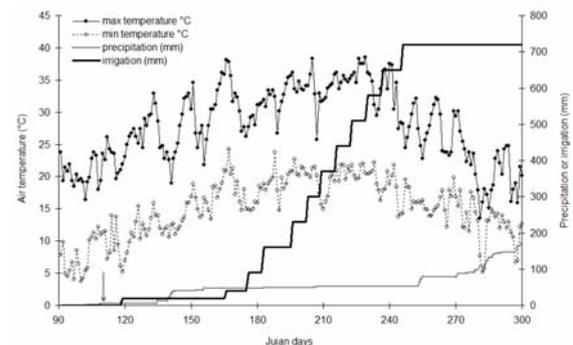


Fig. 1: Air temperature (maximum and minimum) and cumulative precipitation and irrigation water applied at the experimental area of Velestino (central Greece) in 2010. Vertical arrow shows the date of sowing the crops.

3.2 Growth characteristics and biomass production

No significant interactions ($P>0.05$) between row crop type (M and S_I) and N-fertilization (N_1 , N_2 , N_3) were found on plant height, SLA, LAI, leaf/stem ratio, total fresh and dry biomass.

3.3 Plant height

M and S_I crops followed similar patterns of increase in plant height. Maize reached maximum plant height of 227 cm during mid-August, while sorghum reached a maximum plant height of 235 cm during mid-September. No effect of N fertilization was found on plant height of M and S_I crops ($P=0.4$). A significant effect of nitrogen fertilization was found in S_2 crops, during the 2nd and the 3rd growth cycle ($P=0.04$; data not shown).

3.4 Specific leaf area (SLA)

Nitrogen application did not affect SLA in any of the crops ($P=0.5$; Fig. 2). We found higher SLA values at S_2 crops (35 to 20 m² kg⁻¹) than for M and S_I crops (25 to 15 m² kg⁻¹; Fig. 2), because of the higher competition for light between leaves (see plant density in Table II).

3.5 Leaf area index (LAI)

Maize showed a faster development of the LAI during initial stages than sorghum, reaching a maximum value of 3.5 m² m⁻² in the middle of July. On the other hand, S_I crops maintained LAI values of 3.9 m² m⁻² for a longer period than maize (Fig. 2). No effect of N fertilization on M and S_I crops was found ($P=0.7$). In contrast, N fertilization significantly affected LAI during the 2nd growing cycle of the S_2 crops ($P=0.017$; Fig. 2f). In that treatment, (note 155 shoots m⁻²) LAI reached maximum values of 6–8 m² m⁻² within a very short time period. This is attributed most to the high plant density, the ample irrigation water applied and the favourable climatic conditions (temperature; Fig. 1).

3.6 Leaf/stem ratio

Leaf/stem ratio decreased over time in all crops (data not shown), with some difference observed between M and S_I crops. The effect of N-fertilization was not significant ($P=0.29$). It is important to note that in S_2 crops, leaves comprised 50% of the harvestable biomass, while in M and S_I crops leaves comprised less than 15% of the harvestable biomass.

3.7 Nitrogen concentration

Leaf and stem nitrogen concentration (N%) reached maximum values during the first days of growth (4.0, 3.3, 4.3 and 3.5% for M leaves, M stems, S_I leaves and S_2 stems, respectively) and then decreased over time. Maize kernels and cobs averaged 2.0 and 0.6%, respectively. N_3 -plants showed higher N% values compared to N_1 -plants, but this difference proved significantly only a few times (data not shown).

3.8 Fresh biomass weight

Fresh biomass comprises the commercial product for forage purposes, thus it is important to present values. Biomass increase over time for the M and S_I crops was described by Eq. (1); model parameters are presented in Table III. The maximum fresh biomass yields were 4922 and 4826 g m⁻² and were obtained on 121 and 133 DAE for M and S_I crops, respectively (average values across N-levels; Table III; Figs. 3a and b). The effect of N-fertilization on fresh biomass productivity was not significant ($P=0.27$; Figs. 3a, b). S_2 crops fresh biomass productivity increased much faster compared to row crops (Fig. 3). The effect of N fertilization on fresh biomass yield was significant for the 2nd and 3rd growth cycle ($P=0.031$; Fig. 3c), but not during the 1st cycle ($P=0.126$). This management technique (S_2) resulted in much higher cumulative fresh biomass yields (5824 + 6859 + 2368 = 15052 g m⁻²) compared to S_I crop (fresh yield of 4886 g m⁻²; Figs. 3a, b and c).

Table III:

Parameters of Eq. (1) used to describe fresh and dry matter evolution (Fig. 3). CGR is the maximum absolute growth rate (g m⁻² d⁻¹) calculated from Eq. (2).

	Maize (M)			Sorghum (S_I)		
	N_1	N_2	N_3	N_1	N_2	N_3
<i>Total fresh biomass</i>						
W_{max}	4508	5042	5219	4605	4771	5286
t_e	120	122	122	134	133	136
t_m	74	74	76	91	89	92
r^2	0.897	0.887	0.882	0.956	0.947	0.962
P	***	***	***	***	***	***
<i>Total dry biomass</i>						
W_{max}	1795	2015	2061	1685	1575	1760
t_e	136	139	140	137	138	139
t_m	97	99	99	104	99	102
r^2	0.986	0.986	0.982	0.994	0.960	0.990
P	***	***	***	***	***	***
CGR	25.6	27.8	28.3	26.2	22.2	25.5

3.9 Dry biomass weight

Figs. 3 d, e and f illustrate total dry biomass increase over time for M and S_I crops. Table III shows the parameters of Eq. (1) that described biomass increase. From 167 Julian days onwards, M and S_I crops did differ significantly in terms of biomass accumulation. Maize performed 14.4% higher dry biomass yields compared to S_I crop (1956 vs. 1672 g m⁻²) and this is also reflected by the higher growth rates (viz. 27.2 vs. 24.6 g m⁻² d⁻¹; Table III). Nitrogen application rates did not affect the dry weight in M and S_I crops significantly ($P=0.745$; Fig. 3d, e), although higher values were found for the N_3 compared to N_1 crops (Figs. 3d, e and Table III). In contrast, N-fertilization affected dry weight of the S_2 crops ($P=0.03$; Fig. 3f). S_2 crops showed growth rates from 23.3 to 34.4 g m⁻² d⁻¹ reaching total dry biomass weight of 1018, 1232 and 1424 g m⁻² for N_1 , N_2 and N_3 , respectively (2nd growth cycle).

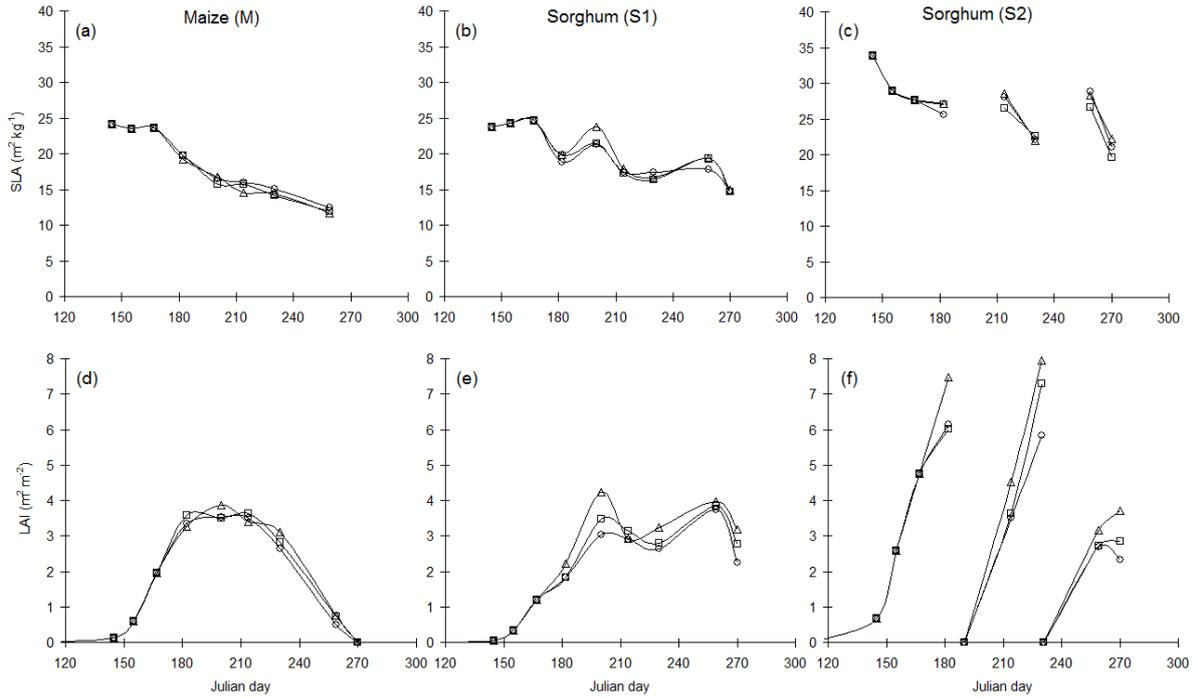


Fig. 2: Time course of specific leaf area (SLA, panels a, b and c) and leaf area index (LAI, panels d, e and f) in relation to nitrogen fertilization (N_1 : \circ ; N_2 : \square and N_3 : Δ) for all crops (M: left; S_1 : middle; and S_2 : right panels).

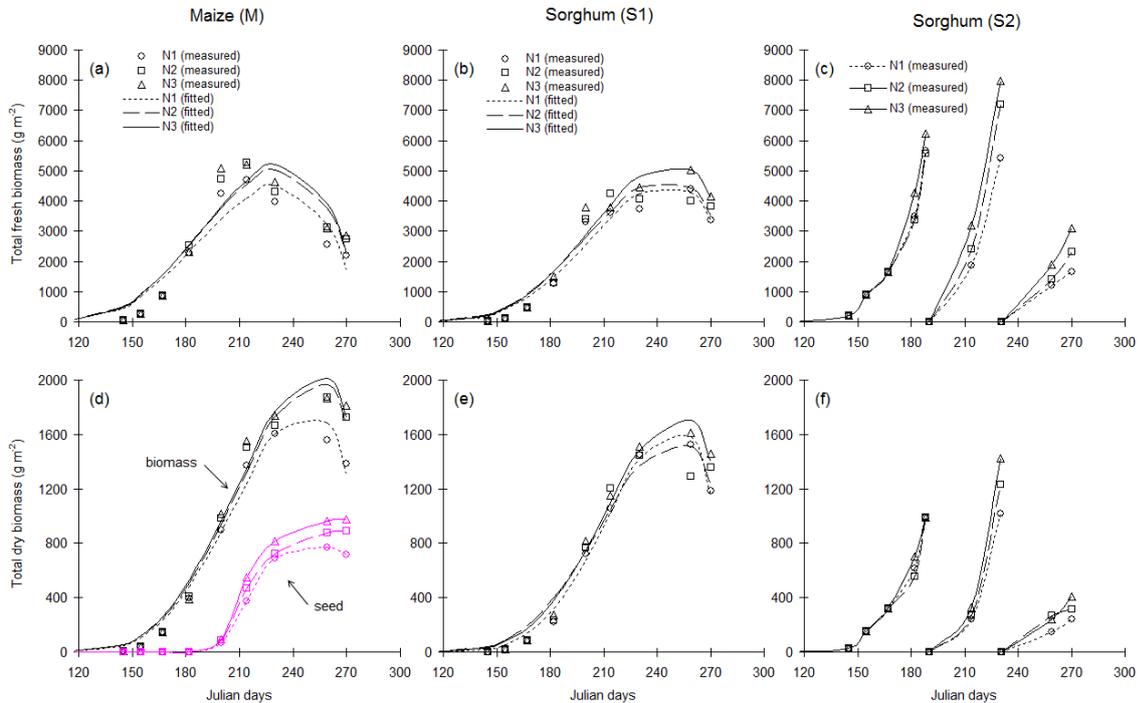


Fig. 3: Time course of total fresh (panels a, b and c) and total dry biomass (panels d, e and f) in relation to nitrogen fertilization (N_1 : \circ ; N_2 : \square and N_3 : Δ) for all crops (M: left; S_1 : middle; and S_2 : right panels). M and S_1 time course was described by Eq. (1); model parameters are given in Table III. Sorghum S_2 time course (panel c) maize kernel dry matter accumulation in relation to nitrogen (panel d; pink colour and symbols) were not described by Eq. (1).

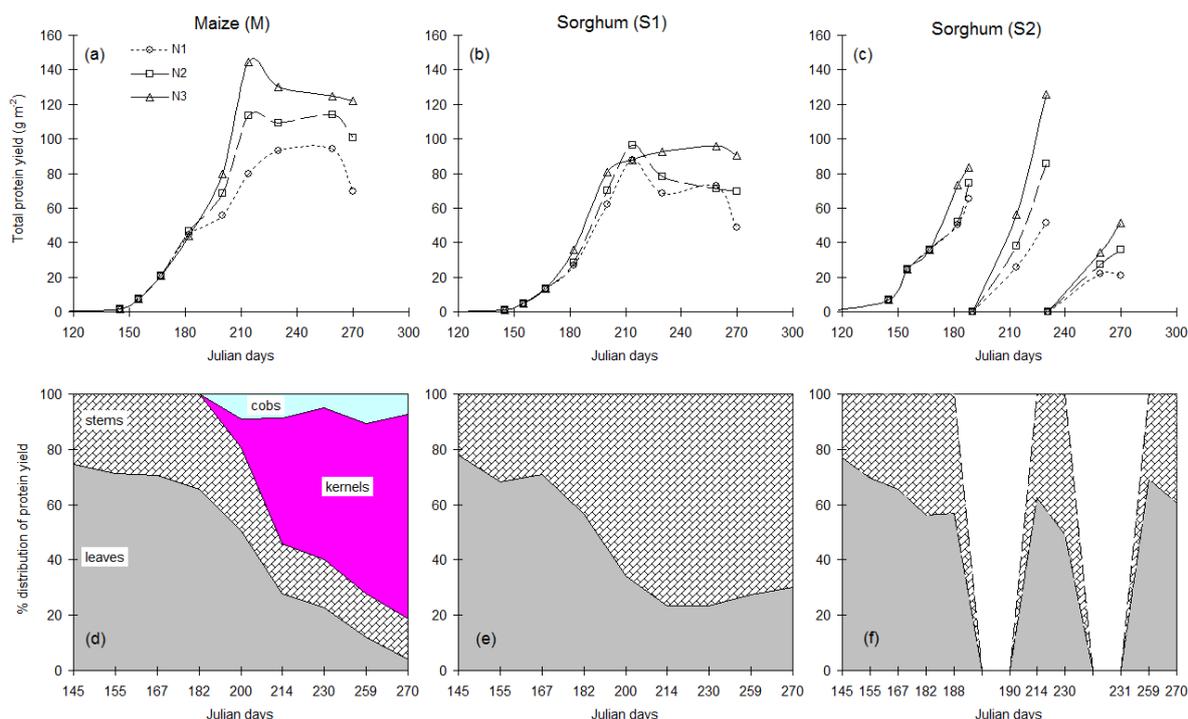


Fig. 4. Panels a, b and c indicate the accumulation of proteins over time for all crops in relation to nitrogen application. Panels d, e and f indicate the distribution of protein yield to different plant components (average values across three nitrogen levels).

3.10 Protein yield

Fig. 4 illustrates total crude protein yields as well as the relative contribution of each plant component to total protein yield. This plot shows the proper harvesting time for forage purposes: first week of August for both row-crops. At that time total protein yield was 112 and 90 g m⁻² for the *M* and *S₁* crops, respectively (average values across N-levels; Figs. 4a, b). Protein yield was higher in plants that had received higher nitrogen fertilization rates (Figs. 4a, b). The higher protein yield of *M* compared to the *S₁* crop was due to the contribution of kernels to total protein yield. The *S₂* crops reached much higher protein yields compared to row-crops (almost double; see Fig. 5). This was because of the higher contribution of the nitrogen-rich leaves to total biomass (Fig. 4).

3.11 Ethanol yield

Theoretical ethanol yields for sorghum crops (*S₁* and *S₂*) were calculated based on literature coefficients (see Materials and Methods). Results indicated that the *S₁* crops reached maximum ethanol yield of 2838 l ha⁻¹; a value that was more than double compared to *S₂* treatment (1300 l ha⁻¹; data not shown). This was due to higher stem biomass yield (data not shown).

4 DISCUSSION

4.1 Crop morphology

Observed plant height for the studied sorghum hybrid Honey graze (*S₁*) was considerably lower compared to studies [3, 4], for the hybrids Dale and H133

(viz. 350–380 cm; hybrid: H133). Apart from the paramount effect of the hybrid used, it should be noticed that abovementioned studies were carried out on aquatic soils, while our study was carried out on a dry soil. Josef et al. [9] studied FS-5 hybrid on a dry soil and also found higher values for sorghum height (viz. 323 cm) than we did for our hybrid.

Leaf area index is of great importance in many eco-physiological and modelling studies. While comparing LAI values of *S₁* (27 shoots m⁻²) and *S₂* (155 shoots m⁻²) crops, one can easily realize the great importance of plant density for obtaining high values of LAI. Actually this is a main reason for the higher yields observed for the dense planting compared to the row planting (Fig. 5). Moreover, we observed a surprisingly rapid development of LAI for the *S₂* crop during the second growth cycle under the highest N-application rate (Fig. 2; 7.96 m² m⁻²; 230 Julian days). Such rapid development of LAI within a short time period is difficult to explain. However, this sorghum crop grew at very high plant densities, with adequate nitrogen fertilization and adequate irrigation (Fig. 1).

The higher SLA values found for the *S₂* crops compared to *M* and *S₁* crops were due to competition among individual plants for light, with the *S₂* leaves expanding in size in an effort to capture more light [10, 11]. Present findings agree well with previous studies on plant density effects on SLA. For instance, Lafrage and Hammer [12] compared different sorghum hybrids under low and high plant densities and found different SLA values (range: 33 to 38 m² kg⁻¹, respectively). However, across different experimental sites it is very difficult to compare the magnitude of the SLA because factors such as radiation and temperature that change with geographical position are also involved [13]. For

instance, in Switzerland, Hund et al. [14] reported for maize SLA values of $51.2 \text{ m}^2 \text{ kg}^{-1}$. This value is almost double compared to our findings for maize (Fig. 2d). Leaf/stem ratio is an index that characterizes forage quality since leaves and stems have different protein concentrations. The decline of the leaf/stem ratio with increasing crop maturity is very common [15]. Bahrani and Deghani [16] reported higher leaf/stem ratio for the dense planting compared to the wide planting, in line with our results.

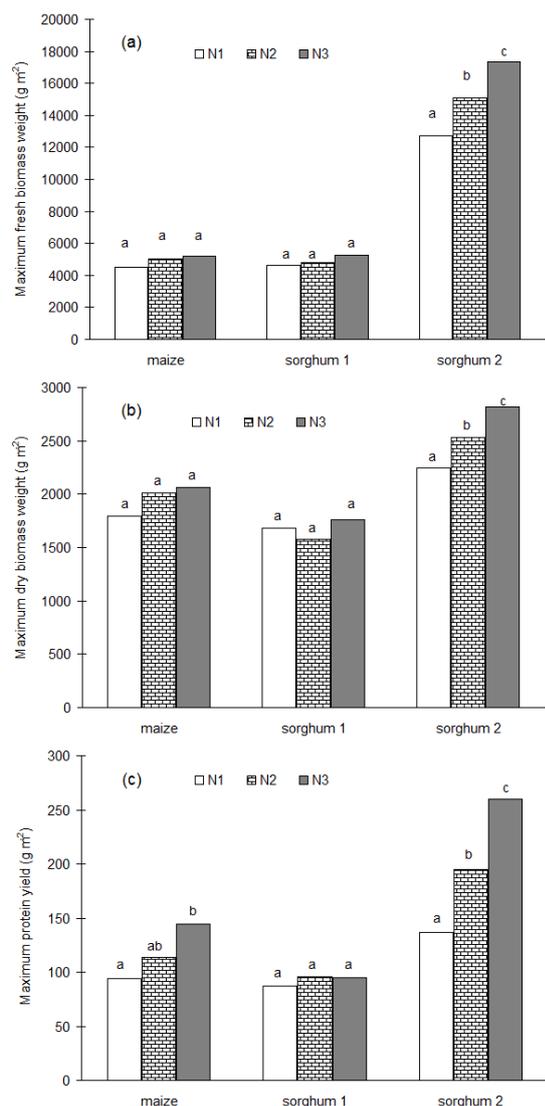


Figure 5: Maximum fresh (a), dry (b), and protein biomass yield (c) for all crops under different N-levels. Different letters within a crop indicate statistically significant differences among nitrogen application rates.

4.1 Crop yields

Among cropping strategies tested we obtained the highest dry matter and protein yields in S_2 crops (Fig. 5). Thus this cultivation strategy is preferable for forage purposes compared to the traditional row planting (e.g. of maize). The large difference in biomass yield between S_2 and maize was due to the higher LAI values of the S_2 crop (higher light interception and more canopy

photosynthesis and thus higher growth rates). Present yielding data in response to plant density (S_1 vs. S_2) agree well with earlier literature findings [16, 17].

Between row crops, S_1 vs. M_1 we found similar productivities (Fig. 5). However, literature reports are very variable in this matter, with some studies reporting higher yields for sorghum [3, 4, 18, 19, 20] and others the opposite [21, 22, 23]. This is mainly due to genotype used (early, medium or later maturity) and secondly due to growing environment (soil type) and management practices (irrigation and fertilization).

In this study the effect of N-fertilization on biomass productivity was low for the row crops (M and S_1 ; Fig 5). This is because the initial fertility of the study soil that was enriched with 6 g N m^{-2} as fertilization was adequate to support growth. In case of the S_2 crops, which showed the highest growth rates and largest biomass yields (Fig. 5), soil fertility was not adequate to support such high growth rates and the effect of nitrogen was proven statistically significant (Fig. 5).

This study provided also important information for the proper harvesting time of the S_1 and M crops for forage purposes. According to our results, these crops maximized their protein yields during the first week of August (central Greece, Fig. 4). Actually, central Greek farmers harvest maize crops for forage purposes at the end of August, to obtain a product with lower moisture content. Beyond August, protein yield declined due to loss of leaf mass (see LAI reduction, Fig. 2) and also due to a decline in protein concentration in all plant tissues.

Our results suggest that dense sorghum plantations and multiple harvests per year is the best strategy to maximize forage yields. However, there are also studies that report the opposite: under high plant densities crude protein decreases [20, 24, 25].

In terms of bioethanol yields, present estimates are very low compared to literature findings for sweet and fibre sorghum (6750 l/ha; [26]).

5 CONCLUDING REMARKS

Cultivating Sudan sorghum at high plant densities ($155 \text{ shoots m}^{-2}$) and harvesting the crop three times during the growing season resulted in 67, 23 and 40% higher fresh, dry, and protein biomass yields, respectively compared to a maize crop. However, to support such intensive cultivation strategies large amounts of nitrogen are needed. Sudan sorghum appears a very good option for forage production in Greece alternative to maize. We strongly believe that future cost/benefit studies will confirm the viability of sorghum cultivation for multiple harvest for forage purposes in Greece.

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