Yields and quality of biomasses and grain in *Cynara cardunculus* L. grown in southern Italy, as affected by genotype and environmental conditions

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Abstract

Cardoon is a crop well adapted to Mediterranean climatic conditions that is able to grow also in marginal lands thus reducing competition for land with food crops. It is considered a key crop for bio-refinery since it allows producing different interesting molecules for industrial application. From stems it is possible to obtain large amounts of cellulose, grains are a good source of oil and proteins and roots can be a source of inulin. The aim of this research has been to evaluate the productive levels of different genotypes of cardoon in two different climatic conditions of Mediterranean cropland (a site in the Vesuvius plain and a site in the internal hilly cropland). In both the sites, during 3 years (from 2012-2013 to 2014-2015), three genotypes (Altilis, Gigante e Trinaseed) were cultivated with 2 planting densities (4 and 8 plants per m^2). A low input cropping system was adopted (no irrigation and 150 kg ha^{-1} of N supplied as ammonium nitrate). In flat site (NA-Ac.), lignocellulosic biomass yield was 19 t ha^{-1} d.m. and grain yield 2.7 t ha^{-1} on the average of the 3 years period. In the hilly site, biomass yield was similar (20 t ha^{-1} d.m.) while grain yield was higher (3.9 t ha^{-1} on the average) as compared to the flat site. As regards biomass composition, an increase of hemicellulose and a decrease of cellulose content were measured in the flat site, maybe as a response of plant to the higher drought stress.

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Introduction

Cardoon (*Cynara cardunculus* L.) is a perennial species with origin in the Mediterranean basin (Gatto *et al*., 2013; Raccuia *et al*., 2004a). The interest in the cultivation of this crop is growing in European Countries such as Spain, France and Italy, because it is particularly adapted to low rainfalls and very warm conditions during summer period (Raccuia *et al*., 2004).

In recent years, cardoon has been considered as a key crop in biorefineries because it is able to produce a series of molecules interesting for industrial applications. Its adaptability to climatic limitations of Mediterranean areas also allows its cultivation in marginal lands, thus reducing competition for land between food and non-food crops (Fagnano *et al*., 2015).

The lignocellulosic (LC) biomass of stems can be used as solid biofuel for renewable energy production by combustion, pyrolysis and gasification (Gonzales *et al*., 2004a, 2004b; Ochoa and Fandos, 2004). The theoretical caloric value is from 16,500 to 17,028 kJ kg^{-1} of dry matter (Piscioneri *et al*., 2000; Encinar *et al*., 2002a, 2000b). It can also be uses for paper pulp (Antunes *et al*., 2002; Gominho *et al*., 2001) or pellet for domestic heating (Gonzalez *et al*., 2004a; Toscano *et al*., 2016) or raw material for bioethanol production or co-digestion for methane production (Kalamaras *et al*., 2014).

Cynara is botanically related to the sunflower and like sunflower it produces oil fruits, which are usually known as *seeds*. The parameters that show the potential of cynara as an oil crop are: seed yield, seed oil content, fatty acid profile and heating value. The seed yield of cynara crop has been estimated at 1.36 t ha^{-1}year^{-1} in Central Spain (Fernandez and Curt, 2004). Seed oil content and fatty acid profile have been studied by Maccarone *et al*. (1999) and Curt *et al*. (2002). A maximum seed oil content of 32.47% has been reported (Curt *et al*., 2002). The oil composition is similar to common sunflower oil: 11% palmitic, 4% stearic, 25% oleic and 60% linoleic fatty acids, on average.

Cynara seed oil can be easily extracted by cold pressing (20/25°C); in this way, the oil composition is not altered and the product can be used for food applications. However, the oil from cynara is also recommended for energy purposes. So, if the LC biomass is used as a solid biofuel and the seeds for oil production, the crop costs can be shared between these two products; in this way the oil cost would be lower than the cost of sunflower oil (Fernandez and Curt, 2004).

Also oleaginous grains can be used for different purposes. Cardoon oil can be used for human consumption, since it is characterised by optimal linoleic/oleic ratio (about 1:8), absence of erucic acid and high levels of alfa-tocopherol, which offers stability against oxidation (Maccarone *et al*., 1999). Oil can also be used for biodiesel production (Encinar *et al*., 1999; Ma and Hanna, 1999) or for biopolymer production (Fagnano *et al*., 2015).

Due to high protein content, the residual flour after oil extraction from grain can be used for animal feed (Fernández and Manzanares, 1990; Foti *et al*., 1999). Fresh biomass is suitable to be used as winter forage for livestock feeding (Cravero *et al*., 2012).

Furthermore, from the roots it is possible to extract inuline (Raccuia and Melilli, 2004, 2010) that is very interesting for functional foods for diabetics (it reduces the absorption of glucose) or celiacs (it can substitute gluten thanks to its rheological properties (Sillitti *et al*., 2016) Agronomic experiments aimed to analyse the behaviour of the new genotypes of this species or of different plant density (Raccuia *et al*., 2011) in different Mediterranean cropping systems are scarce and very recent.

Therefore the aims of this work were: i) to evaluate yield potential of three genotypes in two contrasting environmental conditions; ii) to compare different plant densities; iii) to evaluate the quality of the LC biomass and grain.

Materials and methods

Experiment set-up and crop management

The objective of this study was to evaluate the interaction Cultivars by Locations and the influence of grain density on biomass and grain production of cardoon (*Cynara cardunculus*). In particular, the experiments were made in two contrasting environments: Sant'Angelo dei Lombardi, Avellino (AV), a hilly area subjected to soil erosion (700 m a.s.l.) and characterized by cold and rainy winters and low-fertility soils (Fagnano *et al*., 2015) and Acerra, Naples (NA), a flat area (28 m a.s.l.) characterised by dry and very warm spring-summers and by very fertile soils (Fiorentino *et al*., 2013).

Three genotypes (Altilis, Gigante and the new cultivar Trinaseed) were compared during 3 seasons (from 2012-2013 to 2014-2015), with two-plant density: 4 (0.75 x 0.33 m) and 8 (0.75 x 0.17 m) plants per m^2.

Low input crop management was applied every year (no irrigation and 150 kg ha^{-1} of N supplied as ammonium nitrate); only a supplemental watering was made after transplanting for support the establishment of the crop.

Seedlings at the stage of three-four leaves were transplanted in open field on May 2012. The harvests were made on: September 5, 2013, September 1, 2014, and September 10, 2015 in AV and on August 12, 2013, August 1 2014, and August 4, 2015 in NA.

At harvest, plant height, number of plants per m^2 and number of heads per plant were measured.

The plants were cut at ground level and separated in stems and heads. Heads were then threshed with a specific mini-thresher for separating grains.

Chemical analyses

In laboratory, moisture content of biomass components (stalks, leaves, heads and grains) was measured by weighing 150 g of the different biomasses and drying them in oven a 50 °C until constant weight.

In the last year, dry samples were then used for analysing their chemical composition: Neutral-detergent fibre (NDF), Acid-detergent fibre (ADF) and Acid-detergent lignin (ADL) were determined using an Ankom 200 Fibre Analyser, following sequential extractions in hot neutral- detergent solution (100°C for 1 h), hot acid-detergent solution (100°C for 1 h) and incubation in 72% H_2SO_4(3h) (Goering and Van Soest, 1970). The content in lignin was considered corresponding to ADL, the content of cellulose was calculated as ADF-ADL, the content in hemi-cellulose was calculated as NDF-ADF.

Nitrogen content of biomass and grains was also measured by the Kjeldahl method. Oil was extracted from grains through cold pressing. During the experimental period, soil samples of two layers (0-20, 20-40 cm) were collected from each plot in the same dates of biomass samplings, for measuring organic N and C by the Kjeldahl and Walkley-Black methods, respectively. Concentrations of NH_4^+-N and NO_3^--N were measured according to the Hach® method, and the extracts were analysed by spectrophotometry (Hach DR 2000, Hach Company, Loveland, CO, USA).

Statistical analysis

All data were subjected to analysis of variance by using the software MSTAT- C (Crop and Soil Science Department, Michigan State University). Two Factor Randomized Complete Block Design with Split Plot Combined over Locations and Years was used.

As regards biomass composition, which was measured only in the last year, the design Two Factor Randomized Complete Block Design with Split Plot Combined over Locations was used.

In main plots three cultivars were randomized in three blocks, two seed densities were randomized in sub-plots. All means were separated by using the LSD test at the 0.05 probability level.

Characteristics of study sites

The site of Sant'Angelo dei Lombardi (AV) has a clay-loam (USDA), sub-alkaline soil with a low content of N and organic matter (Table 1). At sowing, the NO_3-N/NH_4-N ratio is 1.25, thus

Table 1. Physical and chemical soil properties in the two sites (0-20 cm).

	S. Angelo (AV)	Acerra (NA)
Clay (%)	38.5	14.4
Silt (%)	25.0	22.6
Sand (%)	36.5	63.0
pH	8.1	7.3
N-NO_3 (ppm)	20	26
N-NH_4 (ppm)	16	17
OM (%)	1.3	2.6
OC (%)	0.8	1.5
C/N	8.0	9.0
Total N (%)	0.1	0.2

OM, organic mass; OC, organic carbon; C/N, carbon-to-nitrogen ratio.

indicating moderate oxygen availability in the soil, and consequently a moderate aptitude to nitrification (Fiorentino *et al.*, 2016).

The site of Acerra (NA), has a sandy loam, neutral soil, with a good contend of N and organic matter (Table 1). Initial NO_3-N/NH_4-N ratio is 1.53, thus indicating higher oxygen availability in the soil, and consequently a higher aptitude to nitrification.

Agro-meteorological conditions

During the 3 years of the experiment, temperature and rainfall were measured by meteorological stations. Reference evapotranspiration was calculated by Hargreaves and Samani (1985) method.

The hilly site (AV) is characterised by very low minimum temperatures in winter (-3 to 1.5°C). Summer temperatures reached 32°C. The mean annual amounts of rainfall were 1200, 890, 983 mm in the three years, respectively (Figure 1). Summer (May-August) water deficits (Rainfall – ET_0) were 524, 580,781 respectively in 2013, 2014 and 2015.

The flat site (NA) (Figure 2) was mainly characterised by air temperatures increasing from April to August with minimum monthly values ranging from 5 (Dec.-Mar.) to 19°C (Jun.-Sep.) and maximum values ranging from 16 (Dec.-Mar.) to 32°C (Jun.-Sep.).

The mean annual amount of rainfall observed in 2013, 2014 and 2015 were 852, 1243, 653 mm, respectively. Summer (May-August) water deficits (Rainfall - ET_0) were 530, 683,689 respectively in 2013, 2014 and 2015.

In June 2014 there was a violent windstorm event with about 30 mm of rain in 1 hour and wind gusts exceeding 100 km h^{-1}.

As expected, the hilly site (S.Angelo-AV) was less warm (-10% in average temperatures) and more rainy (+12% in average annual rainfalls), thus indicating lower drought conditions, as compared with plain site (Acerra-NA).

Results and discussion

Biomass and grain yield

Results of the analysis of variance (Table 2) did not show significant differences for most parameters.

Only two interactions were significant: the interaction Years x Locations for all parameters and the interaction Localitions × Cultivars only for seed yield.

As regards main factors, from Table 3 it is possible to notice a decreasing trend from 1st to 3rd year for all the parameters. The average yield of the 3 year cropping period was 3.3 t ha^{-1} of grains and 13.0 t ha^{-1} of LC residues.

Also Gherbin *et al*. (2001) reported a decreasing cardoon yield from the first to the last season (5th in that case) for all the 17 cultivars cultivated under Mediterranean climate. In that work, the Authors found that yield of the different cardoon cultivars had a very large variability, ranging between 5 and 14 t ha^{-1} $year^{-1}$ in environmental conditions characterized by mean annual rainfall of 500 mm.

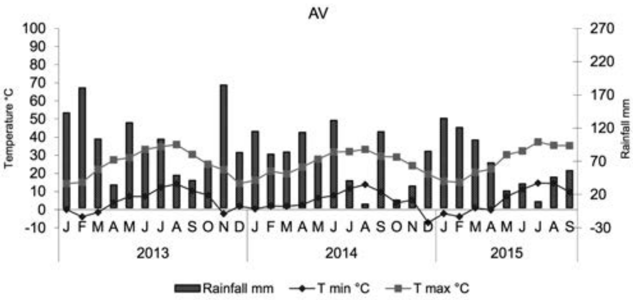

Figure 1. Agro-meteorological parameters of the different years (2013-2014-2015) in hilly site. Values with different letters indicate significant differences per P≤0.05.

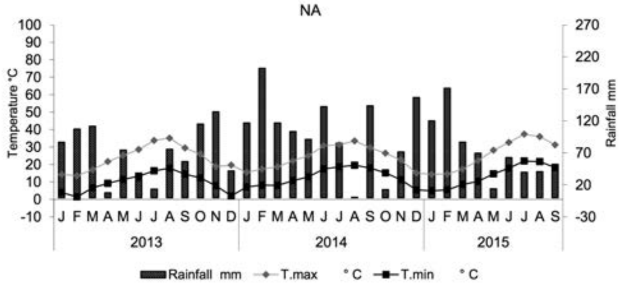

Figure 2. Agro-meteorological parameters of the different years (2013-2014-2015) in flat site.

Table 2. Analysis of variance of yields parameters: significance of main factors and interactions.

	Plant density (num./m^2)	Total biomass (t ha^{-1} d.m.)	Crop residues (t ha^{-1} d.m.)	Grains (t ha^{-1} d.m.)	HI (%)	N content (%)	N uptake(kg ha^{-1})
Year	0.01	0.01	0.01	0.01	0.01		
Location	0.01			0.01	0.01		0.01
Y×L	0.01	0.01	0.01	0.01	0.01		0.01
Cultivar					0.05		
Y×C							
L×C				0.05			0.05
Density	0.01						
CV	16.8%	27.7%	34.2%	34.3%			25.2%

HI, harvest index; N, nitrogen; CV, cultivar.

On the contrary, yield resulted increasing or stable in the first years of other experiment in very different environment: Raccuia and Melilli (2007) reported increasing values of total biomass from 1st to 3rd year (from 7.5 to 20.2 t ha^{-1}). In that study a supplemental irrigation with 50 mm (at flowering on May) was applied. Ierna *et al*. (2012) reported almost constant yield levels (total biomass was 15.4, 21.9 and 22.9 t ha^{-1} in the first three years respectively) in experiments made in Southern Italy.

The average values were higher in AV as compared to NA mainly as regards grain yield (4.0 *vs* 2.6 t ha^{-1}) suggesting higher plant fertility in hilly site, confirmed by the higher harvest index (HI) (20% *vs* 14% in hilly and plain site, respectively). The Trinaseed cultivar confirms the higher HI (18.3%) than the other two genotypes, with a mean yield of 3.5 t ha^{-1} d.m.

Lower yield values were reported by Curt *et al*. (2002) and Fernández *et al*. (2005) with average values of different Mediterranean not irrigated sites of 1.3 t ha^{-1} of grain yield and 14 t ha^{-1} of biomass yield.

Values of the three cultivars were not different on the average, but the significant interaction Locations × Cultivars for grain yield could suggest a different attitude to produce grains of the three genotypes in the different environmental conditions (Figure 3).

The cultivars Trinaseed and Gigante showed higher grain productivity in the hilly site (AV-S.An), while Altilis showed a more stable grain yield without significant difference between hilly and plain sites.

The effect of seed density was significant only for plant density at harvest, but values were lower that those planned at sowing, suggesting that a high mortality rate of plants could represent a strategy of self-regulation of plant population, aimed to reduce intra-crop competition.

The interaction Years × Locations, that was significant for all the measured parameters, is related to the different meteorological conditions in the two sites, as also reported by Raccuia *et al*. (2011).

The number of plant per m^2 decreased from 1st to 3rd year in both the sites (Figure 4A), although with a different trend between the two locations.

In the plain site (NA-Ac.) total biomass (Figure 4B), LC residues (Figure 4C) and grain yield (Figure 4D) were very low in the 2nd year because of the injuries caused by the violent storm event on June 14, 2014. In the 3rd year plant growth recovered and biomass and grain yields were higher and similar to the ones of the 1st year.

In hilly site, biomass and grain yield (Figure 4C and D) were higher in the first two years and decreased in the 3rd year, mainly for a stunted plant development (total biomass per plant was only 266 g d.m. per plant, data not shown). This was due to a more severe water deficit than the two previous years (781 *vs* 524 and 580 mm in the first 2 years). Similar results were reported also by Fernandez *et al*. (2006) that related yield variability to different water availability due to rain distribution.

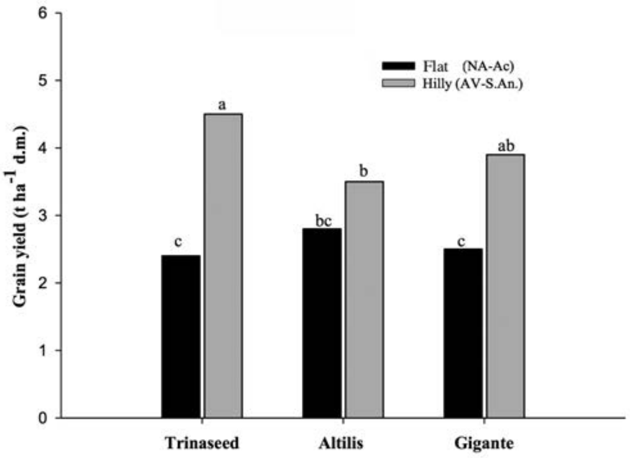

Figure 3. Interaction Locations × Cultivars: grain yield.

Table 3. Average values of main factors: yield parameters.

	Plant density (num./m^2)	Total biomass (t ha^{-1} d.m.)	Crop residues (t ha^{-1} d.m.)	Grains (t ha^{-1} d.m.)	HI (%)	N content (%)	N uptake(kg ha^{-1})
Year							
2013	3.9	24.5	17.8	4.4	18.0	0.35	87.0
2014	2.4	20.3	13.2	3.6	17.7	0.35	67.2
2015	1.8	13.4	8.0	1.8	13.4	0.36	52.9
Location							
NA-Ac	2.5	18.9	13.1	2.6	13.8	0.35	65.5
AV-S.An.	2.8	20.0	12.9	4.0	20.0	0.35	72.5
Cultivar							
Trinaseed	2.6	19.1	12.3	3.5	18.3	0.35	69.5
Altilis	2.6	19.3	13.0	3.2	16.6	0.35	66.6
Gigante	2.8	19.9	13.7	3.2	16.1	0.36	71.0
Seed density							
4/m^2	2.4	19.2	13.0	3.2	16.7	0.35	69.0
8/m^2	2.9	19.7	13.0	3.3	16.8	0.35	69.3
Average	2.7	19.4	13.0	3.3	17.0	0.35	69.0

HI, harvest index; N, nitrogen; NA-Ac, Acerra (NA); AV-S.An., S. Angelo (AV).

The effects of the experimental factors on N content in whole plants of cardoon were not significant (0.35% on the average, Tables 2 and 3). Therefore, N uptake (Table 4) reflected yield differences (Figure 4), with values from 30 kg ha^{-1} to 150 kg ha^{-1}, in relation to the more or less unfavourable environmental conditions. In the two years in which plant growth and yield were affected by very unfavorable meteorological conditions, N uptake was very low (31-32 kg N ha^{-1}), while in the normal years N uptake was 88 kg N ha^{-1} on the average.

Table 4. Interaction Years × Locations: nitrogen uptake by cardoon plants.

	N uptake (kg ha^{-1} tot) biomass 2013	2014	2015
NA-Ac	90.1^{b}	31.6^{d}	74.8^{c}
AV S.An.	83.9^{b}	102.8^{a}	31.0^{d}

NA-Ac, Acerra (NA); AV-S.An., S. Angelo (AV). $^{a-d}$Values with different letters indicate significant differences per P≤0.05.

Table 5. Correlation matrix between yields components.

	Total biomass	Grain yield	Crop residues	Plant density	HI	N content	N uptake
Total biomass	-						
Grain yield	0.91*	-					
Crop residues	0.96*	0.81*	-				
Plant density	0.23	0.38	0.32	-			
Harvest index	0.63	0.87*	0.48	0.54	-		
N content	-0.06	0.26	-0.10	0.37	0.34	-	
N uptake	0.53	0.24	0.51	-0.35	0.03	-0.75	-

HI, harvest index; N, nitrogen. *P≤0.05.

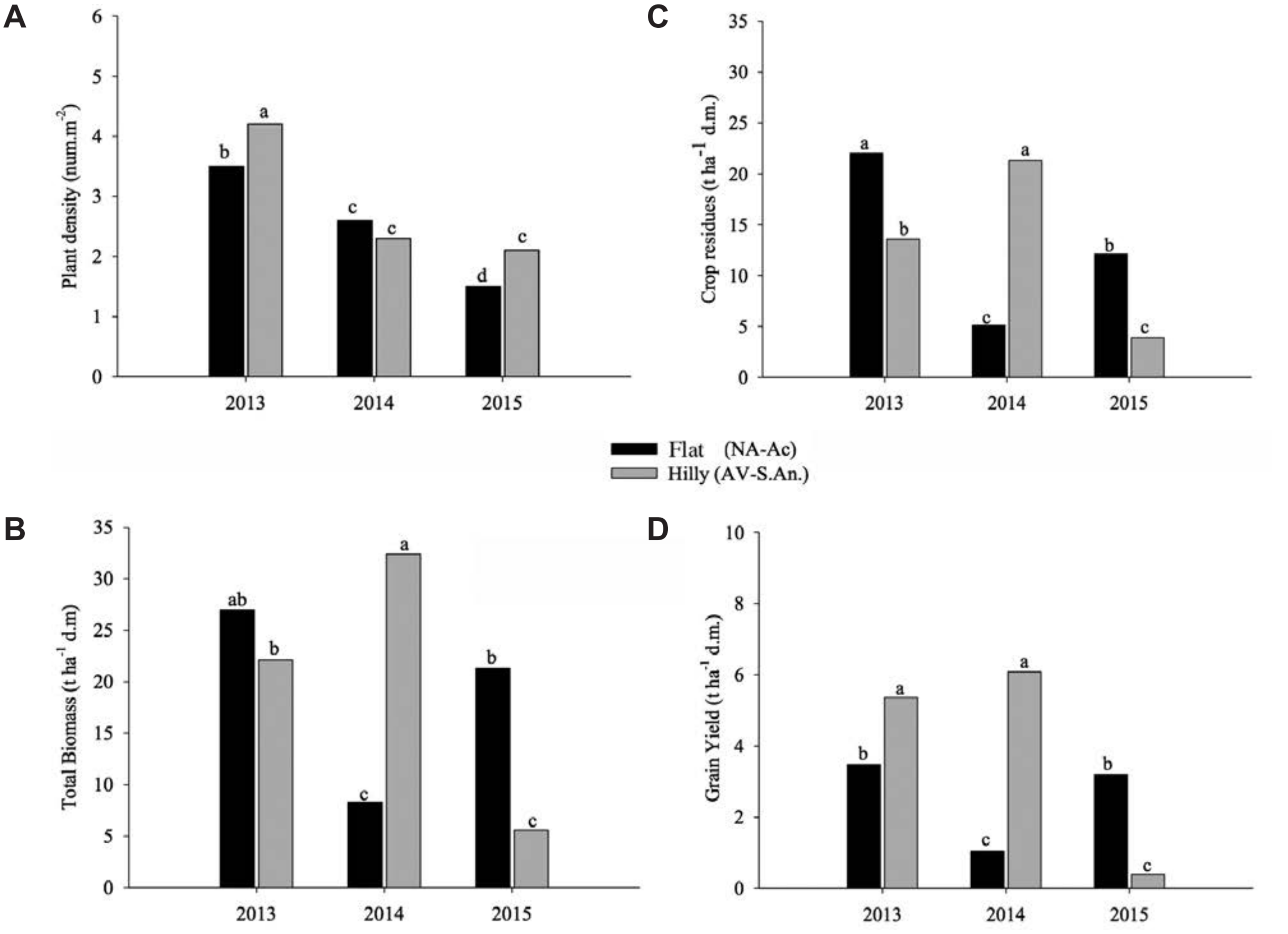

Figure 4. Interaction Years × Locations: A) plant density; B) total biomas; C) crop residues; D) grain yeld. Values with different letters indicate significant differences per P≤0.05.

Grain yield was correlated with total biomass yield and harvest index (Table 5) and not with plant density (number per m^2), suggesting that grain yield is higher when environmental conditions allow a regular plant growth, irrespective of the number of plants.

Soil carbon and nitrogen

From the analysis of variance, no difference was significant as regards C and N into the soil.

C and N in the soil did not show clear trends (Figure 5), maybe for the short term of experimental period, since only long-term experiments allow measuring the impact of cropping systems on soil features. Anyway, in this three years period, the cardoon cropping system didn't show the positive effect on C storage into the soil that we could expect considering the absence of soil tillage. On the contrary other perennial crops, such as giant reed, showed a soil C increase that was considered an effect of the contribution of leaf fall, root exudates and turnover (Fagnano *et al*., 2015; Harper *et al*., 2012; Felten and Hemmerling, 2012; Chimento *et al*., 2016).

Composition of lignocellulosic biomass

As regards, biomass and grain composition measured in the last year, from the analysis of variance (Table 6) the interaction Locations × Cultivars was significant for cellulose and hemicellulose content (Table 7).

As regards main factors (Table 8), in the plain site there was higher hemicellulose content and a lower cellulose and protein content in residual biomass as compare to hilly site, while biomass and grain composition were not different between the cultivars.

A lower cellulose content and a higher hemicellulose content was measured in the flat site, as a response to the higher drought condition as also reported by Emerson *et al*. (2014) and Van der Weide *et al*. (2016); these authors related the decrease in cellulose content to the formation of osmolytes, aimed to the maintenance of osmotic equilibrium in the cell under dry conditions, at the expense of cellulose biosynthesis.

The increase in hemicelluloses content could be related to the exigence of plant to enable cell walls to uphold their structural rigidity without compromising plasticity (Le Gall *et al*., 2015;

Table 6. Analysis of variance of biomass composition: significance of main factors and interactions.

	Crop residues				Grains
	Hemicellulose	Cellulose	Lignin	Proteins	Proteins
Location	0.01	0.01	-	0.01	-
Cultivar	-		-	-	-
L×C	0.01	0.05	-	-	-
Density	-	-	-	-	-
L×D	-	-	-	-	-
L×C					
L×C×D	-	-	-	-	-

Table 7. Interaction Locations × Cultivars: biomass composition.

	Hemicellulose			Cellulose		
	TRI	GIG	ALT	TRI	GIG	ALT
NA-Ac	21.32^{a}	19.18^{b}	21.57^{a}	45.74^{c}	43.69^{d}	46.55^{c}
AV-S.An	12.55^{c}	11.11^{d}	11.19^{d}	50.00^{a}	46.60^{bc}	48.33^{ab}

TRI, Trinaseed; GIG, Gigante; ALT, Altilis; NA-Ac, Acerra (NA); AV-S.An., S. Angelo (AV). $^{a\text{-}d}$Values with different letters within columns indicate significant differences per P≤0.05.

Table 8. Average values of main factors: biomass composition and grain protein (g 100 g^{-1}).

	Crop residues				Grains
	Hemicellulose	Cellulose	Lignin	Proteins	Proteins
Location					
NA-Ac	20.7	45.3	12.9	2.2	15.0
AV-S.An.	15.0	48.3	12.3	2.5	14.0
Cultivar					
Trinaseed	19.7	47.9	13.3	2.4	14.0
Altilis	17.4	45.1	11.8	2.5	15.0
Gigante	16.4	47.4	12.7	2.4	14.0
Seed density					
4/m^2	18.2	46.7	12.8	2.2	14.8
8/m^2	17.4	47.3	12.4	2.3	14.0
Average	17.8	46.8	12.6	2.3	14.4

NA-Ac, Acerra (NA); AV-S.An., S. Angelo (AV).

Tenhaken, 2015). These authors reported that hemicelluloses contribute to cell wall rigidity by reinforcing the cell wall matrix through cross-linking other fibres that could be more easily broken thus ensuring cell wall plasticity.

Lignin content was not different between sites and cultivars (12.6% on the average), confirming the findings of Van der Weide *et al*. (2016).

Protein and oil content of grains were not different between the factors analysed (14% and 23% on the average, respectively).

Protein value was slightly lower than that reported by Genovese *et al*. (2016) (16% in cv. Altilis), while it was much lower than those (21-22%) reported by other studies made on different genotypes (Raccuia and Melilli, 2007). Oil content of seeds is similar to that reported by other Authors (Raccuia and Melilli, 2007).

Conclusions

Cardoon resulted a suitable crop for producing LC biomass and grains with low inputs in different Mediterranean croplands. Grain yield was higher in the hilly than in the flat site (3.9 *vs* 2.7 t ha^{-1} on average of the 3-years experiment). On the contrary, LC biomass yield was more stable in relation to the environmental conditions of the two sites (20.1 t ha^{-1} d.m. *vs* 19.0 t ha^{-1} in hilly and flat sites, respectively). Biomass and grain composition were not very different. Only a decrease of cellulose and an increase of hemicellulose were measured in the flat site, maybe as a response of plants to the more drought conditions.

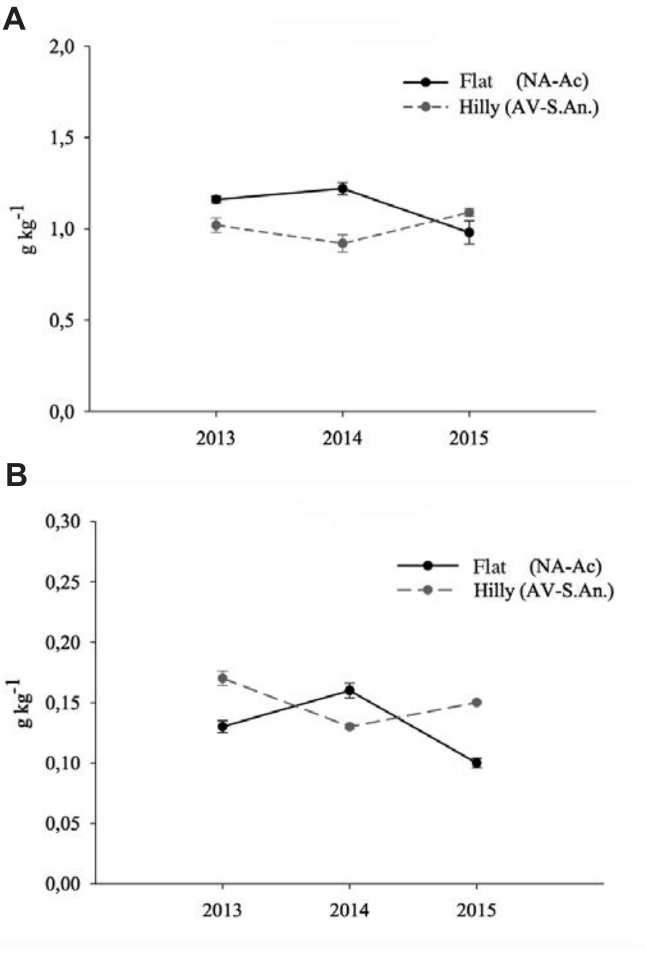

Figure 5. Trend of organic carbon (A) and nitrogen (B).

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