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Effects of fertilization and tillage system on growth and crude protein content of quinoa (*Chenopodium quinoa* Willd.): An alternative forage crop

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Abstract

A field experiment was conducted to determine the effects of fertilization and tillage on growth, yield and quality of quinoa crop (*Chenopodium quinoa* Willd.). The agronomic performance and nutritive value of quinoa was analyzed in order to define alternatives to local forages for dry-season feeding of ruminants in the Mediterranean region. The experiment was laid out in a split-plot design with two replicates, two main plots [conventional tillage (CT) and minimum tillage (MT)] and sub-plots (fertilization treatments: control, cow manure, inorganic fertilization 100 kg ha^{-1} (N1) and inorganic fertilization 200 kg ha^{-1} (N2)). The results indicated that quinoa growth was influenced by both tillage and fertilization. The lowest height and dry weight were found under MT. Moreover, the lowest height and dry weight (8205 kg ha-1 and 8020 kg ha^{-1} for CT and MT, respectively) were found under control treatment (no-fertilization). Concerning the nitrogen content there were no significant differences between CT and MT systems. In addition, the highest quinoa nitrogen content was observed for N2 treatment (200 kg N ha^{-1}). Moreover, the highest nitrogen content was measured at 150 DAS. Concerning the crude protein content, there were no significant differences between CT and MT systems. In addition, there were significant differences in crude protein between fertilization treatments. The greatest value was observed for N2 treatment. Moreover, the highest crude protein yield (2481 kg ha^{-1} and 2356 kg ha^{-1} for CT and MT, respectively) and acid detergent fibre (ADF) were found under N2 treatment. In addition, ash was not influenced by both tillage systems and fertilization treatments. Data indicate that quinoa crop could be used as alternative to legumes for protein production to feed ruminant animals.

Key words: Crude protein, Quinoa, Soil properties, Tillage system, Yield

Introduction

Quinoa (*Chenopodium quinoa* Willd.), is an Amaranthacean, stress-tolerant plant, cultivated along the Andes, for the last 7000 years. Its grains have higher nutritive value than traditional cereals and it is a promising worldwide plant for human consumption and nutrition (Vega-Gálvez et al., 2010). Quinoa is one of the main food crops in the Andean mountains, but during recent times there has been increased interest for the product in the United States, Europe, and Asia (Jacobsen, 2003). Quinoa is a good source of protein and can be used as a nutritional ingredient in food products (Gonzalez et al., 2012). The Organization of the United Nations for Food and Agriculture (FAO) has declared the year 2013 as the year of the quinoa.

Moreover, limited data are available regarding the fodder quality of quinoa crop. Bhargava et al. (2010) reported that the foliage of many species of Chenopodium (*C. album, C. berlandieri, C. bushianum, C. giganteum, C. murale, C. quinoa, and C. ugandae*) is a rich source of minerals like potassium, sodium, calcium and iron. The foliage of quinoa is rich in protein, carotenoid and ascorbic acid (Bhargava et al., 2007). Therefore, members of the genus *Chenopodium* are used as a foliage crop and fodder in many parts of the world (Bhargava et al., 2010).

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In temperate regions, white clover (*Trifolium repens* L.) is the predominant species used for grazing, whereas red clover (*Trifolium pratense* L.) and alfalfa (*Medicago sativa* L.) are cultivated mainly for hay and silage making (Krawutschke et al., 2013). Drought is an adverse factor that forages must cope with in the Mediterranean region. Quinoa is able to tolerate drought (Razzaghi et al., 2011; Razzaghi et al., 2012). Jacobsen (2003) reported that quinoa can grow with only 200 mm of rainfall. Therefore, the aim of this study was to determine the effects of tillage system and organic fertilization on growth and fodder quality of quinoa crop, under Mediterranean semi-arid conditions.

Material and Methods

A quinoa crop (*Chenopodium quinoa* Willd.) was established in the area of Agrinio (western Greece, Lat: 38.35, Long: 21.25) in 2012. The soil was a clay loam (24.9% clay, 61.2% silt, and 13.9% sand) with pH 7.4, organic matter 1.45% and EC 0.63 mS cm^{-1}. Some meteorological data of the experimental sites are presented in Figure 1. The sites were managed according to organic agriculture guidelines (EC 834/2007). The experiments were set up on an area of 650 m^2, according to the split-plot design with two replicates, two main plots (conventional tillage: CT, moldboard plowing at 25 cm, followed by one rotary hoeing at 10-15 cm; minimum tillage: MT, chiseling at 25 cm depth followed by chiseling at 10-15 cm) and four sub-plots (fertilization treatments: control, cow manure (2000 kg ha^{-1}, 1.24% N, inorganic fertilization (fertilizer 26-0-0) with 100 kg N ha^{-1} and 200 kg N ha^{-1}). The main-plot size was 300 m^2. The crop was cultivated before quinoa becomes durum wheat. Quinoa was sown by hand in rows 30 cm apart at a depth of 2-3 cm. Quinoa was sown on 5th of May 2012 at a rate of 10 kg ha^{-1}. Overhead sprinkler system was set up on the field. The total quantity of water was 180 mm.

The organic matter was measured by the Walkey-Black method, for 0-15 cm depth for every plot (Walkey and Black, 1934). Furthermore, the total nitrogen was determined by the Kjeldahl method (Bremner, 1960) using a Buchi 316 device in order to combust and extract the soil samples.

For the computation of dry weight, height and LAI, 10 plants were randomly selected in each plot. The dry weight was determined after drying for 72 h at 70ºC. Leaf area was measured using an automatic leaf area meter (Delta-T Devices Ltd). Root samples were collected 150 DAS (days after sowing) and from the 0–25 cm layer by using a cylindrical auger (25 cm length, 10 cm diameter) at the midpoint between successive plants within a row. For each sample, roots were separated from soil after being in water + $(NaPO_3)_6$ + Na_2CO_3 for 24 h. For the determination of the root density, the root samples were placed on a high-resolution scanner using DT software (Delta-T Scan version 2.04; Delta-T Devices Ltd, Burrwell, Cambridge, UK). The total nitrogen was determined by the Kjeldahl method. Moreover, total protein content was calculated from the nitrogen content using a conversion factor of 6.25 (AOAC, 2009). Acid detergent fibre (ADF) was determined according to the methods of Van Soest et al. (1991).

The data were subjected to statistical analysis according to the split-plot design. The statistical analysis was performed with STATGRAPHICS Plus 5.1 logistic package. Differences between treatment means were compared at P=5% with ANOVA in order to find the statistically significant differences.

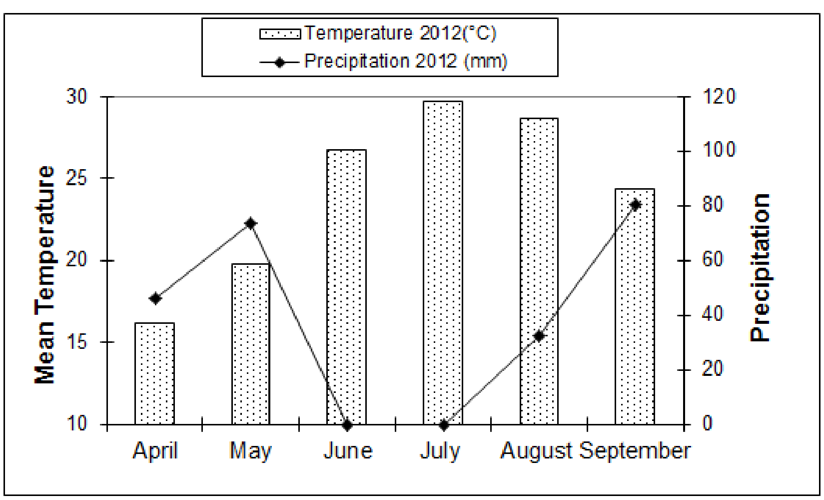


Figure 1. Meteorological data (temperature: °C and precipitation: mm) for the experimental sites during the experimental period (April-September).

Table 1. Effects of tillage system (conventional tillage: CT, minimum tillage: MT) and fertilization (control, cow manure, inorganic fertilization 100 kg ha^{-1} (N1), inorganic fertilization 200 kg ha^{-1} (N2)) on soil porosity (%), soil total nitrogen (%), root density (cm cm^{-3}) and LAI of quinoa crop.

Fertilization	Tillage system			
	CT	MT	CT	MT
Experiment 2011	Organic matter		Soil total N	
Control	1.78	1.62	0.132	0.134
Cow manure	2.69	2.51	0.172	0.195
N1	1.97	1.70	0.144	0.131
N2	1.89	1.62	0.149	0.127
F_{illage}	10.76**(LSD=0.155)		0.13^{ns}	
$F_{fertilization}$	39.45***(LSD=0.219)		16.47***(LSD=0.019)	
Experiment 2012	Root density		LAI	
Control	1.01	0.88	4.30	4.45
Cow manure	1.21	1.11	4.70	4.65
N1	1.15	1.18	4.75	4.35
N2	1.21	1.23	4.25	4.45
$F_{tillage}$	1.20^{ns}		0.03^{ns}	
$F_{fertilization}$	4.67*(LSD=0.219)		1.32^{ns}	

F-test ratios are from ANOVA. Significant at *p=0.05, **p=0.01, ***p=0.001, ns: not significant. The LSD (p=0.05) for tillage systems and organic fertilization are also shown.

Results and Discussion

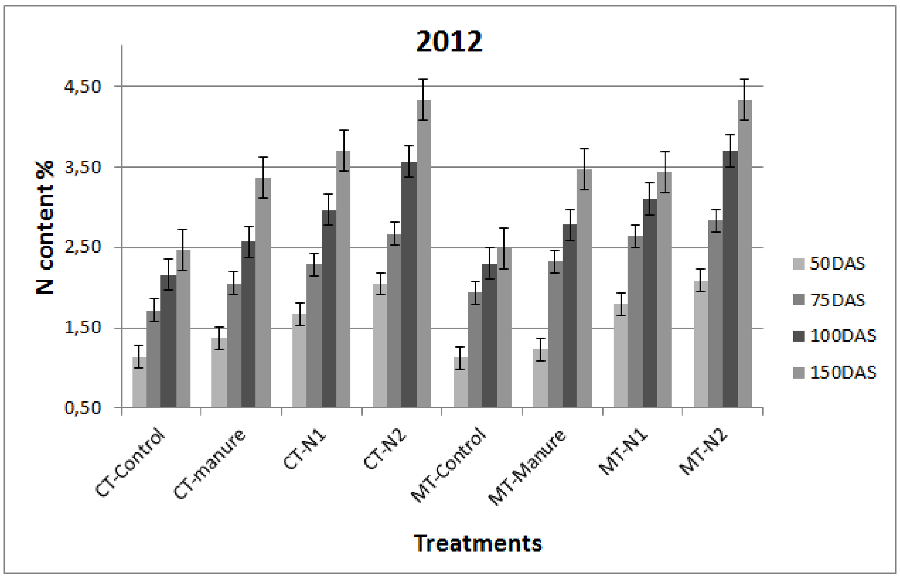
Tillage and fertilization influences the soil properties. The lowest soil organic matter was found under MT (Table 1). There were statistically significant differences between MT and CT systems. In addition, earlier studies have shown that the adoption of minimum tillage system lead to improvement of soil properties (i.e. organic matter, porosity and total N) (Bilalis et al., 2010, 2012). Furthermore, there were no significant differences in soil total N among tillage systems.

Moreover, there were significant differences between fertilization treatments concerning the soil organic matter and total N. Inorganic N fertilizer had no effect on soil organic matter. Tueche and Hauser (2011) reported that N fertilizer had no effect on soil physical properties. The highest organic matter and total nitrogen content were found under cow manure treatment. Efthimiadou et al. (2010) also observed that organic soil amendments increased the level of soil organic matter and total nitrogen. Furthermore, according to López-Espinosa et al. (2013) the use of organic fertilizers can be considered as an alternative fertilization method for organic crop production. Finally, in soil properties, no tillage x fertilization interaction was found.

Concerning the root density, there were no significant differences between CT and MT systems (Table 1). In a previous study, it was shown that the quinoa root density (1.03-1.21 cm cm^{-3}) was higher in soils subjected to minimum tillage than to conventional tillage (Bilalis et al., 2012). Also, there were significant differences in root growth between fertilization treatments. The lowest root diameter was found under control treatment. Leaf area index (LAI) was not influenced by both tillage systems and fertilization. In addition, Bilalis et al. (2012) observed that the highest LAI (4.47-5.03) and dry weight (8650-9290 kg ha^{-1}) were found in MT. Quinoa responds well to nitrogen fertilization (Berti et al., 2000; Schooten and van Pinxterhuis, 2003; Schulte auf'm Erley et al., 2005). The lowest height (165 cm and 155 cm for CT and MT, respectively) and dry weight (8205 kg ha^{-1} and 8020 kg ha^{-1} for CT and MT, respectively) was found under control treatment (no-fertilization). Dry weight and height had positive and significant correlation with root density (r=0.741, p<0.05 and r=0.842, p<0.001, respectively).

Concerning the quinoa nitrogen content there were no significant differences between CT and MT systems (Figure 2). In addition, there were significant differences in quinoa N content between fertilization treatments. All fertilization treatments resulted in values higher than those of the control. The greatest value was observed for N2 treatment (200 kg N ha^{-1}). Schulte auf'm Erley et al. (2005) observed that the N uptake (161 kg ha^{-1}) was nearly doubled by a fertilization of 120 kg N ha^{-1} compared to N 0 (82.6 kg ha^{-1}). The highest nitrogen content was measured at 150 DAS (Figure 1). Quinoa nitrogen content had positive and significant correlation with root density (r=0.821, p≤0.05). Finally, in quinoa growth traits, no tillage x fertilization interaction was found.

The assessment of quinoa crude protein content is of a great importance for the fodder industry. Forage nutritive value is primarily determined by concentrations of crude nitrogen N protein. There are few data available regarding the fodder quality of quinoa crop. Concerning the crude protein content, there were no significant differences between CT and MT systems (Figure 2). N fertilization consistently had a positive impact on quinoa forage quality. There were significant differences in crude protein between fertilization treatments. The greatest value (27% or 270 g kg^{-1} dry matter) was observed for N2 treatment (200 kg N ha^{-1}). In this context, Kering et al. (2011) reported that application of N increased crude protein of both spring and summer harvested bermuda grass forage. The lowest crude protein yield was found under MT (Table 1). There were no statistically significant differences between MT and CT systems. Crude protein content had positive and significant correlation with root density (r=0.798, p≤0.05).



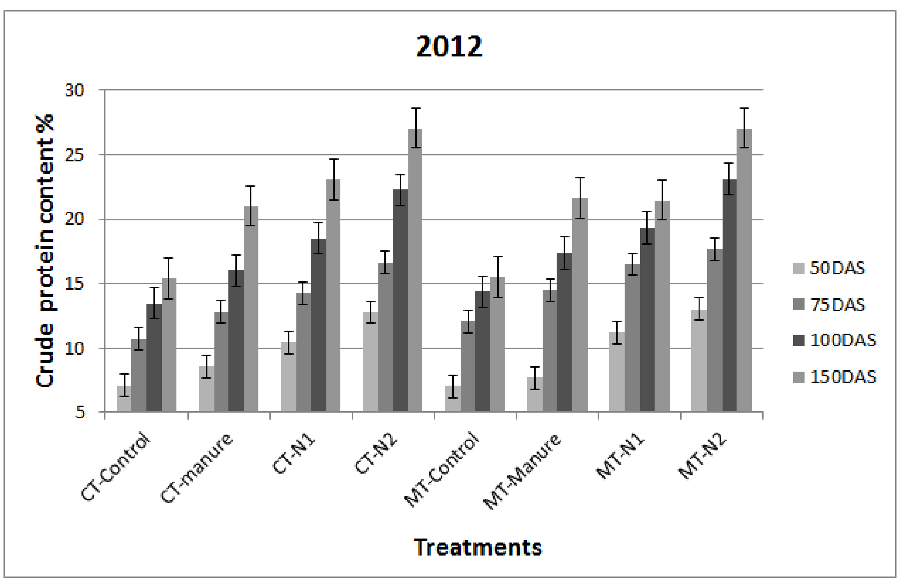


Figure 2. Effects of tillage system (conventional tillage: CT, minimum tillage: MT) and fertilization (control, cow manure, inorganic fertilization 100 kg ha^{-1} (N1), inorganic fertilization 200 kg ha^{-1} (N2)) on nitrogen (%) and crude protein content (%) of quinoa plants (50, 70, 100 and 150 days after sowing (DAS)). Error bars indicate standard error.

There were significant differences between fertilization treatments concerning the crude protein yield. The highest crude protein yield (2481 kg ha^{-1} and 2356 kg ha^{-1} for CT and MT, respectively) was found under N2 treatment. Crude protein yield had positive and significant correlation with root density and soil total N ($r=0.835$, $p\leq0.01$ and $r=0.832$, $p\leq0.05$, respectively).

Forage species differ in their crude protein content. Dugalić et al. (2012) reported that crude protein content in alfalfa plants ranged between 21.7% - 25.9%. The nutritional value of forage crops is also influenced by stage of maturity (Krawutschke et al., 2013). Our results indicated that the highest crude protein content was measured at 150 DAS (Figure 1). In addition, Schooten and van Pinxterhuis (2003) reported that quinoa crude protein content decreased from 190 g crude protein kg^{-1} dry matter at 70 DAS to 155 g at 98 DAS. Additionally, organic matter digestibility increased with growing period, with maximal values of 63.5% for Atlas cultivar and 68.5% g/Kg for Ras1 cultivar at 112 days (Schooten and van Pinxterhuis, 2003). As the plant ages, its morphological and histological development decreases the amount of cell content, which is soluble, and increases the amount of cell walls (Baumont et al., 2000).

Forage intake is affected by crude protein, fibre and ash content (Ibrahim et al., 2012). Acid detergent fibre (ADF) is a major indicator of digestibility, negatively affects feed quality (Han et al., 2003). Therefore, forage with a low ADF content is higher in quality than one with a high ADF content. ADF content in quinoa plants ranged from 24.78% to 39.45% (Table 2). There were significant differences between fertilization treatments concerning the ADF content. The highest ADF content (35.80% and 39.45% for CT and MT, respectively) was found under N2 treatment. In addition, Kering et al. (2011) reported that N fertilization consistently decreased ADF content in berrnuda grass forage. Furthermore, ash was not influenced by both tillage systems and fertilization treatments (Table 2). Finally, in quinoa quality traits, no tillage x fertilization interaction was found.

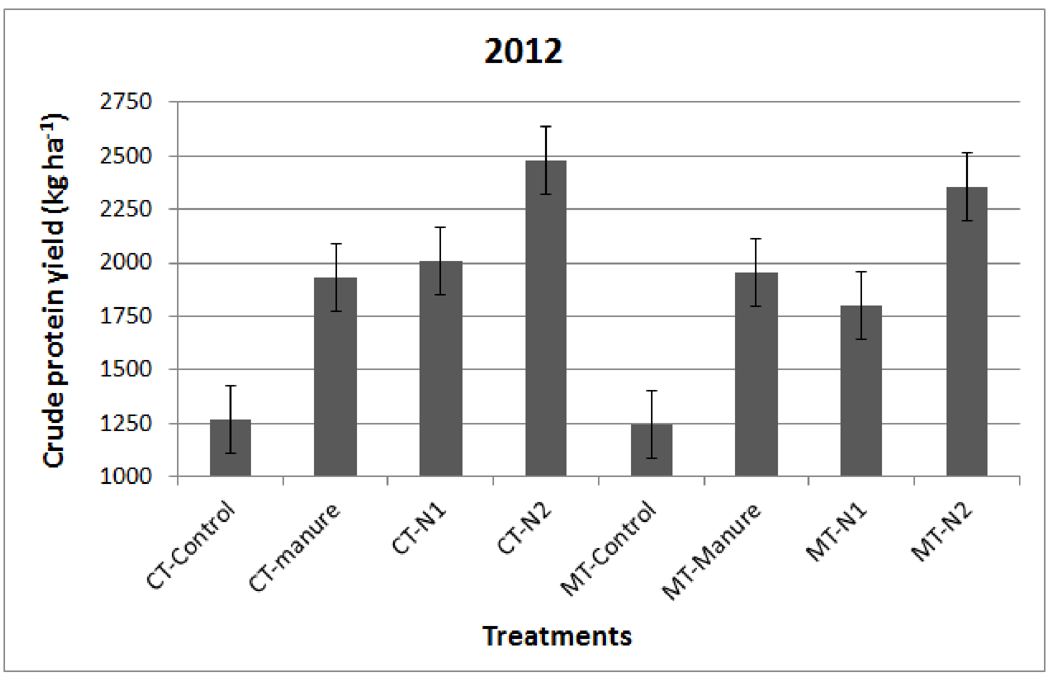


Figure 3. Effects of tillage system (conventional tillage: CT, minimum tillage: MT) and fertilization (control, cow manure, inorganic fertilization 100 kg ha^{-1} (N1), inorganic fertilization 200 kg ha^{-1} (N2)) on crude protein yield (kg ha^{-1}) of quinoa crop. Error bars indicate standard error.

Table 2. Effects of tillage system (conventional tillage: CT, minimum tillage: MT) and fertilization (control, cow manure, inorganic fertilization 100 kg ha^{-1} (N1), inorganic fertilization 200 kg ha^{-1} (N2)) on dry weight (kg ha^{-1}), height (cm), total ash (%) and acid detergent fibre: ADF (%) of quinoa crop.

Fertilization	Tillage system			
	CT	MT	CT	MT
Experiment 2011	Dry weight		Height	
Control	8205	8020	165	155
Cow manure	9170	9010	177	172
N1	8725	8390	171	173
N2	9165	8705	171	168
$F_{tillage}$	5.26*(LSD=143)		10.01** (LSD=5.14)	
$F_{fertilization}$	31.42***(LSD=229)		11.21**(LSD=5.89)	
Experiment 2012	Total ash		ADF	
Control	14.11	14.41	29.72	27.78
Cow manure	13.08	13.86	24.78	27.95
N1	13.88	14.71	30.23	36.80
N2	14.65	14.65	35.80	39.45
$F_{tillage}$	1.23^{ns}		4.96^{ns}	
$F_{fertilization}$	1.34^{ns}		15.25***(LSD=4.19)	

F-test ratios are from ANOVA. Significant at *p=0.05, ***p=0.01, ***p=0.001, ns: not significant. The LSD (p=0.05) for tillage systems and organic fertilization are also shown.

Conclusions

Our results indicate that quinoa growth was influenced by both tillage and fertilization. The lowest height and dry weight were found under MT. The lowest height and dry weight was found under control treatment. N uptake increased with N fertilization. Concerning the crude protein content, there were no significant differences between CT and MT systems. In addition, there were significant differences in crude protein between fertilization treatments.

The highest crude protein yield (2481 kg ha^{-1} and 2356 kg ha^{-1} for CT and MT, respectively) was found under N2 treatment. Our data indicated that quinoa crop could be used as alternative to legumes for dry-season feeding of ruminants in the Mediterranean region. N fertilization consistently had a positive impact on quinoa forage quality.

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