

# Maize Biomass Production, N-Use Efficiency and Potential Bioethanol Yield, Under Different Cover Cropping Managements, Nitrogen Influxes and Soil Types, in Mediterranean Climate

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## Abstract

To evaluate the effect of cover cropping faba bean with maize, compared to maize monocrop cultivation, on yield (dry matter), nitrogen utilization efficiency (NUE) and N fertilizer recovery fraction of maize, field experiments were carried out over a period of three years. Experimental sites were located in central Greece, on a fertile, clayey soil and on a sandy soil of moderate fertility. A factorial combination of four nitrogen dressings (0, 80, 160, 240 kg ha<sup>-1</sup>) and three legume treatments (incorporated into the topsoil or harvested before the sowing of maize and mono-cropping) were tested in a split plot design in three blocks. Results showed a substantial importance of the legume cover crop for both soil types, for all studied factors. Maize total dry biomass yield fluctuated from 13.4 to 20.3 Mg ha<sup>-1</sup> for the control plots, from 15.1 to 21.6 Mg ha<sup>-1</sup> when faba bean was harvested and from 15.3 to 22.4 Mg ha<sup>-1</sup> when incorporated, for clayey soil and from 12.4 to 16.9 Mg ha<sup>-1</sup> for the control plots, from 14.9 to 19.4 Mg ha<sup>-1</sup> when faba bean was harvested and from 14.5 to 19.6 Mg ha<sup>-1</sup> when incorporated, for sandy soil. The NUE was estimated at 56 kg kg<sup>-1</sup> and 55 kg kg<sup>-1</sup> for clayey and sandy soil, respectively. The N recovery fraction was enhanced by 10-15% after faba bean cover cropping, for both soil types. Such systems should be seriously considered in future land use planning, with respect to the sustainable cultivation of maize.

**Keywords:** maize, faba bean, cover crop, nitrogen utilization efficiency, biomass, bioethanol

## 1. Introduction

Maize gathers a lot of research interest, in comparison with other energy crops, as it is the dominant raw material (along with sugarcane) for bioethanol production (Coyle, 2007), the most common and widespread biofuel (Singh et al., 2010). The participation of bioethanol in the global production of biofuels is over 94% (Renewable Fuels Association, 2007), as many countries are moving to replace fossil fuels with biofuels, according to international regulations. In the EU, the European directive (2009/28/CE) imposes the participation of biofuels at 10% of all transport fuel by 2020 (Rutz & Janssen, 2008; Xavier, Correia, Pereira, & Evtuguin, 2010).

There are several concerns around energy crops and bio-energy produced from biomass because of the direct competition with food crops for land and resources and the low efficiency of current conversion technologies. For example, in the EU the bioethanol demand amounted to 12.7 billion liters in 2010, with domestic production capacity limited to just 2 billion liters per year (Zarzycki & Polska, 2007). This imbalance means that to meet the demand with raw materials grown within the EU, around 13% of total arable land should be devoted to energy crops (Magar, Pelkonen, Tahvanainen, Toivonen, & Toppinen, 2011). According to the Department of Energy (DOE) in USA, the Net Energy Balance (NEB) in the whole system of bioethanol production from maize is currently positive (Lavigne & Powers, 2007). However, the energy efficiency of the system is reported to be low; as expressed by the typical NEB ratio of 1.34 (Shapouri, Duffield, & Wang, 2002), although it is possible to increase the NEB ratio by the development of agricultural and conversion technologies (e.g. better maize variety,

improvement of efficiency in fertilizer use, appropriate co-products management). At the same time, the raw materials used for the production of first generation biofuels, derive by crops that are traditional foods for humans and animals, resulting in a dangerous food-energy competition for land availability (Bacovsky, Mabee, & Worgetter, 2010).

Recent technological developments in making ethanol from cellulosic biomass have also identified crop residues as an ethanol source. Most of the crop residues have been unutilized, for example, around 90% of maize stover is unused and remains in the field (Kim & Dale, 2004). Because tremendous amounts of crop residues are annually produced in the world (Kim & Dale, 2004), it is logically to consider them as materials of bioethanol. In fact, utilization of maize stover for bioethanol production has been actively studied in the USA (Sheehan et al., 2004; Varvel, Vogel, Mitchell, Follett, & Kimble, 2008). However, utilization of crop residues should be carefully considered because they are well known to be important to maintain sustainability of crop production through prevention of soil degradation, improvement of soil water balance, maintenance of soil organic carbon content and soon (e.g. McAloon, Taylor, Yee, Ibsen, & Wooley, 2000; Allmaras, Schomberg, Douglas, & Dao, 2000; Clapp, Allmaras, Layese, Linden, & Dowdy, 2000).

Improvement to problems that exist around energy crops and bio-energy produced, can surely arise from maximization of crop production and/or minimization of required inputs during their cultivation. Several traditional practices have been adopted for the conservation of energy, which are generally considered more environmentally friendly, such as intercropping, crop rotation, reduced soil tillage (Tilman, Hill, & Lehman, 2006), with the greater energy savings achieved by the techniques aimed at reducing inputs of mineral nitrogen, such as legumes cover crops (Crews & Peoples, 2004; Jensen, Peoples, & Hauggaard-Nielsen, 2010). The latter is responsible for a large share of the energy consumed by arable crops reaching 50% of the total energy input (Kuesters & Lammel, 1999).

Legume cover crops are grown during the winter before the cultivation of the energy crop and are either harvested after completing their biological cycle, contributing to soil nitrogen of biological fixation by the nodules of the roots or incorporated into soil as a green manure (usually close to blossoming), contributing organic nitrogen as well. This alternative nitrogen supply collects a great deal of interest, as it is renewable, clean and environmentally friendlier than the industrial nitrate fertilizers (Giller, 2001; Jensen & Hauggaard-Nielsen, 2003), while at the same time doesn't require fossil energy to manufacture, transport and disperse, as industrial nitrate fertilizers (Smil, 2001). Legume cover cropping or legume green manuring are widely accepted as sustainable practices due to yield advantage, as well as to high utilization efficiency of light, water, and nutrients (Willey, 1979) for the subsequent crop, and have gained the interest of researchers also because these advantages are combined with improved reliability from season to season (Helenius, 1990), reduced inputs, better quality silage (Prithiviraj, Carruthers, Fe, Cloutier-Martin, & Smith, 2000) and weed control (Bilalis, Sidiras, Economou, & Vakali, 2003). On sandy soils, the main aims of these practices are also combined with the effort to control soil erosion and maintain acceptable organic matter levels in the topsoil, especially when legumes are used as green manure (Salmeron-Miranda, Bath, Eckersten, Forkman, & Wivstad, 2007), allowing the exploitation of crop residues for bioethanol production with less environmental risk. Legume crop residues are effective sources of N (Bremer & van Kessel 1992; Haynes, Martin, & Goh, 1993), and when released in synchrony with crop N demand, crop residue N is a particularly desirable source of N, as losses to the environment are minimized (Stute & Posner, 1995; Soon, Clayton, & Rice, 2001). Faba bean seems an excellent candidate for a legume-maize cover cropping system, as it is grown during winter in temperate climates, on residual moisture after crops such as maize (Jensen et al., 2010). Using the faba bean biomass of 5-13 t DM ha<sup>-1</sup> as a green manure results in large inputs of organic N and carbon (C) to the soil, which in the long-term significantly stimulates microbial activity, enhance soil fertility, influence soil structure and water-holding capacity, improve the supply of mineral N, and improve the yield potential of crops compared to continuous cereal systems relying on N-fertilizers (e.g. Wani, McGill, Haugenkozyra, Robertson, & Thurston, 1994).

In order to incorporate cover crops into existing cropping systems, it is essential to evaluate several eco-physiological traits, such as dry matter production and nutrient-use efficiency of the sequential main crop (Daimon, 2006). Thus, the efficiency of legume contribution, such as faba bean, in these systems could be estimated both by biomass production and N use of the energy crop. Information on dry matter production and partitioning between various plant parts should permit better analysis and interpretation of the results and also allow a better understanding of processes and resource exploitation for crop production (Williams, Nageswara, Rao, Doughtedji, & Talwar, 1996). Crop N use is usually evaluated by the N-use efficiency index, or their components: N-uptake efficiency and N-utilization efficiency (Moll, Kamprath, & Jackson, 1982). N-uptake efficiency refers to the quantity of N absorbed by the plant roots relative to the available soil N, while N-utilization

efficiency, quantifies the amount of total biomass produced per unit of N uptake. N uptake along the biological cycle reflects quite closely plant growth, as  $N \text{ uptake} = \text{biomass} \times N \text{ concentration}$ . N-utilization efficiency is influenced by the transportation, partitioning and remobilization of N within the plant or within the cell, as well as by the specific metabolic processes at the cellular level (Masclaux, Quilleré, Gallais, & Hirel, 2001).

In that respect, the main objective of this study was the quantification of basic (control) N uptake, N-uptake efficiency and N-utilization efficiency, as well as total biomass production, of Mediterranean land use systems, involving the cultivation of maize, pre-cropped with *Vicia faba*, either harvested (cover crop) or incorporated in the topsoil before the sowing of maize (green manure). It was hypothesized that a) cover crops will increase NUE and main crop yield in sandy soils and b) the positive cover crop impacts found in regions with temperate climate will be also evident in regions with Mediterranean climate. Additionally, data from field experiments on the two farming systems were analyzed in combination with the bioethanol convention of maize biomass described in the literature, in an effort to obtain the integrated assessment of the potential bioethanol production.

## 2. Materials and Methods

### 2.1 Materials Studied and Area Descriptions

Field experiments were carried out in central Greece, involving the cultivation of *Zea mays* cv PR36k67 (FAO 430) as main crop after the cultivation of *Vicia faba* cv Extra Precocce, as cover crop in the period 2009-2011. The study area was consisted by two experimental fields in two locations of the Thessaly Plain, with similar climate (Figure 1) but different soil characteristics. The first experimental field was located in the Trikala area (coordinates: 39°32'17.08"N, 21°46'19.17"E, elevation 120 m ASL). The soil was Typic Xerofluvent (USDA, 1975; Soil Survey Staff, 2010), with a calcareous sandy-clayey-loamy texture (average soil particle size distribution: clay 22%, silt 18%, sand 60%), pH of 7.7, and organic matter content of 1.3% in the topsoil. The second field was located in the Sotirio area (coordinates: 39°30'02.85"N, 22°42'50.37"E, elevation 60 m ASL), and the soil was silty-clayey to clayey, classified as Vertisol (USDA, 1975; Soil Survey Staff, 2010), with average soil particle size distribution of clay 63%, silt 35%, sand 2%, pH of 7.9, and 1.7% organic matter content in the topsoil.

### 2.2 Field Experiments and Management

In each site, the experimental design was a factorial split plot in three replicates. The main factor was nitrogen dressings (in 4 treatments: 0, 80, 160, 240 kg N ha<sup>-1</sup> for maize). The sub-factor was cover crop management (in 3 treatments: I = incorporated into the topsoil upon full flowering (flowers open on 5 racemes per plant), H = harvested before the sowing of the main crop, and C = control, mono-crop). All fertilization plots were maintained at the same spots during the entire experimental period. Planting arrangement was 75 cm x 20 cm for maize and 25 cm x 15 cm for faba bean. Plots size was 9 m<sup>2</sup>, consisted of four rows 75 cm apart and an average plant density for maize of 10 plants m<sup>-2</sup>. Control plots were left fallow (no cover crop) during winter, while the same growing techniques and irrigation schedule (Table 1) were followed for all plots, except for the N dressings. Fertilization was applied in two doses. The first was applied at sowing as basal dressing with 80 kg N ha<sup>-1</sup> (nitrate form) in all plots, except control plots in which only P and K fertilization was applied. The second dose (ammonium form) was applied on the onset vegetative phase of maize, when plant height was approximately at 50 cm. Sowing dates and other relevant agronomic data are summarized in Table 1. The amount of dry biomass of faba bean incorporated in the topsoil was measured directly by means of destructive sampling upon full flowering each year. In all years/sites combinations, the mean cover crop biomass amount incorporated was 9-9.5 Mg ha<sup>-1</sup>. Maize was grown under optimum water and nutrient availability, and no macro-nutrient deficit, or water stress, were observed throughout the crop cycle.

### 2.3 Methods and Techniques

At harvest (Table 1), all plots were sampled (1.5 m<sup>2</sup> of the two middle rows of 1 m each), for plant dry weight and yield components. All plant samples were cut near the soil surface and the belowground fractions were left in the field. Dry matter of seed, stems and leaves were determined by drying sub-samples in a convection oven at 70°C until constant weights. The samples were then milled to a fine powder with particle size of <1 mm to determine the total N content, using the standard Kjeldahl method (Nelson & Sommers, 1973). N-uptake was determined by multiplying the measured dry matter weights by their respective N concentrations. Total potential bioethanol production was estimated by multiplying the measured dry matter weights by their respective potential bioethanol production, viz. 360 lt Mg<sup>-1</sup> for maize seed and 280 lt Mg<sup>-1</sup> for maize stover (Linoj Kumar, Dhavala, Goswami, & Maithel, 2006).

Weather data for the Sotirio experimental field, were recorded hourly on an automatic meteorological station installed next to experimental site. Incident solar radiation, air temperature, rainfall and class-A pan evaporation

rate were monitored. For the Trikala experimental field, actual weather data were obtained from the nearest meteorological station of the National Meteorological Service situated 200 m far from the field.

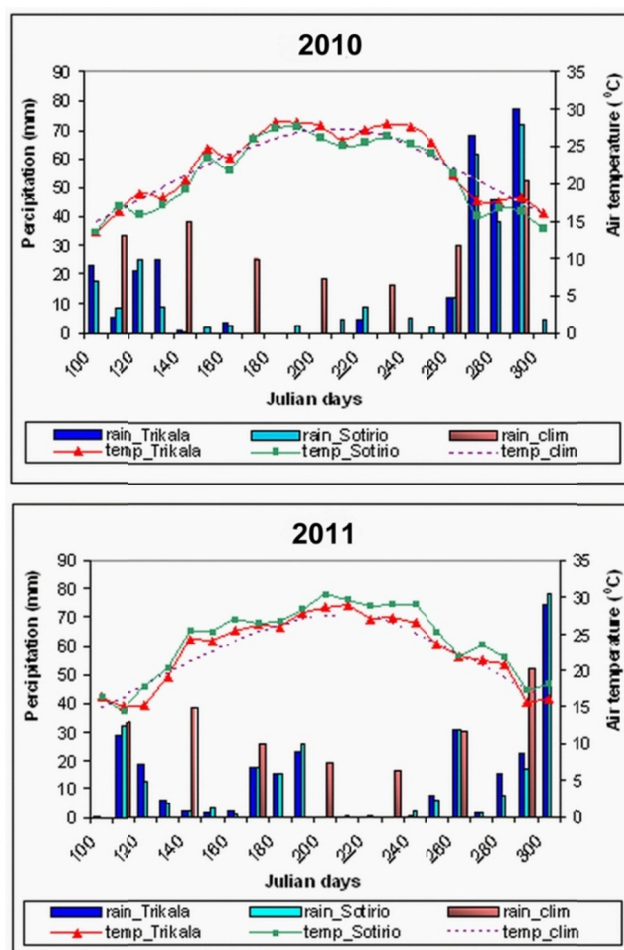


Figure 1. Temperature and precipitation (10-days mean values), occurring in the study areas during the growing period of maize, in 2010 (above) and in 2011 (below). Climatic air temperature (dashed line) and monthly total precipitation are also presented (data acquired from N. M. S. for 1955 to 2005)

Table 1. Agronomic data for the two experimental areas and two cultivation periods

	Trikala (sandy soil)		Sotirio (clayey soil)	
	2010	2011	2010	2011
<i>Vicia faba</i>				
Sowing	04/02	02/02	07/01	03/01
50% emergence	18/02	13/02	04/02	31/01
Incorporation	09/05	25/04	08/04	03/04
Harvest	05/06	27/05	29/05	25/05
<i>Zea mays</i>				
Sowing	18/06	17/06	08/06	12/06
50% emergence	26/06	22/06	14/06	18/06
Harvest	16/10	13/10	12/10	15/10
Irrigation (mm)	350	300	450	400
Irrigation method	sprinkler	sprinkler	drip	drip
Fertilization (kg ha <sup>-1</sup> )	P=50, K=50	P=50, K=50	P=50, K=50	P=50, K=50

All measured and derived data (from the calculations mentioned) data were subjected to analysis of variance (ANOVA), using SPSS18 software, following the experimental design and the GLM method. As test criterion for detecting differences between means the  $LSD_{0.05}$  was used (Steel & Torrie, 1982). The data presented in this paper refer to the second and third year of the experiment. Soil-plant N relationships are presented with the use of 'three quadrant diagrams' (Van Keulen, 1982; De Wit, 1992). With such diagrams, yield response to nutrient input, "agronomic response", (quadrant I) is dissected into their components: the relation between N uptake and yield (physiological efficiency, quadrant II), which is primarily determined by the physiology of the plant and the relation between N supply and N uptake (quadrant III) which is governed by processes in the soil.

### 3. Results

#### 3.1 Weather Conditions

In general both study areas are characterized by a typical Mediterranean climate with hot, dry summers and cool, humid winters. Figure 1 illustrates the mean air temperature and precipitation for both experimental areas in 2010 and 2011. Mean air temperature reached a value of  $26^{\circ}\text{C}$  and maintained at this level in both years despite the higher fluctuation over the climatic average in 2010. Total precipitation from April to October was 286 and 271 mm for Trikala and Sotirio respectively in 2010, while 269 and 258 mm in 2011. During the vegetative phase of the crop, precipitation was higher in 2011, and as a result less irrigation was needed. In all experimental years, average relative humidity during the growing season was higher in the Trikala area. Average relative humidity was 61.44% in Trikala, while 58.67% in Sotirio. Solar radiation was similar for both study areas, but reached higher values in 2010. During the period that maize was cultivated reached average of  $263.41 \text{ W m}^{-2}$  in 2010, over an average of  $204.52 \text{ W m}^{-2}$  in 2011.

#### 3.2 Biomass Production

The results allocated considerable increments in seed production, as well as in total dry biomass production and a significant effect ( $P < 0.05$  and  $P < 0.01$ ) of faba bean treatment at almost all cases (years and soil types) and for all N fertilization levels. Significant differences ( $P < 0.05$  and  $P < 0.01$ ) on seed production, total dry biomass production and total N uptake were also observed between the N fertilization levels but no interactions faba bean treatment  $\times$  N fertilization were found for any of the monitored parameters.

In the sandy soil (Table 2), plots treated with faba beans and maximum inorganic fertilization, exhibited higher seed and total biomass production ( $12.24 \text{ Mg ha}^{-1}$  seed and  $19.66 \text{ Mg ha}^{-1}$  biomass respectively), compared to the control unfertilized plots ( $7.82 \text{ Mg ha}^{-1}$  seed and  $12.43 \text{ Mg ha}^{-1}$  biomass respectively, without any inorganic or organic influx). In the clayey soil (Table 3), the results also confirmed the positive effect of faba bean cover cropping, as total dry biomass productivity reached a mean of  $19.75 \text{ Mg ha}^{-1}$  and  $18.75 \text{ Mg ha}^{-1}$  when faba bean was used as green manure and as winter cover crop, respectively, and  $17.71 \text{ Mg ha}^{-1}$  without previous legume cultivation, on the second year of the experiment. Despite the lower productivity of the third year (measured data of solar radiation and class-A pan evaporation rate suggest lower rates of photosynthesis for this year), a mean difference of  $1.52 \text{ Mg ha}^{-1}$  in total dry biomass in the case of sandy soil and of  $2.22 \text{ Mg ha}^{-1}$  in the case of clayey soil was equally present between the cover cropping management practices and mono-cultivation of maize.

Table 2. Seed dry biomass ( $\text{Mg ha}^{-1}$ ) and seed N uptake ( $\text{kg ha}^{-1}$ ), total dry biomass ( $\text{Mg ha}^{-1}$ ) and total N uptake ( $\text{kg ha}^{-1}$ ) of maize, as affected by the three faba bean treatments (incorporated or harvested before sowing of the main crop, or fallow-control) in the Trikala area (sandy soil)

Trikala (sandy soil)				2010				
	Incorp.	Harvest.	Contr.	LSD <sup>a</sup>	Incorp.	Harvest.	Contr.	LSD <sup>a</sup>
	Total dry matter ( $\text{Mg ha}^{-1}$ )				Dry seed ( $\text{Mg ha}^{-1}$ )			
N <sub>0</sub>	14.59	14.91	12.43	2.48*	9.22	8.99	7.82	1.17*
N <sub>1</sub>	17.22	15.34	14.93	2.29*	10.71	9.57	8.75	1.97*
N <sub>2</sub>	18.29	18.40	15.81	ns <sup>b</sup>	11.58	11.31	9.56	1.75*
N <sub>3</sub>	19.66	19.48	16.90	2.57*	12.24	11.77	10.19	1.58*
Mean	17.44	17.03	15.02	2.42*	10.94	10.41	9.08	1.33*
	Total N uptake ( $\text{kg ha}^{-1}$ )				Seed N uptake ( $\text{kg ha}^{-1}$ )			
N <sub>0</sub>	138.96	127.63	94.03	44.93*	102.38	98.17	69.97	28.20*
N <sub>1</sub>	147.19	134.92	115.75	31.44**	111.94	104.07	86.54	17.53*
N <sub>2</sub>	165.74	164.92	123.13	41.75*	122.13	121.38	93.38	27.00*
N <sub>3</sub>	194.05	180.72	134.88	45.84*	138.96	127.46	101.43	37.53*
Mean	161.48	152.04	116.95	35.09**	118.85	112.77	87.83	24.94**
2011								
	Incorp.	Harvest.	Contr.	LSD <sup>a</sup>	Incorp.	Harvest.	Contr.	LSD <sup>a</sup>
	Total dry matter ( $\text{Mg ha}^{-1}$ )				Dry seed ( $\text{Mg ha}^{-1}$ )			
N <sub>0</sub>	13.61	13.42	11.45	1.96**	7.91	7.59	6.82	1.09*
N <sub>1</sub>	14.83	14.77	13.18	1.59*	8.80	8.57	7.73	0.84*
N <sub>2</sub>	15.16	15.38	13.95	1.20*	8.94	8.90	8.15	0.75*
N <sub>3</sub>	16.24	16.37	15.19	1.05**	9.39	9.49	8.56	0.84**
Mean	14.96	14.98	13.44	1.51**	8.76	8.64	7.81	0.83**
	Total N uptake ( $\text{kg ha}^{-1}$ )				Seed N uptake ( $\text{kg ha}^{-1}$ )			
N <sub>0</sub>	114.29	105.60	90.94	14.65*	75.00	71.61	60.73	ns <sup>b</sup>
N <sub>1</sub>	131.49	120.51	99.73	20.78*	84.65	82.23	68.04	14.18*
N <sub>2</sub>	142.81	136.52	107.01	29.52*	92.97	90.05	70.96	19.09**
N <sub>3</sub>	165.18	155.94	118.36	37.58**	110.32	107.64	73.07	34.57**
Mean	138.45	129.64	104.01	25.63**	90.74	87.88	68.20	19.68**

N<sub>0</sub>: 0, N<sub>1</sub>: 80, N<sub>2</sub>: 160, N<sub>3</sub>: 240  $\text{kg ha}^{-1}$ .

<sup>a</sup> LSD: least significant difference at  $P < 0.05$ (\*) και  $P < 0.01$ (\*\*).

<sup>b</sup> ns: no significant.

Table 3. Seed dry biomass ( $\text{Mg ha}^{-1}$ ) and seed N uptake ( $\text{kg ha}^{-1}$ ), total dry biomass ( $\text{Mg ha}^{-1}$ ) and total N uptake ( $\text{kg ha}^{-1}$ ) of maize, as affected by the three legume treatments (incorporated or harvested before sowing of the main crop, or fallow-control) in the Sotirio area (clayey soil)

Sotirio (clayey soil)				2010				
	Incorp.	Harvest.	Contr.	LSD <sup>a</sup>	Incorp.	Harvest.	Contr.	LSD <sup>a</sup>
	Total dry matter ( $\text{Mg ha}^{-1}$ )				Dry seed ( $\text{Mg ha}^{-1}$ )			
N <sub>0</sub>	15.33	15.13	13.42	1.71*	8.32	8.27	7.42	0.85*
N <sub>1</sub>	19.88	18.87	17.38	ns <sup>b</sup>	10.27	9.81	9.17	1.09*
N <sub>2</sub>	21.36	19.33	19.69	1.66*	11.66	10.91	9.68	1.22*
N <sub>3</sub>	22.42	21.68	20.33	1.34*	12.50	12.38	10.54	1.84**
Mean	19.75	18.75	17.71	2.04*	10.69	10.34	9.20	1.14**
	Total N uptake ( $\text{kg ha}^{-1}$ )				Seed N uptake ( $\text{kg ha}^{-1}$ )			
N <sub>0</sub>	123.08	114.28	100.64	22.44*	85.30	81.88	72.56	12.74*
N <sub>1</sub>	143.11	139.35	120.37	ns <sup>b</sup>	101.22	98.44	87.06	14.17*
N <sub>2</sub>	164.77	159.32	134.24	30.52*	118.89	115.25	95.16	23.72*
N <sub>3</sub>	181.05	172.33	138.73	33.60*	129.54	124.41	98.58	25.83*
Mean	153.00	146.32	123.49	22.82**	108.74	104.99	88.34	16.66**
2011								
	Incorp.	Harvest.	Contr.	LSD <sup>a</sup>	Incorp.	Harvest.	Contr.	LSD <sup>a</sup>
	Total dry matter ( $\text{Mg ha}^{-1}$ )				Dry seed ( $\text{Mg ha}^{-1}$ )			
N <sub>0</sub>	13.65	13.27	11.84	1.43**	7.29	7.08	6.24	0.84**
N <sub>1</sub>	14.72	14.12	12.59	1.53**	8.12	7.85	6.95	0.90**
N <sub>2</sub>	15.86	15.55	13.72	1.83*	8.94	8.90	7.73	1.17*
N <sub>3</sub>	16.94	16.19	14.14	2.05**	9.39	9.25	8.07	1.18**
Mean	15.29	14.78	13.07	1.71**	8.44	8.27	7.25	1.02*
	Total N uptake ( $\text{kg ha}^{-1}$ )				Seed N uptake ( $\text{kg ha}^{-1}$ )			
N <sub>0</sub>	114.87	105.23	90.44	14.79*	73.32	64.80	53.92	10.89*
N <sub>1</sub>	136.02	133.64	104.10	29.54**	85.19	82.41	63.24	19.17**
N <sub>2</sub>	159.47	149.26	113.61	35.65**	98.09	96.49	70.35	26.13**
N <sub>3</sub>	177.96	168.12	123.42	44.70**	108.34	104.75	81.02	23.73**
Mean	147.08	139.06	107.89	31.17**	91.24	87.11	67.13	19.98**

N<sub>0</sub>: 0, N<sub>1</sub>: 80, N<sub>2</sub>: 160, N<sub>3</sub>: 240  $\text{kg ha}^{-1}$ .

<sup>a</sup> LSD: least significant difference at  $P < 0.05$ (\*) and  $P < 0.01$ (\*\*).

<sup>b</sup> ns: no significant.

### 3.3 Seed Yield and N Availability

Seed dry biomass yields of maize under four N-dressings for the two faba bean treatments, plus control (mono-cropping), are schematically presented in quadrant I of Figure 2 for 2010 and Figure 3 for 2011, for sandy soil and in Figures 4 and 5 for 2010 and 2011, respectively, for clayey soil.

In case of sandy soil, as illustrated in quadrant I of Figures 2 and 3, N-dressing had a positive effect on seed productivity in control plots, which fluctuated from  $7.82 \text{ Mg ha}^{-1}$  to  $10.19 \text{ Mg ha}^{-1}$  in 2010 and from  $6.82 \text{ Mg ha}^{-1}$  to  $8.56 \text{ Mg ha}^{-1}$  in 2011. No significant differences ( $P > 0.05$ ) were observed between 0 and 240  $\text{kg N ha}^{-1}$  application input levels in green manured and cover cropped plots, indicating that both faba bean managements are equally efficient in respect of seed productivity.

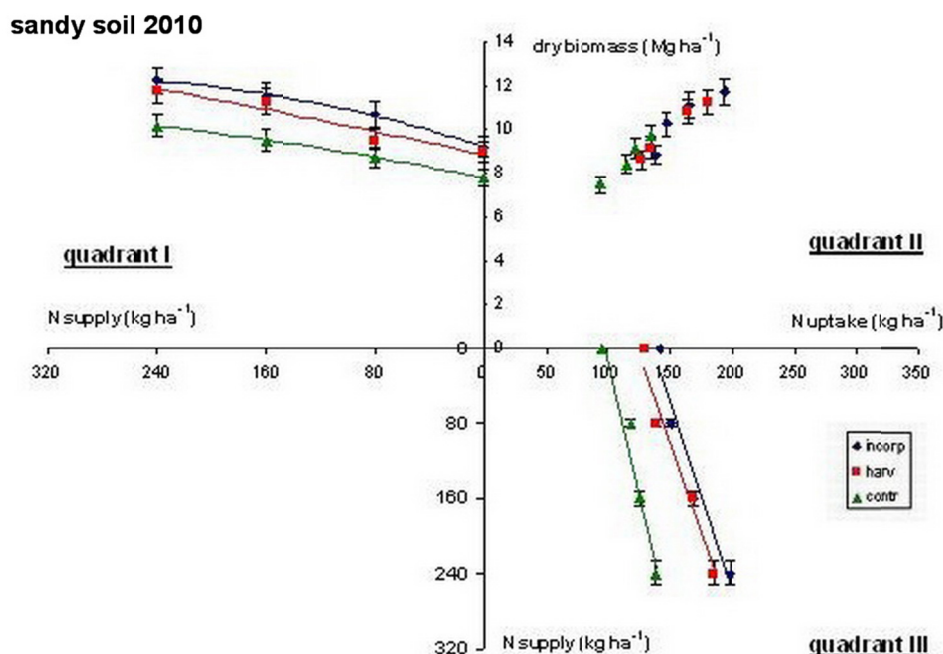


Figure 2. Three-quadrant diagram of maize seed yield response to N application and three faba bean cover cropping practices, in Trikala area (sandy soil), in 2010. Vertical bars indicate standard error of means

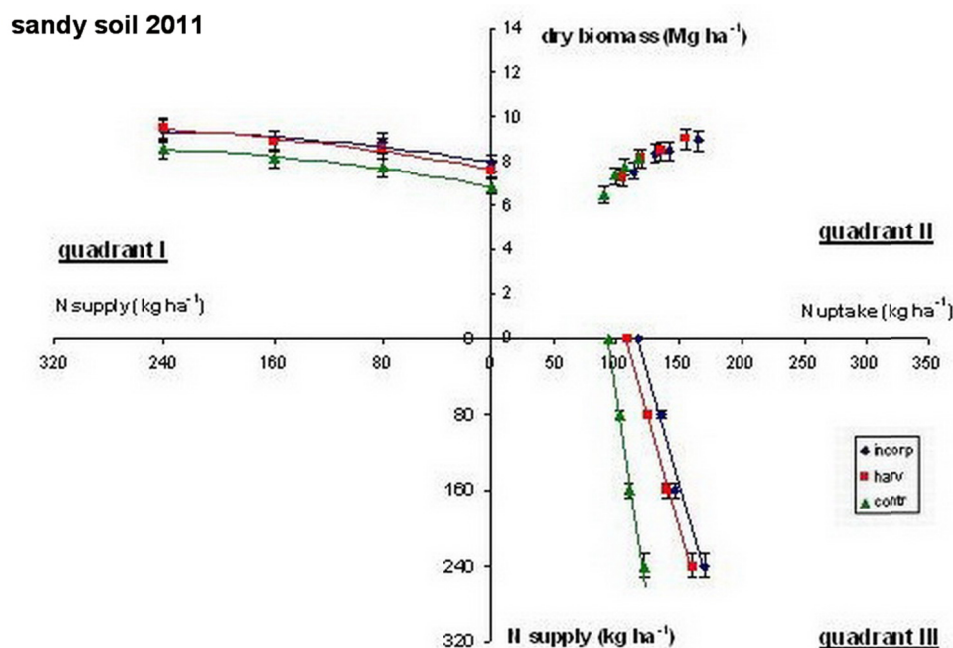


Figure 3. Three-quadrant diagram of maize seed yield response to N application and three faba bean cover cropping practices, in Trikala area (sandy soil), in 2011. Vertical bars indicate standard error of means

As depicted from Figures 4 and 5, quadrant I, seed yield was increased in response to nitrogen application in all cases, in clayey soil also. Actually, in the case of faba bean incorporation, a remarkable yield increase was recorded in all cases, and yield of  $8.32 \text{ Mg ha}^{-1}$  for maize seed was possible under no fertilization (control). Maximum yields reached as much as  $12.50 \text{ Mg ha}^{-1}$ , but very high yields were reached upon modest fertilization also, as maize seed yield reached  $10.27 \text{ Mg ha}^{-1}$  with N-dressing of  $80 \text{ kg ha}^{-1}$ .



### 3.4 Biomass Yield and NUE

Nitrogen use efficiency (NUE) is defined as Agronomy efficiency = yield/supply or physiological efficiency = yield/uptake (Van Keulen, 1982), and also as the maximum economic yield produced per unit of N applied, absorbed or utilized by the plant to produce grain and straw (Moll et al., 1982). As already mentioned, this NUE can be divided into two processes: uptake efficiency (NUpE; the ability of the plant to remove N from the soil as nitrate and ammonium ions) and the utilization efficiency (NUtE; the ability to use N to produce grain or biomass yield). When dry matter or grain yield is multiplied by N concentration, the results are a measure of nutrient uptake and expressed in accumulation or uptake units, become useful indicators of soil fertility depletion and are related to crop yield levels. Nitrogen accumulation patterns in crop plants follow dry matter accumulation, as the amount of N accumulated generally parallels dry matter accumulation and increases with plant age (Miller, Gan, McConkey, & McDonald, 2003).

#### 3.4.1 N supply-N Uptake Relations

At harvest, the relations of plant N uptake to the N supplied by organic or inorganic fertilization reflect the efficiency of the applied nitrogen fertilization, known as the recovery fraction (Rf). This fraction can be calculated as the ratio: of (N uptake at  $N_x$  - N uptake at  $N_0$ ) to applied N at  $N_x$ , and is geometrically presented by the slope of the lines in quadrant III in Figures 2, 3, 4 and 5. The x-axis intercepts represent the basic uptake, viz. the N uptake under no fertilization. This basic N uptake is a good indicator of N (annually) mineralized and therefore of the inherent fertility of the particular soil (organic matter content, C/N ratio, soil structure, soil physical properties, residual N, etc) and the external weather conditions. Such information is very useful for N fertilization recommendations.

For sandy soil (Figures 2, 3, quadrant III), mineral N uptake (basic uptake), ranged from 94 kg ha<sup>-1</sup> for the control plots, to 139 and 127 kg ha<sup>-1</sup> with legume cover cropping. This means that 33 kg ha<sup>-1</sup> and 45 kg ha<sup>-1</sup> N may have been additionally mineralized, in the case of cover crop and green manure respectively, as net mineralization during the growing season can vary from 0.25 to 1.50 kg N per ha per day, depending on weather, soil conditions and the nature and management of crop residues and cover crops (Schröder, Neeteson, Oenema & Struik, 2000). Although lower base N uptake values (in accordance to the lower biomass productivity) were found on the following year (Figure 3, quadrant II), e.g. 90, 105 and 114 kg ha<sup>-1</sup> for control, cover crop and incorporated cover crop, respectively, the differences of basic N uptake remained at high values e.g. 15 kg ha<sup>-1</sup> and 24 kg ha<sup>-1</sup> verifying the beneficial contribution of faba bean as cover crop and green manure on the fertility of poor sandy soils.

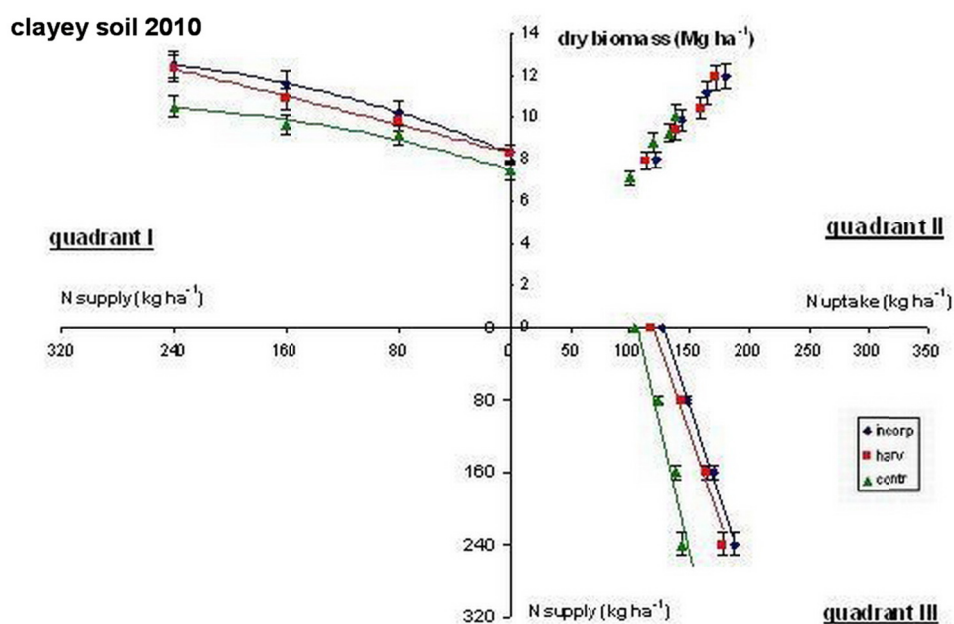


Figure 4. Three-quadrant diagram of maize seed yield response to N application and three faba bean cover cropping practices, in Sotirio area (clayey soil), in 2010. Vertical bars indicate standard error of means

Similar results were found for clayey soil (Figures 4, 5 quadrant III), e.g. 100, 114 and 123 kg ha<sup>-1</sup> soil mineral N uptake for control, cover crop and incorporated cover crop, respectively, meaning that 14 kg ha<sup>-1</sup> and 23 kg ha<sup>-1</sup> N may have been additionally mineralized, in the case of cover crop and green manure, on the second year, and 15 kg ha<sup>-1</sup> and 24 kg ha<sup>-1</sup> N on the following year.

Accordingly to base uptake rates, the N-Rf was also affected by faba bean rotation, as N-Rf mean values for sandy soil were increased from an average of 15-20% for control plots to an average of 30% for plots with previous faba bean cultivation (for both management practices), while in the case of clayey soil, the increase in plots with previous faba bean cultivation was even higher reaching 30-35% N recovery, probably due to also higher soil moisture.

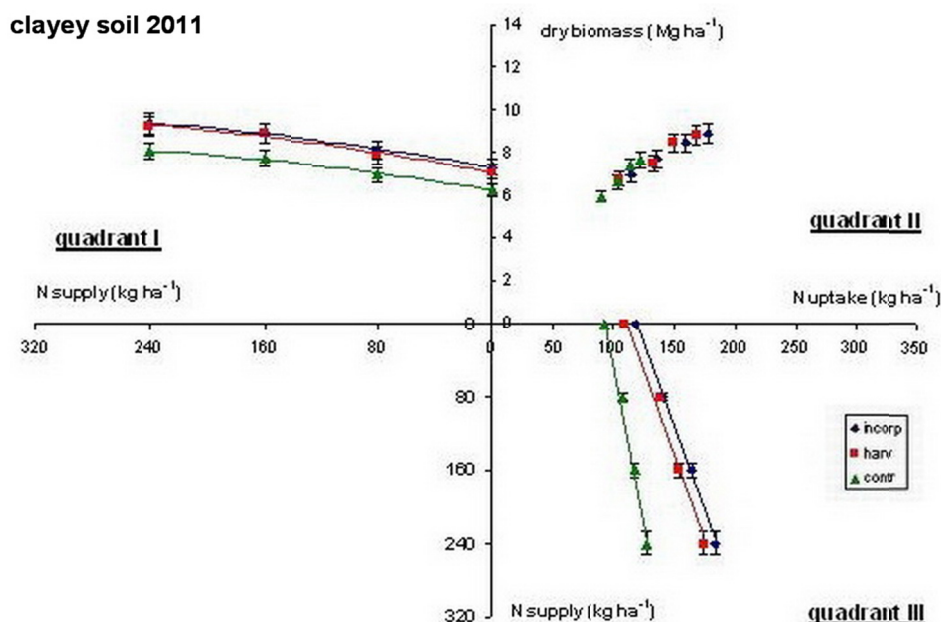


Figure 5. Three-quadrant diagram of maize seed yield response to N application and three faba bean cover cropping practices, in Sotirio area (clayey soil), in 2011. Vertical bars indicate standard error of means

### 3.4.2 N Utilization-Yield Relations

N-utilization efficiency quantifies the amount of grain/biomass produced per unit of N uptake. NUtE is calculated as the ratio of dry grain yield to total N uptake for maize, and is represented by the slopes of the curves of the diagrams in quadrant II in Figures 2, 3, 4 and 5, with mean values 55-56 kg dry grain produced per kg N taken up and 54-55 kg dry grain produced per kg N taken up for Trikala and Sotirio area respectively. The linear yield-uptake relations found in the case mono-cropping (control plots) (Figures 2, 3, 4, 5, quadrant II), are connected to the minimum nitrogen concentrations in the plant tissue upon stress conditions, meaning that potential productivity was not yet obtained. Oppositely, as shown in Figures 2,3,4,5, quadrant II, for the cover cropping managements and especially for the incorporation of faba bean as green manure, with increasing N rates applied, NUtE declines from linearity, signaling an increase in N concentrations in the tissues (luxurious growth).

### 3.5 Potential Energy Yield

A theoretical way of calculating the bioethanol production from dry biomass of maize is formulated as:

$$\text{Total bioethanol production (lt ha}^{-1}\text{)} = 360 (\text{lt Mg}^{-1}) \times \text{dry seed biomass (Mg ha}^{-1}\text{)} + 280 (\text{lt Mg}^{-1}) \times \text{dry stover biomass (Mg ha}^{-1}\text{)} \quad (1)$$

Figure 6, schematically illustrates the mean values of bioethanol production of each treatment per ha, when equation 1 is applied to the values of maize seed harvested from each experimental plot, in combination with the use of maize crop residues for the production of second generation bioethanol (lignocellulosic bioethanol). Those values fluctuated from 3750, 4360 and 4440 lt ha<sup>-1</sup>, for incorporated, harvested and control treatments, without N inputs, up to 6530, 7060 and 7280 lt ha<sup>-1</sup> for incorporated, harvested and control treatments, with high N inputs, respectively. It is clearly seen that exploitation of crop residues for the production of second generation bioethanol

can theoretically subsist from 1500 to 2500  $\text{lt ha}^{-1}$  additional biofuel, which as depicted from Figure 6 seems to be unaffected by the level of inorganic N applied.

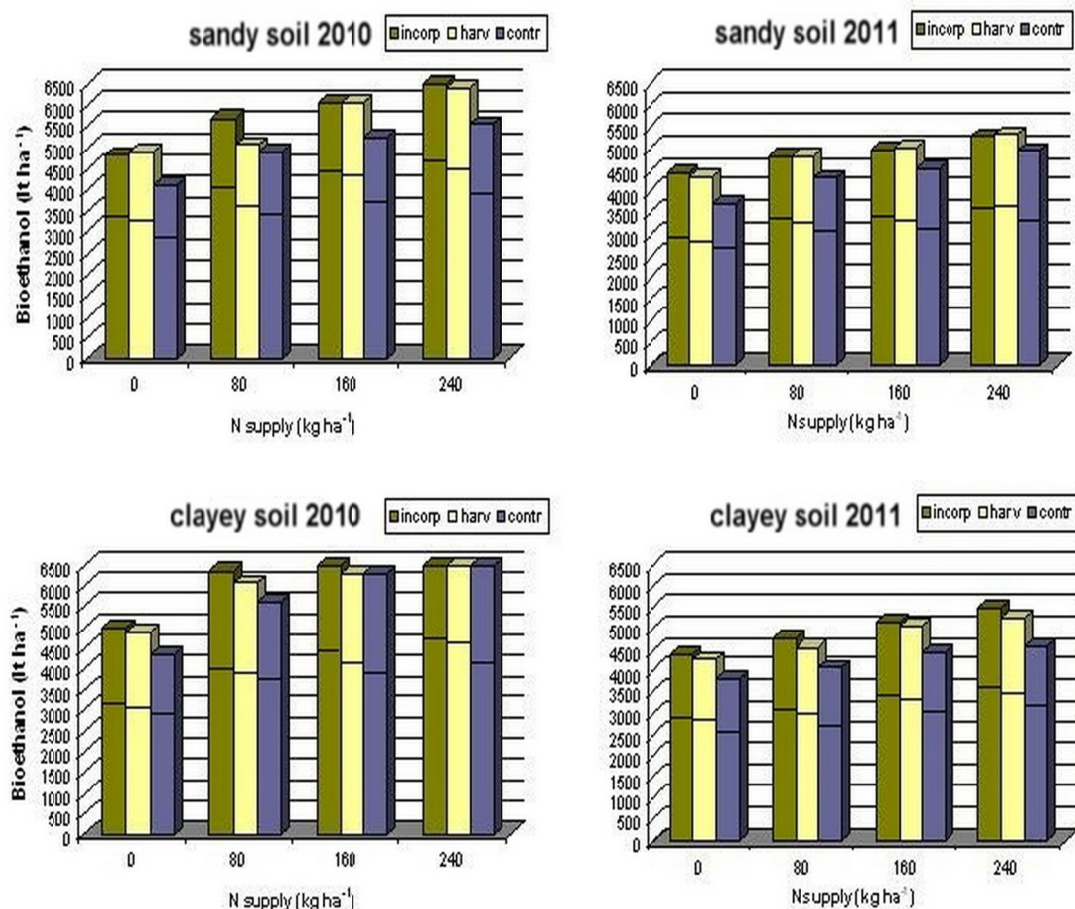


Figure 6. Potential bioethanol production from maize total dry biomass in 2010 (left) and 2011 (right), to four nitrogen application (0, 80, 160 and 240  $\text{kg ha}^{-1}$ ) and three faba bean cover cropping practices (incorporated in the topsoil, harvested before sowing of maize and control), on sandy soil (above) and clayey soil (below). The lower section of each column represents potential bioethanol production from maize seeds and the upper section potential bioethanol production from maize crop residues

#### 4. Discussion

This experiment attempts to evaluate the potential effect of cover cropping faba bean on maize yield and nitrogen utilization efficiency, compared to maize monocrop, in two years field experiments conducted in two soils of contrasting texture, central Greece. Productivity and N utilization efficiency of maize, the dominant source of biomass-based liquid transportation fuels, are investigated under cropping practices that may result in greater efficiencies in N management and use in agroecosystems and reduce N-related environmental problems.

This two years study showed that both cover cropping managements can lead to substantial increments on maize seed and total biomass production, compared to traditional mono cropping system. At the same time although maximum dry biomass yield was obtained on both soil types under maximum fertilizer rate, as in maize, the amount of biomass accumulated increases with increasing N supply (Muchow, 1988), when comparing the effect of fertilization treatments among faba bean cover cropping treatments, notably larger yields were harvested at even modest N fertilizer levels. Particularly faba bean, as green manure, increased maize yields when used together with half the recommended rate of inorganic fertilizers, which is consisted with previous studies (Bunch, 1995). Results suggested that in all cases (sites and years), the combined application of faba bean residues and inorganic N fertilizer, could be an effective strategy of achieving larger biomass yield at smaller fertilizer rates, as maize

following leguminous crops can show improved root growth and root vigour, which can have consequences for the uptake of nutrients and water (Van Zeeland, Lamers, & Van Dijk, 1999). So cover cropping could be a potentially useful practice for obtaining comparable or even higher maize yields with less fertilizer N. This is reflected by different yield responses of maize in monoculture and maize grown in rotation to N fertilization (Lory, Russelle, & Randall, 1995; Omay, Rice, Maddux, & Gordon, 1998; Peterson & Varvel, 1989; Varvel & Peterson, 1990). The decreasing positive effect of cover cropping with increasing N fertilizer level indicated that raising the N fertilizer rate could easily offset the negative effects of maize in monoculture. In other words, to reach maximum economical profits, lower levels of N fertilizer had to be applied in cover cropped grown maize. This should be attributed both to the additional N provided by the legume crop residues and to additional N immobilized and gradually N mineralized by the legume, through biological nitrogen fixation and organic legume N (Burgess, Mehuys, & Madramootoo, 2002). During the mineralization of leguminous materials, up to 50% of the amount of N can be released within two months of incorporation into topsoil depending on the prevailing soil temperature and moisture conditions (Kirchmann & Bergqvist, 1989).

Cover cropping faba bean had the same beneficial effect on N uptake as well as on N-Rf, on both study soil types and years. The fraction of total plant N uptake allocated to maize aboveground plant parts is known to depend on environmental factors such as N availability (Gleeson, 1993) and cultivar (Fichtner & Schulze, 1992), and consequently the N content in aboveground plant parts varies greatly, with values between 50 kg N ha<sup>-1</sup> and 300 kg N ha<sup>-1</sup> (Fageria & Baligar, 2005; Bilalis et al., 2005). In that respect, cover-cropping managements had a very distinctive influence on N uptake values, in all cases. An increment of basic N uptake fluctuating from 15 to 45 kg ha<sup>-1</sup> was recorded, as expected. Similarly to total N uptake, cover cropping managements had a positive effect to the fraction of N uptake by the seeds, as in maize, 45–65% of the grain N is provided from pre-existing N in the stover before silking, while the remaining 35–55% of the grain N originates from post-silking N uptake (Gallais & Coque, 2005). Positive effect of cover-cropping management to N uptake, was recorded for all N fertilizer rate treatments as well. Fertilizer recovery is the result of the balance between crop N uptake and N immobilization by microbial processes in soils of different compositions. Therefore, the concept of the NUE of a crop should also be considered as a function of soil texture, climate conditions, interactions between soil and bacterial processes (Walley, Yates, van Groenigen & van Kessel, 2002; Burger & Jackson, 2004), and the nature of organic or inorganic N sources (Schulten & Schnitzer, 1998). The enhanced N-Rf could assist apart from economic viability, especially in the case of sandy soil, in limiting nitrate leaching to deep soil layers and avoiding ground water pollution with environmentally noxious chemicals, as earlier mentioned.

Furthermore, as illustrated in quadrant II of Figures 2, 3, 4 and 5, a strong proportional relation existed between biomass yield and N uptake. The almost linear relationships presented in these figures define the minimum N requirement and the maximum NUE at a given yield level. The higher slope of the linear relationship for maize cover cropping than for mono cropping indicates that the NUE increases more with increasing seed yield in cover cropping than in mono cropping. Nitrogen uptake in excess of that required for yield determination results in lower NUE than the maximum value for a given yield level. This luxury N uptake is associated with increased stem and seed N concentration (Muchow, 1988). Our results, as well as other studies (Bänziger, Beärn & Lafitte, 1997; Bertin & Gallais, 2000; Laffite & Edmeades, 1994), prove that maize can become specifically adapted to environments with a reduced input of N.

As far as potential bioethanol productivity, it is obvious that in full fertilized treatments the production of bioethanol was higher than the unfertilized treatments, corresponding to the relation of total fresh and dry biomass production. However, the high potential production of cover cropped treatments, even without N influx must be noticed, taking into consideration that apart from net energy content, cover cropping managements assist in higher energy yield (the difference between the energy content of the harvested biomass and the energy used throughout the whole growing season for soil tillage, sowing, fertilizers, chemicals, etc.) and higher energy efficiency (the ratio between the entire energy content of biomass yield and the energy utilized in the cropping system) (Cosentino, Copani, Patanè, Mantineo, & D'Agosta, 2008). At the same time, faba bean cover cropping, can assist in enhancing the maize bioethanol production system Net Energy Balance via enhancing NUE and N-Rf (Hattori & Morita, 2010), but even more notably, also allows the exploitation of maize crop residues for bioethanol production, without environmental risks. It is known that the utilization of crop residues should be carefully considered because they are well known to be important to maintain sustainability of crop production through prevention of soil degradation, improvement of soil water balance, maintenance of soil organic carbon content (e.g. McAloon et al., 2000; Allmaras et al., 2000; Clapp et al., 2000), aspects that legume cover cropping could counter balance.

Despite many studies with faba bean in cropping systems (e.g. Pare, Chalifour, Bourassa, & Antoun, 1993; Rochester, Peoples, Hulugalle, Gault, & Constable, 2001; R. J. Lopez-Bellido, Lopez-Bellido, L., Benitez-Vega, & F. J. Lopez-Bellido, 2007; Walley, Clayton, Miller, Carr, & Lafond, 2007), there seems to be a serious knowledge gap concerning what management practices are able to create the most suitable environment for a succeeding faba bean-maize cover cropping system. Hence, the investigation of the sustainability of bio-energy plants in low input-cropping systems, along with the estimation of macro-nutrient utilization efficiency, is necessary.

## 5. Conclusions

Results demonstrated that cover cropping systems, especially involving the use of faba bean as cover crop or green manure, deserve increased attention and tend to be superior to the traditional monocrop systems in quantitative and qualitative characteristics. They substantially increased the base uptake and the recovery fraction of nitrogen, resulting in large increment of the final yield productivity of maize. Due to their low inherent fertility, light textured soils may produce satisfactory biomass yields up to 17 Mg ha<sup>-1</sup> under high N-dressings that impose nitrification hazards. Alternatively, cover cropping, or incorporation in the soil, of *V. faba*, before the sowing of maize may result in equal biomass production levels, making the effort to reduce both economical inputs as well as environmental impacts, possible. On fertile alluvial soils, high total biomass yields (15 Mg ha<sup>-1</sup>) could be obtained even under no N fertilization when faba bean is incorporated in the topsoil, while yields of 21 Mg ha<sup>-1</sup> could be obtained with modest N-dressing of 160 kg ha<sup>-1</sup>.

Secondly, significant differences were observed among treatments, both for cover cropping management and for N fertilization, in final biomass yield, grain yield, NUE and N-Rf. Higher biomass production levels may result for both soil types mainly due to the increase in N-mineralization (base uptake) and the enhanced fertilizer recovery fraction (10-15%).

Furthermore, considering the attained maximum biomass and average bioethanol convention for maize, cover cropping practices could provide up to 1500-2000 lt ha<sup>-1</sup> of additional second generation biofuel, by permitting the exploitation of crop residues with smaller environmental risk.

The review on agronomic and energetic aspects of maize when subsumed in an appropriate legume cover cropping scheme, shows that this strategy may result in greater efficiencies in N management and use in agro-ecosystems, reduction of N-related environmental problems, without an appreciably great loss in productivity. Such systems should be seriously considered in future land use planning, with respect to the sustainable cultivation of maize in Mediterranean agro-ecological zones.

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