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RESEARCH ARTICLE

Application of liquid cattle manure and inorganic fertilizers affect dry matter, nitrogen accumulation, and partitioning in maize

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Abstract Efficient use of N applied in the form of organic and inorganic fertilizers is important in maize (*Zea mays* L.) production to maximize producer's economic returns and maintain soil and water quality. A field study was conducted for three consecutive years (2003–2005) in Thessaloniki, Greece to investigate whether liquid cattle manure can be used to replace inorganic fertilizers and also whether inorganic fertilizer can be applied preplant or as a combination of preplant and sidedress and can affect maize growth, development and N use efficiency. The treatments were control (unfertilized), liquid dairy cattle manure (Manure), application of 260 kg N ha^{-1} $year^{-1}$ as basal dressing (N-single), application of 130 kg ha^{-1} $year^{-1}$ N as basal dressing before sowing and 130 kg N ha^{-1} when plants were at the eight-leaf stage (V8) (N-split). In 2 out of the 3 years of the study there was a significant positive effect of fertilizer application on maize growth, development, N uptake, and partitioning compared with the control. Dry matter production was increased by an average of 39% during the 2 years in plots fertilized either with manure or inorganic fertilizers than the control plots. Also from the yield components kernel weight per ear and number of kernels per ear were increased by an average of 35% and 32%, respectively in the fertilized plots compared with the control plots. Chlorophyll level was affected as it was increased by an average of 18%, 14%, and 18% at the ten-leaf stage (V10), silking and milk stage, respectively in the fertilization treatments compared with the control. Similar trend was observed in the other parameters that were studied. No differences were found between the manure and the different times of N application which indicates that manure can be used to replace inorganic fertilizer. Applying N either preplant in a single application or in split application (half of N preplant and half as sidedress) did not have any effect on any characteristics that were studied indicating that preplant application can be used as it is more cost effective. The present study indicates that liquid cattle manure can be used to replace inorganic fertilizers and also that there was no difference between preplant and sidedress application of N.

Keywords Chlorophyll · Dry matter · Inorganic fertilizer · Nitrogen · Organic fertilizer · Partitioning

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Introduction

In recent years the increase in fossil fuel prices led to the increase in fertilizer cost. In addition, the economic and environmental costs of excessive N fertilization have risen as one of the most important issues. There is also a great interest in identifying suitable combinations of time of N application and N amendments that could result in efficient N use. Alternative forms of fertilizers such as manures (liquid manure, farmyard manures, composts and green manures) can be used as sources of plant nutrients and at the same time increase N use efficiency and crop yield (Eghball 2002; Fageria and Baligar 2005). Despite their significant advantages, manures also have disadvantages, which must be considered before using them in agricultural production systems. The disadvantages include odour, high fly breeding potential, transfer of pathogens and weeds, NO_3–N leaching, methane emission that contribute to the greenhouse gases, and increase of soil salinity.

The use of dairy manure not only increases the soil inorganic N pool, but also increases the seasonal soil N mineralization available to the crops (Chang et al. 1993; Murwira and Kirchmann 1993; Ma et al. 1999a, b). Crop yield is usually increased by manure application because of the increased nutrient availability and the improved soil structure (Chang et al. 1993; Matsi et al. 2003). Eghball and Power (1999) found that application of beef feedlot manure and composted feedlot manure resulted in maize yield similar to that from commercial fertilizer application. In addition, manure increased maize yield significantly (Jokela 1992; Zebarth et al. 1996). However, Schlegel (1992) found that composted manure plus fertilizer addition resulted in greater grain sorghum (*Sorghum bicolor* L.) yield than either source applied alone. In contrast, there is also evidence that application of dairy manure before sowing is not as effective as inorganic N fertilizer in increasing crop yields (Murwira and Kirchmann 1993; Paul and Beauchamp 1993). Manure application in excess of crop needs can cause a significant build up of P, N, other ions and salts in soils which can reduce seed germination and may slow the growth of maize (Murphy et al. 1972).

Nitrogen is one of the most important nutrients for maize production as it affects dry matter production by influencing leaf area development and maintenance as well as photosynthetic efficiency (Muchow 1988). N level has a direct effect on chlorophyll content and many times chlorophyll content is used to determine the N status of the plant (Eghball and Power 1999). Moreover, N deficiency delays both vegetative and reproductive phenological development, reduces yield, and yield components such as the number of kernels per ear (Uhart and Andrade 1995a, b). Maize dry matter (DM) production increased linearly with N application (O'Leary and Rehm 1990) and also maize silage quality up to 200 kg N ha^{-1}. Higher N rates can lead to a significant increase in residual NO_3–N concentrations and to underground water contamination. There is a need to minimize drainage water NO_3–N and at the same time to maintain or improve crop yield. Timing of N application and form of N can be an adequate strategy to ensure N availability when crops need it and minimize N losses.

Nitrogen use efficiency (NUE) defined as grain yield produced per kg of N applied is essential in modern crop production systems as it can provide a balance between nutrient inputs and outputs over the long term and also it can improve crop yield and prevent NO_3^- pollution of the underground water (Fageria and Baligar 2005). One way to increase N use efficiency is to apply it at different times in the growing season as it is needed by the crop (Marschner 1995; Fageria and Baligar 2005). This minimizes the opportunity for N loss because the plant can rapidly take it up. Many farmers use this management practice by applying N as sidedress or through the irrigation systems later in the growing season. Maize begins to rapidly take up N during the middle of vegetative growth period with the maximum rate of N uptake occurring near silking (Hanway 1963). Thus, applying N as sidedress (V8-V10) should be one of the best ways of providing the crop with N to meet this high demand (Binder et al. 2000). This is supported by experimental evidence which showed that sidedressing of N resulted in greater grain yield and fertilizer use efficiency than that produced after preplant application (Welch et al. 1971; Miller et al. 1975; Binder et al. 2000). However, it is important not to delay N application for too long as it can reduce yield and N fertilizer recovery (Jung et al. 1972).

Nitrogen fertilization of maize is one of the most important management practice which affects growth and yield of the crop (Welch et al. 1971; Jung et al. 1972; Miller et al. 1975; Ma et al. 1999a, b; Binder et al. 2000). However, there is limited published work about the effect of application of liquid cattle manure compared with the inorganic fertilizer and also the effect of the different time of N application (preplant and sidedress) on maize growth and development. In addition any crop practice such as the application of liquid cattle manure should be evaluated for its suitability in the cropping system before its adoption by the farmers. The objective of this study was to determine the effect of liquid manure application and N fertilizer applied preplant or as sidedress on plant growth, yield and dry matter production, physiology, and N use efficiency in maize.

Materials and methods

Study site

The experiment was carried out at the experimental farm of the Aristotle University of Thessaloniki, in northern Greece (22°59'6.17" E, 40°32'9.32" N) during 2003, 2004, and 2005 growing seasons. The hybrid was Cecilia (Pioneer Hi-bred, Inc), which is a widely used hybrid in Greece. The soil type was a calcareous loam (Typic Xerorthent) as described previously (Matsi et al. 2003). Briefly, the soil contained an average of 265 g kg^{-1}, clay, 476 g kg^{-1} silt, and 259 g kg^{-1} sand, with a pH 8.3 (1:2 water), $CaCO_3$ content 71 g kg^{-1}, EC_{se} 0.58 dSm^{-1}, organic C 7.5 g kg^{-1}, Kjeldahl N 1 g kg^{-1}, NO_3–N 22 mg kg^{-1}, Olsen P 12 mg kg^{-1}, and exchangeable K 93 mg kg^{-1}. The preceding crop was wheat (*Triticum aestivum* L.). Maize was sown on 5 June 2003, 21 May 2004, and 28 May 2005. The distance between rows was 80 cm and the plant density was around 80000 plants per ha at sowing. Weather data (rainfall, maximum, minimum, and average temperatures) were recorded daily in the experimental area and are given in Table 1 (reported as mean monthly data for each of the 3 years of the study).

The staging system used in this study divides plant development into vegetative (V) and reproductive (R) stages. Subdivisions of the V stages are designated numerically as V1, V2, V3, etc. through V(n), where (n) represents the last leaf stage before tasseling (VT). The six subdivisions of the reproductive stages are designated numerically with their common names (R1—silking, R2—blister, R3—milk, R4—dough, R5—dent, R6—physiological maturity) (Ritchie et al. 1993)

Table 1 Monthly means of maximum (Max), minimum (Min) and average (Aver) air temperatures and rainfall for 2003, 2004, 2005 years at Thessaloniki, Greece

Month	Max. (°C)	Min. (°C)	Aver. (°C)	Rainfall (mm)	Max. (°C)	Min. (°C)	Aver. (°C)	Rainfall (mm)	Max. (°C)	Min. (°C)	Aver. (°C)	Rainfall (mm)
	2003				2004				2005			
January	18	–1	8.3	106	23.0	–6.0	5.6	5	21	1	5.6	43
February	17	–3	4.5	0	21.0	0.0	10.6	5	22	2	5.3	20
March	22	–3	9.6	4	26.0	2.0	13.4	86	26	4	9.8	54
April	27	–2	12.9	31	24.0	1.0	13.1	63	31	7	13.8	5
May	34	11	22.6	77	32.0	8.0	19.9	25	36	12	19.8	0
June	38	15	26.6	15	42.0	11.0	27.8	19	40	16	24.2	32
July	40	16	27.8	23	40.0	18.0	29.0	87	42	18	26.5	36
August	41	18	29	0	40.0	18.0	28.0	23	39	17	25.8	21
September	38	11	23	21	31.0	12.0	22.3	79	36	14	21.8	26
October	32	6	18.3	81	25.0	6.0	17.2	34	31	12	16.0	51
November	25	6	14.5	36	20.0	2.0	12.0	29	28	8	10.9	61
December	18	0	8.4	70	19.0	–4.0	6.8	140	20	3	6.7	64
Total				464				595				413

Crop management and experimental design

The experiment was laid out in a completely randomized block design with six replications. The treatments were the following: (1) no fertilization (control), (2) injection of 80 Mg ha^{-1} $year^{-1}$ (wet weight basis) liquid dairy cattle manure before sowing (Manure), 3. application of 260 kg N ha^{-1} $year^{-1}$ as basal dressing before sowing (N-single), (4) application of 130 kg ha^{-1} $year^{-1}$ N as basal dressing before sowing and 130 kg N ha^{-1} when plants were at the V8 growth stage (on 7 July 2003, 8 July 2004, and 14 July 2005) (N-split). P was applied at the rate of 130 kg ha^{-1} $year^{-1}$ as basal dressing before sowing. The exchangeable K was 93 mg kg^{-1}, which is considered to be at the adequate range (Borges and Mallarino 2001). The experimental plots were 6 by 8 m and were separated by 2 m buffering zone to avoid any horizontal N movement. The treatments were established at the same plots each year. The liquid manure was applied with a liquid applicator (Zunhammer-Gülle Technik, Munich, Germany). Inorganic fertilizers were applied on 2 June 2003, 21 May 2004, and 24 May 2005, and manure was applied on 28 May 2003, 20 May 2004, and 24 May 2005. The total mineral N in the manure did not varied from year to year averaging 3 g kg^{-1} (wet weight basis). The chemical properties of manure were as follows pH 7.8, dry matter 80 g kg^{-1}, organic matter 56 g kg^{-1}, P 0.68 g kg^{-1}, K 2.5 g kg^{-1}. The manure was incorporated with a tandem harrow disc to a depth of 12–15 cm within 2 h after application.

The experimental area was irrigated with overhead sprinklers with a 400-mm total amount of water. First irrigation took place within the first week after corn sowing for all years. Weed control was achieved with atrazine (6-chloro-N2-ethyl-N4-isopropyl-1,3,5-triazine-2,4-diamine) applied pre emergence at 2.25 kg active ingredient (a.i.) ha^{-1} and also with rimsulfuron, 1-(4,6-dimethoxypyrimidin-2-yl)-3-(3-ethylsulfonyl-2-pyridylsulfonyl)urea, (Rush 25 WG at 50 g a.i. ha^{-1}) applied post emergence. Additional mechanical weeding was performed to control escaped weeds in all years.

Measurements

Total above ground DM in stalk, leaves and tassels or bract leaves at silking and harvest were measured. Also yield components (number of ears per m^2, number of kernels per ear, and kernel weight per ear), one thousand kernel weight (TKW), morphological parameters (plant height, number of leaves per plant, ear leaf area, and ear length), chlorophyll content, N uptake and partitioning into the different plant organs at silking and at harvest were determined.

Crop DM partitioning was measured twice during each growing season at silking (R1), and at harvest. At each sampling 10 plants were randomly selected and separated into leaves, stalks, reproductive components (tassels and cobs), and kernels which were dried at 80°C until a constant weight.

At physiological maturity, the numbers of ears per m^2 was determined by measuring the number of ears in two rows. The number of kernels per ear was determined by counting the number of rows per ear and multiplying the number of kernels per row from five rows per ear and from 10 ears per plot. Kernel weight per ear was determined by weighing the kernel weight from each ear at harvest. One thousand kernel weight was determined by weighing the weight of 200 randomly selected kernels from each plot and multiplying it by 5 to express it to 1000 kernels.

Plant height was determined by measuring the height of 20 randomly selected plants per plot from the soil to the top of the tassel at silking and getting an average value for each plot. The number of leaves was determined by counting the number of leaves in 20 plants per plot at silking. The ear leaf area was determined by measuring the width and length of each ear leaf and multiplying by 0.75 (length $\times$ width $\times$ 0.75) (Montgomery 1911) at silking.

Nitrogen uptake and partitioning between the different plant parts were measured twice during each growing season at silking (R1) and at harvest. The dry samples were ground to pass a 30 mesh screen. Total N concentration was analyzed using Kjeldahl method (Bremner 1996), and the N uptake and partitioning were determined for each component.

Chlorophyll measurements

Chlorophyll content readings were taken with a hand-held dual-wavelength meter (SPAD 502, Chlorophyll meter, Minolta Camera Co., Ltd., Japan). For each plot 30 younger fully expanded leaf blades per plot were used when the plants were at V10 stage, at silking (R1), at milk stage (R3), and at physiological maturity (R6). The instrument stored and automatically averaged these readings to generate one reading per plot.

Nitrogen use efficiency and its components

Nitrogen remobilization from the different plant organs was calculated according to Uhart and Andrade (1995b) as follows:

$$N_{\text{stem remobilization}}\ (\text{kg ha}^{-1}) = N_{\text{stem silking}} - N_{\text{stem harvest}}$$

$$N_{\text{leaf remobilization}}\ (\text{kg ha}^{-1}) = N_{\text{leaf silking}} - N_{\text{leaf harvest}}$$

$$N_{\text{total remobilization}}\ (\text{kg ha}^{-1}) = N_{\text{stem remobilization}} + N_{\text{leaf remobilization}}$$

$$N_{\text{absorption}}\ (\text{kg ha}^{-1}) = N_{\text{total}} - N_{\text{total remobilization}}$$

where $N_{\text{stem remobilization}}$, $N_{\text{leaf remobilization}}$, is stem and leaf N remobilization between silking and harvest, $N_{\text{stem silking}}$, $N_{\text{stem harvest}}$, is stem N uptake at silking and harvest, respectively, $N_{\text{leaf silking}}$, $N_{\text{leaf harvest}}$ is leaf N uptake at silking and harvest, respectively. $N_{\text{absorption}}$ is the post-silking N uptake and N_{total} is the total N uptake at harvest. $N_{\text{total remobilization}}$ is the total N remobilization from leaves and stems between silking and harvest.

Nitrogen use efficiency (NUE) and its components, N uptake efficiency, and N utilization efficiency, were calculated according to Moll et al. (1982) on kg ha^{-1} basis. These indices were calculated as follows:

- N use efficiency, as G_w/N_s (grain produced per unit of N supplied), where G_w is the grain dry weight and N_s the N that was supplied where in the case of manure N_s was 240 kg ha^{-1}.
- N uptake efficiency, as N_t/N_s, where N_t is the total N that was taken up.
- N utilization efficiency was calculated as G_w/N_t.

Physiological efficiency (PE) was calculated from the formula:PE $= (Yf - Yu)/(Nf - Nu)$, where Y_f is the total yield (seeds + biomass) of the fertilized, Y_u is the total yield (seeds + biomass) of the unfertilized, N_f is N uptake in the fertilized plot in grain and straw, N_u is N uptake in the unfertilized plot in grain and straw (Fageria and Baligar 2005).

Statistics

Analyses were performed with a personal computer using the SPSS™ (SPSS Inc., IL, USA) (blocks × treatments). A combined analysis of variance (ANOVA) over years was performed for all parameters. The LSD ($P = 0.05$) test was used to find significant differences among means.

Results

The weather conditions during each growing season were quite different (Table 1). The first growing season was characterized by a warm and dry summer, the second growing season was characterized by a quite mild spring with significant rainfall during the summer, and the third growing season was warm during the summer with low amount of rainfall during the spring. Also the non-homogeneous variation of the data across years reflected climatic fluctuations and prevented a combined analysis.

Dry matter accumulation and partitioning at silking and harvest

Dry matter accumulation and partitioning at silking and at harvest are shown on Table 2. There were no significant differences between the different treatments in the first year of the study. However, during the second year dry matter of leaves and bract leaves at harvest in the manure treatment was higher by 58% compared with the control treatment. In addition, stem biomass at harvest was higher by 21% and 27% in manure and N-split treatments, respectively than the control treatment, whereas the total biomass was higher in manure treatment by 25% compared with the control treatment. However, in the third year there was a clear trend between the control treatment and the other treatments at both growth stages (silking and harvest) and in all parts of the plant (Table 2). In particular, the total biomass at silking was higher by 61%, 72%, and 81% in manure, N-single, and N-split treatments, respectively compared with the control

Table 2 Dry matter partitioning at silking (R1) and at harvest in maize fertilized with manure, and inorganic nitrogen (applied in two different growth stages), during the 2003, 2004, and 2005 growing seasons

Treatments	Silking (R1)			Harvest				
	Leaves + Flowers	Stalks	Total biomass	Leaves + Bract leaves	Stalks	Cob	Grain yield	Total biomass
Mg ha^{-1}								
				2003				
Control	6.3a*	9.3 a	15.6 a	6.3 a	8.0 a	18.2 a	16.6 a	32.5 a
Manure	6.7 a	8.9 a	15.7 a	5.9 a	8.4 a	18.8 a	17.0 a	33.1 a
N-single	6.2 a	8.9 a	15.1 a	5.5 a	7.5 a	17.4 a	16.1 a	30.5 a
N-split	6.8 a	9.7 a	16.6 a	5.9 a	7.3 a	17.2 a	16.0 a	30.4 a
				2004				
Control	5.7 a	7.4 a	13.2 a	4.7 a	6.2 a	19.4 a	15.4 a	30.2 a
Manure	6.2 a	8.5 a	14.7 a	7.4 b	7.5 b	21.5 a	18.0 a	36.3 b
N-single	6.1 a	7.8 a	14.0 a	5.9 ab	6.6 ab	20.0 a	16.6 a	32.5 ab
N-split	7.3 a	7.7 a	15.0 a	6.1 ab	7.3 b	22.1 a	18.1 a	35.6 ab
				2005				
Control	3.9 a	5.6 a	9.5 a	5.8 a	5.5 a	11.3 a	8.7 a	22.6 a
Manure	7.0 b	8.3 b	15.3 b	7.8 ab	9.3 b	16.9 b	13.7 b	34.0 b
N-single	7.8 b	8.6 b	16.4 b	10.3 b	9.2 b	19.9 b	14.9 b	39.5 b
N-split	8.0 b	9.2 b	17.2 b	9.3 ab	8.3 b	16.6 b	13.4 b	34.3 b

* Means in the same column and year followed by the same letter do not differ significantly according to the LSD test ($P = 0.05$)

whereas at harvest the corresponding values of total biomass were higher by 50%, 74%, and 51% (Table 2). Similarly, in the third year the grain yield was higher by an average of 60% in the fertilizer treatments compared with the control (Table 2). Also the different form of fertilizer (organic or inorganic) did not affect the biomass production and partitioning. In addition there was no difference between the different times of fertilizer application as single and as split N application.

Yield components

The number of ears per m^2 was increased during the third year of the study in the manure treatment by 6% compared with the control treatment (Table 3). Also during the second year the kernel weight per ear was increased by 19% between the control and the manure treatment. The corresponding increase in the third year was 47%. A similar trend was found for the number of kernels per ear as the manure treatment had 18% more kernels per ear than the control at the second year. In the third year, there was a significant increase in the kernel weight per ear by 47%, 63%, and 47% in manure, N-single, and N-split treatments, respectively, compared with the control treatment. Also, the corresponding increase of the number of kernels per ear was 36%, 46%, and 53% (Table 3). The weight of 1000 kernels was not affected in the first 2 years of the study, but it was affected by the fertilizer treatments in the third year (Table 3). The weight of 1000 kernels was increased by fertilizers 9% in manure and N-single treatments, respectively compared with the control.

Morphological characteristics

In the first year, the number of leaves per plant was not affected by the application of manure and the chemical fertilizers. However, the number of leaves per plant was smaller in the control treatments in the following 2 years. In particular, during the 2004 there

Table 3 Number of ears per m^2, kernel weight per ear, number of kernels per ear, and weight of 1000 kernels in maize fertilized with manure and inorganic nitrogen (applied in two different growth stages) during the 2003, 2004 and 2005 growing seasons

Treatments	Number of ears per m^2	Kernel weight per ear (g)	Number of kernels per ear	Weight of 1000 kernels (g)
		2003		
Control	7.7 a*	218 a	593 a	369 a
Manure	8.1 a	220 a	622 a	356 a
N-single	7.5 a	215 a	599 a	360 a
N-split	7.7 a	214 a	616 a	347 a
		2004		
Control	7.6 a	202 a	551 a	366 a
Manure	7.5 a	241 b	649 b	371 a
N-single	7.6 a	218 ab	590 ab	368 a
N-split	7.8 a	230 ab	623 ab	368 a
		2005		
Control	7.3 a	120 a	355 a	333 a
Manure	7.7 b	177 b	484 b	363 b
N-single	7.6 ab	196 b	520 b	362 b
N-split	7.5 ab	177 b	542 b	339 ab

* Means in the same column and year followed by the same letter do not differ significantly according to the LSD test ($P = 0.05$)

was an increase in the number of leaves per plant by 10% and 8% in manure and N-single treatments, respectively, compared with the control treatment. During the 2005 the difference was much higher as it was 23%, 23%, and 25% higher in manure, N-single, and N-split, respectively compared with the control treatment.

Plant height was increased by 6% in the case of manure and N-split treatment compared with the control in the second year (Table 4). Also during the third year, maize plants were taller by 23%, 20%, and 23% in manure, N-single, and N-split, respectively compared with the control treatment. In both years, plant height decreased in control, which indicates that

Table 4 Number of leaves per plant, height of plants at silking (R1), ear leaf area, and ear length in maize fertilized with manure and inorganic nitrogen (applied in two different growth stages) during the 2003, 2004 and 2005 growing seasons

Treatments	Number of leaves per plant	Height of plants at silking (m)	Ear leaf area (cm^2)	Ear length (cm)
		2003		
Control	12.2 a*	2.1 a	606 a	17.4 a
Manure	12.3 a	2.1 a	630 a	17.5 a
N-single	11.6 a	2.2 a	594 a	18.1 a
N-split	12.7 a	2.1 a	615 a	17.6 a
		2004		
Control	11.2 a	2.1 a	636 a	14.6 a
Manure	12.4 b	2.3 b	635 a	17.2 b
N-single	12.2 b	2.2 ab	599 a	17.8 b
N-split	11.7 ab	2.3 b	602 a	18.6 b
		2005		
Control	10.7 a	2.0 a	326 a	13.3 a
Manure	13.1 b	2.4 b	545 b	16.7 b
N-single	13.1 b	2.5 b	561 b	17.6 b
N-split	13.4 b	2.4 b	555 b	19.1 b

* Means in the same column and year followed by the same letter do not differ significantly according to the LSD test ($P = 0.05$)

application of manure or inorganic fertilizers had also a significant effect on plant height.

Ear leaf area was not affected in the first 2 years of the study (Table 4). However, in the third year, ear leaf area was significantly increased by 67%, 72%, and 70% in manure, N-single, and N-split, respectively, compared with the control treatment. Moreover ear length was affected only during the second and third year of the study as it was lower by an average of 22% and 33% in the control compared with the organic and inorganic fertilization treatments, respectively. The different types and moment of fertilization did not affect the ear length which agrees with the other data presented in this study.

Chlorophyll content

During the first year there were no significant differences between the different treatments in chlorophyll content in the four examined growth stages (Table 5). In all cases chlorophyll was slightly lower during the vegetative stage and increased until the silking stage but decreased again at later growth stages. The same trend was observed during the second year. In this year, in most cases, there were statistical significant differences between the control and the other treatments but not all growth stages (Table 5). At the vegetative stage, the chlorophyll content was higher by an average of 7.5% in fertilized treatments compared with the control. At silking, chlorophyll content followed the same trend as in the vegetative stage. At the milk stage, there was no difference between the control and the N-split treatment, but there was difference between the control and the manure and N-single treatment as it was higher by 6%. At physiological maturity, the only difference was between the N-single treatment and the control and N-split treatments (Table 5). Chlorophyll content was affected by the different treatments especially in the third year of the study. In this year, in all growth stages, control had lower chlorophyll content compared with the other treatments (Table 5). In particular, the differences among the control and manure, N-single, and N-split treatments were 20–26%, 26–38%, and 28–33%, respectively at the four growth stages.

Table 5 Chlorophyll level at vegetative (V10), silking (R1), milk stage (R3), and physiological maturity (R6) in maize fertilized with manure, and inorganic nitrogen (applied in two different growth stages), during the 2003, 2004, and 2005 growing seasons

Treatments	Vegetative stage (V10)	Silking (R1)	Milk stage (R3)	Physiological maturity (R6)
		2003		
Control	52.3 a*	60.0 a	58.8 a	54.8 a
Manure	52.4 a	59.8 a	58.1 a	54.5 a
N-single	51.7 a	59.1 a	58.8 a	54.3 a
N-split	52.5 a	59.5 a	58.7 a	54.3 a
		2004		
Control	51.3 a	55.3 a	54.9 a	50.4 a
Manure	54.2 b	58.2 b	58.3 b	52.0 a
N-single	55.7 b	59.7 b	58.1 b	54.0 b
N-split	55.5 b	59.5 b	56.8 ab	52.0 a
		2005		
Control	41.1 a	45.5 a	41.1 a	38.6 a
Manure	50.5 b	54.8 b	51.1 b	48.7 b
N-single	53.7 b	57.5 b	53.5 b	53.3 b
N-split	53.9 b	58.2 b	54.2 b	51.5 b

* Means in the same column and year followed by the same letter do not differ significantly according to the LSD test ($P = 0.05$)

Nitrogen uptake, partitioning, and remobilization

In the first year, there were no significant differences among the four treatments in N uptake and partitioning, at both growth stages of maize (silking and harvest) (Table 6). However, N uptake was higher in the leaves compared with the stems. In addition, seeds had higher amount of N at harvest compared with the leaves and stems. A similar trend was observed in the next 2 years of the study. However, in the second year, significant differences were found for leaves between manure and N-split treatments at silking. Also, at the same year stem N uptake at silking showed an increase by 55% in manure treatment compared with the control treatment (Table 6). The stem N uptake at harvest was 30% and 39% higher in manure and N-split treatment, respectively compared with the control treatment.

Application of manure and inorganic fertilizer increased N uptake in the third year of the study in both growth stages of maize (Table 6). In particular, at silking leaves N uptake was higher by an average

Table 6 Nitrogen uptake and partitioning at silking (R1) and harvest in maize fertilized with manure and inorganic nitrogen (applied in two different growth stages) during the 2003, 2004, and 2005 growing seasons

Treatments	Silking (R1)			Harvest			
	Leaves + Flowers	Stalks	Total	Leaves + Bract leaves	Stalks	Kernels	Total
kg ha^{-1}							
			2003				
Control	140 a*	84 a	224 a	65 a	51 a	259 a	376 a
Manure	144 a	77 a	221 a	56 a	52 a	240 a	348 a
N-single	134 a	72 a	206 a	56 a	50 a	217 a	323 a
N-split	149 a	81 a	230 a	61 a	49 a	219 a	330 a
			2004				
Control	134 ab	68 a	202 a	55 a	50 a	202 a	307 a
Manure	150 a	105 b	255 ab	86 a	65 b	253 a	405 a
N-single	143 ab	85 ab	228 a	72 a	59 ab	229 a	359 a
N-split	183 b	91 ab	274 b	68 a	69 b	238 a	376 a
			2005				
Control	75 a	48 a	123 a	47 a	41 a	102 a	190 a
Manure	148 b	76 b	224 b	76 b	62 a	187 b	325 b
N-single	164 b	86 b	250 b	107 b	74a	196 b	377 b
N-split	173 b	92 b	265 b	101 b	80 a	186 b	367 b

* Means in the same column and year followed by the same letter do not differ significantly according to the LSD test (P = 0.05)

of 116% in fertilized (manure or inorganic fertilizer) treatments compared with the control treatment. Stems showed a similar increase with the highest difference to be between the control and inorganic fertilization treatments. At harvest, seeds had higher N uptake by an average of 86% in fertilized treatments compared with the control. The corresponding increase for the total N uptake was 71%, 98%, and 93% at the manure, N-single, and N-split, respectively compared with the control (Table 6). The highest N uptake in maize happens around the milk stage and after this there is N remobilization from leaves to the developing kernels.

Nitrogen remobilization was affected by the different treatments during the second and third year of the study (Table 7). Stem N remobilization was lower than leaf remobilization, which agrees with the change in N concentration of the stem and leaves between silking and harvest. In the second year the highest stem N remobilization was found at the manure treatment compared with the other treatments, whereas for the leaf N remobilization it was higher in N-split. However, during the third year the control treatment had lower remobilization of N from leaves and stems than the other treatments (Table 7). N absorption or post-silking N uptake was significant as it was almost half of the total N at harvest. At the last 2 years of the experimentation, in most cases, it was lower at the control treatment. Remobilization efficiency and the proportion of the grain N that comes from remobilization were not affected by the fertilizer treatment during the 3 years and ranged 18–52% and 34–57%, respectively. The amount of N that was remobilized from leaves and stems between silking and maturity was from 18% to 51%.

Nitrogen use efficiency

Nitrogen use efficiency and N uptake efficiency were affected by the three fertilizer treatments over the 2 of the 3 years of the study and it was higher than the control treatment. The N use efficiency and N uptake efficiency were not different between the three

Table 7 Nitrogen remobilization from stems and leaves between silking (R1) and harvest, N absorption, N use efficiency, N uptake efficiency, utilization efficiency and physiological efficiency in maize fertilized with manure, and inorganic nitrogen (applied in two different growth stages), during the 2003, 2004, and 2005 growing seasons

Treatments	$N_{stem\ remobilization}$	$N_{leaf\ remobilization}$	$N_{absorption}$	N use efficiency	Uptake efficiency	Utilization efficiency	Physiological efficiency
kg ha^{-1}							
			2003				
Control	32.5 a*	74.9 a	151.6 a	–	–	44.2 a	–
Manure	24.9 a	88.2 a	126.9 a	70.8 a	1.4 a	48.9 a	14.7 a
N-single	21.9 a	78.0 a	117.0 ab	61.9 a	1.3 a	49.8 a	34.7 b
N-split	31.3 a	88.0 a	100.1 b	61.5 a	1.3 a	48.5 a	38.1 b
			2004				
Control	17.8 a	79.1 a	105.2 a	–	–	50.2 a	–
Manure	40.1 b	63.7 a	149.7 b	75.0 a	1.7 a	44.4 a	59.1 a
N-single	26.7 a	71.1 a	130.8 b	63.8 a	1.4 a	46.2 a	40.4 b
N-split	21.7 a	114.8 b	102.1 a	69.6 a	1.5 a	48.0 a	78.8 c
			2005				
Control	6.9 a	27.7 a	67.5 a	–	–	45.7 a	–
Manure	13.7 b	71.5 b	102.0 b	57.1 a	1.4 a	42.0 a	84.9 a
N-single	12.1 b	56.2 b	127.9 b	57.3 a	1.5 a	39.4 a	90.0 a
N-split	12.1 b	71.7 b	102.0 b	51.5 a	1.4 a	36.4 a	65.9 b

* Means in the same column and year followed by the same letter do not differ significantly according to the LSD test ($P = 0.05$)

fertilizer treatments as there were no differences in N uptake among the three treatments (Table 7). Utilization efficiency was unaffected by the organic or inorganic fertilization over the 3 years and ranged 36.4–50.2 kg ha^{-1}. This indicates that the three fertilizer treatments did not affect N uptake or grain yield. Physiological efficiency was affected by the treatments and it was lower at the manure treatment during the first two growing seasons compared with the N-single treatment, whereas it was similar with the N-single in the third growing season (Table 7).

Discussion

Compared with the unfertilized control treatment both inorganic fertilizer and manure application affected most of the characteristics that were studied during the third year, in some of the characteristics there was an increase in the fertilizer treatments compared with the control in the second year (2004) but during the first year (initial study year, 2003) there was no effect. This is possible due to the high levels of soil mineral N associated with the preceding years. Also 2003 was quite warm and dry year, which can have a significant effect on crop growth and development. Similar response was found by others as the climate affected differently the response of maize to fertilization during the different growing seasons (Ma et al. 1999a, b). Also the non-homogeneous variation of the data across years reflected climatic fluctuations and prevented a combined analysis. Nonetheless, the trend was similar as application of manure favored grain yield and the physiological characteristics in a similar fashion as the inorganic fertilizer treatments.

Dry matter accumulation and partitioning at silking and harvest

Variations in N supply can affect growth and development of maize (Muchow 1988; Muchow and Davis 1988; McCullough et al. 1994; Uhart and Adrade 1995a, b). N shortage and N excess affect assimilate partitioning between vegetative and reproductive organs (Donald and Hambin 1976; Subedi and Ma 2005). The most striking differences were found in the third year of the study, where there were differences in both growth stages and in all plant parts. Moreover, there were differences in the third year as N deficiency in the control treatment delayed phenological stages and especially silking promoting kernel maturation which led to shorter kernel filling period (data not shown). This is in agreement with the findings of other researchers, who reported N deficiency decreased dry matter production and partitioning especially in the reproductive organs resulting in lower grain yield, kernel size and set, and also shorter kernel filling period (Uhart and Andrade 1995a, b). Among the three fertilizer treatments there were no differences in growth and development as the dry matter accumulation and partitioning at the different plant parts was not affected by manure or the different time of N application, which agrees with others (Eghball and Power 1999; Ma et al. 1999a, b; Binder et al. 2000). The fact that there was no difference among the three fertilizer treatments was probably because of the N soil level that was at the adequate range. In contrast, Miller et al. (1975) and Binder et al. (2000) reported that sidedressing of N resulted in greater grain yield than that produced after preplant application. This discrepancy can be because of the climatic conditions since in Mediterranean climates annual rainfall is quite small (400–500 mm) which prevents leaching of NO_3^-. In contrast in climates with higher amount of rainfall there is significant NO_3^- leaching and it is probably important to provide N into different times as sidedress. In addition grain yield was relatively high compared with other studies (Anderson et al. 1984; Ma et al. 1999b). However, this is the case in Greece since the average grain yield for maize is 10 Mg ha^{-1} for the last 5 years (2000–2004) (FAO 2006).

Yield components

Nitrogen fertilization affected the yield components probably because there was lower amount of N in the soil at the control treatment that was taken up by the plant and also the N that was translocated to the kernels or to the ears and also the photoassimilates (Subedi and Ma 2005). Yield components are important as they correlate with the final grain yield. They can be affected by fertilization, source-sink relationships, genotype, and environmental factors, (e.g., drought) (Lemcoff and Loomis 1986; Moll et al. 1994; Uhart and Andrade 1995b). One of the most important yield components is the number of kernels per ear, which was affected more by manure and inorganic fertilizer application. This is possible because of the reduction of the number of kernels which was due to the poor ovule fertilization where N deficiency had a more profound effect and it did not affect the number of ears (Uhart and Andrade 1995b).

Yield components were not affected by the type and moment of fertilization but there were significant differences between the control and the fertilizer treatments. Also sidedress N application has been shown to be beneficial for maize yield (Welch et al. 1971; Jung et al. 1972; Miller et al. 1975; Binder et al. 2000). However, this is not supported by our data, which indicates that there were no significant differences between preplant and sidedress N application. Also at our experiments because of the low rainfall the amount of N leached was quite low and this is possible one explanation for not seeing a difference in yield response between preplant and sidedress N application. Preplant N application has the advantage that can minimize cultivation cost since N sidedress can represent a considerable expense for the farmer (fertilizer and application cost).

Morphological characteristics

The number of leaves per plant was smaller in the control treatments in the last 2 years of the study which indicates that N has a direct effect on leaf emergence, growth, and development which agrees with others (Uhart and Andrade 1995a). Also the different type and moment of fertilization did not affect the number of leaves per plant, which indicates that liquid manure or preplant N application can be used without any significant difference on plant growth. The effect of N deficiency on leaf area is well documented in maize (Marschner 1995; Uhart and Andrade 1995a). The fact that in most characteristics that were studied there was no difference between the manure treatment and the different time of N application indicates that the limiting factor for the observed changes was the N supply from the soil which was covered by the N application. In addition liquid cattle manure can be used to supply maize plants with N. The different type of fertilizer application did not affect the growth of maize plants, which indicates that N-single treatment can be used as it is also more cost effective because the overall expense for the farmer is lower than the split application.

Plant height and ear length was decreased in control, which indicates that application of manure or inorganic fertilizers had also a significant effect on plant height and on ear length (Ma et al. 1999b; Subedi and Ma 2005). Plant height is affected by N levels as when the N level is low to sustain plant growth the plants become shorter; the total biomass and grain yield is lower compared with sufficient N (Marschner 1995; Subedi and Ma 2005). Under low soil N the ears become shorter and their development is seriously affected (Ma et al. 1999b). Also this was the case in our study. The different types and moment of fertilization did not affect the ear length, which agrees with the other data presented in this study.

Chlorophyll content

Chlorophyll meters have been used extensively as a quick and non-destructive way of determining the N level of maize and other cereals (Eghball and Power 1999). It measures the degree of greenness of the leaves and is an indication of plant N concentration during the growing season. It is used to assist in determining the optimum N application time and as an alternative measure to determine the N status of the plant where tissue or soil analysis cannot be used (Schepers et al. 1992). In addition, in most cases, the treatments with manure or chemical fertilizer had higher SPAD readings than that of control at all growth stages during the last 2 years of the experiment. The lower amount of N available in the control was remobilized to support kernel growth, and the leaves senesced quicker compared with the fertilizer treatments. In contrast to the other treatments where there was an adequate N supply, the ear-leaf and all leaves above the ear maintained greenness until physiological maturity. This clearly indicates that when there is adequate N supply in the soil leaf senescence is slower and the plant supplies the kernels with N and photoassimilates for longer time which results in higher yields (Eghball and Power 1999; Subedi and Ma 2005). This finding is also in agreement with Rajcan and Tollenaar (1999) and Subedi and Ma (2005) who found that leaf longevity was enhanced by increasing soil N supply.

Chlorophyll content was not affected by the type and moment of fertilization but there were significant differences between the control and the fertilizer treatments. This indicates that liquid cattle manure when it is applied in a comparable rate to N fertilizers can provide enough N, which can support maize growth. Also sidedress N applications has been shown to increase N concentration and seed protein content (Welch et al. 1971; Jung et al. 1972; Miller et al. 1975; Binder et al. 2000). However, this is not supported by our data which indicates that there were no significant differences between preplant and sidedress N application. Preplant N application has the advantage that can minimize cultivation cost since N sidedress can represent a considerable expense for the farmer (fertilizer and application cost).

Nitrogen uptake, partitioning, and remobilization

Nitrogen uptake was affected by the different treatments during the second and third year of the study. The highest N uptake in maize happens around the milk stage and after this there is N remobilization from leaves to the developing kernels. This increase in N uptake is similar to the increase that was found by others (Anderson et al. 1984; Moll et al. 1994; Ma et al. 1999a, b). In addition, the different type (inorganic or organic) or time of N application did not have any effect on N uptake which agrees with the findings of others (Jokela and Randall 1989).

Nitrogen remobilization is important as it can provide the developing kernels with N when soil N is limited. N remobilization was affected by the different treatments during the second and third year of the study. Stem N remobilization was lower than leaf remobilization, which agrees with the change in N concentration of the stem and leaves between silking and harvest. Remobilization efficiency and the proportion of the grain N that comes from remobilization were not affected by the fertilizer treatment during the 3 years and ranged 18–52% and 34–57%, respectively. The amount of N that was remobilized from leaves and stems between silking and maturity was from 18% to 51% and it was comparable with other studies (Uhart and Andrade 1995b). However, these values may be reduced if volatilization and leaching losses were considered and as Uhart and Andrade (1995b) noted N remobilization should be considered as an apparent remobilization. N remobilization is quite important when maize plants suffer from N deficiency as it can supply with N the developing kernels (Marschner 1995). In addition N remobilization can affect the response of a hybrid to temporary N deficiency and hybrids with higher remobilization efficiency can survive that stress.

Nitrogen use efficiency

Nitrogen use efficiency and N uptake efficiency were not different between the three fertilizer treatments as there were no differences in N uptake among the three treatments. Utilization efficiency was unaffected by the organic or inorganic fertilization over the 3 years and ranged 36–51 kg ha^{-1}. This indicates that the three fertilizer treatments did not affect N uptake or grain yield, which agrees with Fageria and Baligar (2005). In contrast, Miller et al. (1975) and Binder et al. (2000) reported that sidedressing of N resulted in greater fertilizer use efficiency than that observed with the preplant N application. Also physiological efficiency was affected by the treatments and was lower at the manure treatment during the first two growing seasons compared with the N-single treatment, whereas it was similar with the N-single in the third growing season. The lower physiological efficiency in manure treatment over the 2 of the 3 years of the study can be because of the N released from the manure and the increase in N uptake during the exponential growth of maize (Ma et al. 1999a).

Conclusion

Nitrogen fertilization is one of the most critical factors for maximum yield of maize and there is an increased interest in understanding more fully the impact of varied N application and form of fertilization during plant development. From this study it was found that fertilization with manure or with inorganic fertilizers can affect significantly maize growth, development, N uptake and N use efficiency compared with the control treatment. No differences were found in dry matter, N accumulation and partitioning between the fertilizer treatments, which is possible because of the adequate N content in the soil. Application of N preplant or as sidedressing did not seem to affect the characteristics that were studied, indicating that it is more cost effective to incorporate all N as a preplant application. This study provides some useful information about the effect of application of manure and inorganic fertilizer on grain yield, yield components, dry matter and N accumulation and partitioning of maize. This information can be used for better N management which can be used for cost effective N application preplant or using liquid manure thereby leading in lower N loss and pollution of the underground water.

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