

## Nitrogen dynamics during growth of sweet sorghum [*Sorghum bicolor* (L.) Moench] in response to conventional and organic soil fertility management

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### Abstract

The effects of organic and conventional soil fertility management on nitrogen supply and yield of sweet sorghum crop (cv Keller) were studied in two experiments conducted in summer-autumn in W. Greece. Two fertilization treatments, conventional (120 kg N, 120 kg P<sub>2</sub>O<sub>5</sub>, 170 kg K<sub>2</sub>O and 20 kg MgO per ha) and organic (winter cover crop of common vetch, natural white sphagnum peat, 170 kg K<sub>2</sub>O ha<sup>-1</sup>) were applied. Plant dry weight biomass, plant nitrogen content, concentration of NO<sub>3</sub>-N and NH<sub>4</sub>-N in soil and soil solution were measured. Nitrogen uptake, nitrogen dilution curve and nitrogen nutrition index were estimated. Plant dry weight biomass (1.918-2.078 kg m<sup>-2</sup>) and soil NO<sub>3</sub>-N content (0.75-4.74 mg 100 g<sup>-1</sup> soil) were not affected by fertilization treatments. Organic fertilization increased NO<sub>3</sub>-N concentration in soil solution at 30 cm depth during cultivation period (4.12-25.52 mg L<sup>-1</sup>), soil NH<sub>4</sub>-N content 74 days after transplanting - DAT (0.14 mg 100 g<sup>-1</sup> soil), NO<sub>3</sub>-N concentration in soil solution at 60 cm depth 74 DAT and NH<sub>4</sub>-N concentration in soil solution 31 DAT at 30 cm depth (0.10 mg L<sup>-1</sup>), 45 and 59 DAT at 60 cm depth (0.14 and 0.39 mg L<sup>-1</sup>, respectively). Conventional fertilization increased soil NH<sub>4</sub>-N content when estimated at 45 DAT (0.19 mg 100 g<sup>-1</sup> soil). Nitrogen uptake was higher in organic soil fertility management 45 DAT (4.61-5.12 g m<sup>-2</sup>). However, crop nutrition nitrogen index was not affected by treatments. These results indicate that organic soil fertility management provides adequate nitrogen nutrition to sweet sorghum.

**Keywords:** dry weight biomass; nitrogen nutrition index; nitrogen uptake; soil ammonium nitrogen; soil nitrate nitrogen.

**Abbreviations:** CSFT\_conventional soil fertility treatment; DAT\_days after transplanting; DW\_dry weight biomass; NH<sub>4</sub>-N\_ammonium nitrogen; NNI\_nitrogen nutrition index; N\_nitrogen; NO<sub>3</sub>-N\_nitrate nitrogen; Nup\_nitrogen uptake; OSFT\_organic soil fertility treatment.

### Introduction

An interest in the contribution of crop biomass to the energy supply received considerable attention during the 1970s because of the need of achieving energy self-sufficiency. This interest was renewed in the mid-1990s in an effort to mitigate global climate change (Berndes et al., 2003). The production of bioenergy is considered to be important not only for environmental protection (e.g. changes in air and water quality, reduction of greenhouse gas emissions, etc.), but also as an alternative use of arid or marginal land. However, the development of bioenergy cropping system can lead to environmental damage and some important features (e.g. effects on soil and water, changes in land use, etc.) must be taken into account (McLaughlin and Walsh, 1998). Sweet sorghum [*Sorghum bicolor* (L.) Moench] is cultivated for the production of food, feed and fiber (reviewed by Almodares and Hadi, 2009). Nowadays it is also considered as an energy crop, mainly due to its potential to produce high biomass even under conditions of limited water supply. This is particularly important in areas of limited water sources, as in Mediterranean countries. However, it must be mentioned that sorghum genotypes present different sensitivity to drought

stress (Ali et al., 2011). Studies for the cultivation of sweet sorghum as a raw material for ethanol production have been carried out (Dalianis, 1997) and breeding programs have been developed in many countries (e.g. India, Australia). Monti and Venturi (2003) found that sweet sorghum (cv. Keller) is a more potent bioenergy source than wheat. Ture et al. (1997) reported that the production of bioenergy from sweet sorghum can achieve a 90% reduction of CO<sub>2</sub> emissions. Hence, sweet sorghum is cultivated in many countries (e.g. USA, Argentina) for the production of bio-ethanol (Reddy and Reddy, 2003). Although sweet sorghum produces well under low input cultivation conditions, yields are generally higher under conditions of balanced fertilization and adequate irrigation. It is also a crop with good nitrogen (N) use efficiency (Gardner et al., 1994) but inappropriate N fertilization limits yields. Depending on soil fertility, sorghum producers often apply 45 to 224 kg N ha<sup>-1</sup> (Zhao et al., 2005). However, high N fertilization should be avoided since excessive N may reduce crop ethanol yield (Wiedenfeld, 1984) as well as considerably increase production costs and reduce energy efficiency, due to the fact

that N fertilization accounts for up to 50% of the total energy input in arable crops (Kuesters and Lammel, 1999). Nevertheless, the appropriate timing of N application is reported to have a higher effect on plant growth rate (Tsialtas and Maslaris, 2005) and yield (Almodares and Darany, 2006) than the total amount of N applied. Therefore, efficient monitoring of plant N status and appropriate soil fertility management are both essential for balancing critical factors, such as fertilization costs, crop needs, and environmental pollution. Because of the increasing prices of chemical fertilizers and underground water pollution (Jaynes et al., 2001), alternative N sources are required. In addition, a prerequisite for the development of an energy crop is its environmental safety (Barbanti et al., 2006). To this effect, organic farming is considered to be suitable for the cultivation of sweet sorghum, when applying intercropping with legumes (Arshad and Ranamukhaarachchi, 2012) and using winter cover crops. Zegada-Lizarazu and Monti (2012) proposed that sorghum-legume rotations can lead to a reduction of N fertilizer requirements of sorghum. Organically managed soils have been found to have higher total organic matter content (Mader et al., 2002; Pulleman et al., 2003), due to the use of animal and green manures and the efficient cycling of crop residues (Stockdale et al., 2001). However, the comparative effect of organic and inorganic fertilization on growth, biomass yield and nitrogen uptake largely depends on weather conditions (Sow et al., 1996; Unger and Baumhardt, 1999). Since literature concerning organic cultivation of sweet sorghum is limited, especially in the Mediterranean area, the objective of this study is to analyse the dynamics of soil N and N uptake (Nup) of sweet sorghum crop grown under conventional and organic management systems. These results are expected to provide evidence of the adequacy of organic soil fertility management in sweet sorghum cultivated under conditions of limited rainfall and high daily temperatures during summer in western Greece.

## Results and discussion

### Meteorological data

Mean monthly temperature was quite similar in both cropping periods, but there were differences in rainfall distribution, which were recorded (Fig. 1). In the first cropping period (year 1), rainfall was lower (about 18 mm) in September and higher in June and July (about 22.5 mm and 7 mm, respectively) compared with the second cropping period (year 2).

### Dry weight biomass at harvest

The two-year mean value of dry weight biomass of the aboveground part of the plant was 1.918 kg m<sup>-2</sup> in OSFT, and 2.078 kg m<sup>-2</sup> in CSFT. Fertilization treatments did not significantly affect the dry weight biomass produced. Yields obtained in this study were quite similar to those reported by Kavadakis et al. (2001) for sorghum cultivated in Central Greece.

### Inorganic N concentration in soil

Nitrate N (NO<sub>3</sub>-N) content in soil was not significantly affected by fertilization treatments (Fig. 2). Soil ammonium N (NH<sub>4</sub>-N) content ranged between 0.05 and 0.26 mg 100 g<sup>-1</sup> of soil with the highest values measured at the end of the cultivation period. Conventional soil fertility treatment

significantly increased soil NH<sub>4</sub>-N content 45 days after transplanting (DAT), while organic soil fertility treatment significantly increased soil NH<sub>4</sub>-N content 74 DAT (Fig. 2). NO<sub>3</sub>-N concentration in soil solution at 30 cm depth was significantly higher in OSFT compared with CSFT (Fig. 3). However, at 60 cm depth no significant effect of the fertilization treatment was observed up to 74 DAT, whereas at 88 DAT OSFT increased NO<sub>3</sub>-N concentration in soil solution (Fig. 3). It is possible that the mineralization of the green manure and peat incorporated into the soil by OSFT led to increased NO<sub>3</sub>-N concentration in soil solution. Accordingly, Fraser et al. (1988) attributed the higher levels of soil NO<sub>3</sub>-N content to increased organic matter mineralisation in comparison to conventional fertilization. In this study, the higher N-NO<sub>3</sub> concentration in soil solution at 30 cm depth following organic fertilization could also be related to high rainfall levels recorded during the cropping periods. Rainfall favours NO<sub>3</sub> leaching (Barbanti et al., 2006), in particular when inorganic fertilizers are applied to the soil (Errebhi et al., 1998). Irrespective of the fertilization treatment, a decrease in the concentration of NO<sub>3</sub>-N in soil solution was observed during the course of the cropping period, both at 30 and 60 cm depth (Fig. 3). This decrease reflects the rapid N uptake (Nup) by growing plants, suggesting the importance of N feeding to sorghum crop. Type of fertilization did not significantly affect NH<sub>4</sub>-N concentration in soil solution at both 30 and 60 cm depths, with the exception of 31 DAT at 30 cm depth and at 45, 59 DAT at 60 cm depth when OSFT significantly increased NH<sub>4</sub>-N concentration in soil solution (Fig. 4). The insignificance of the type of fertilization applied on the accumulation of NH<sub>4</sub>-N in the soil solution suggests the occurrence of intense nitrification.

### N uptake (Nup) during cropping season

Nup was not significantly affected by the fertilization treatments, except at 45 DAT when OSFT showed significantly higher values in comparison with CSFT in both cropping periods (Fig. 5). Nup ranges were quite similar to those reported by Wiedenfeld (1984) and Curt et al. (1996), 12.52 and 11.42 – 15.71 g N m<sup>-2</sup>, respectively. These values were lower than those reported by Barbanti et al. (2006), i.e. 13 - 20 g N m<sup>-2</sup>. The differences in soil N content observed between organic and conventional fertilization treatments were not reflected in maximum Nup by the crop. However, maximum Nup also depends on several other factors, such as root density and specific root absorption capacity (Lemaire et al., 1996). Moreover, organic and conventional systems differ in fundamental ways and different responses to the application of organic fertilizers have been observed (Azam Shah et al., 2009). These suggest that comparisons between organic and conventional soil fertility management are fraught with difficulty.

### Dilution curve of total N concentration at increasing dry weight (DW)

Greenwood et al. (1990) proposed a N dilution curve ( $\%N = a(DW)^b$ ) where  $a=4.1$  and  $b=-0.5$  for C<sub>4</sub> crops, such as sorghum, when dry weight production (DW) ranges from 1 tn ha<sup>-1</sup> to the beginning of the reproductive stages. Lemaire and Gastal (1997) proposed a general equation for C<sub>4</sub> crops in which  $a=3.6$ ,  $b=-0.34$ , whereas Plenet and Cruz (1997) proposed values equal to 3.9 and -0.39, respectively.

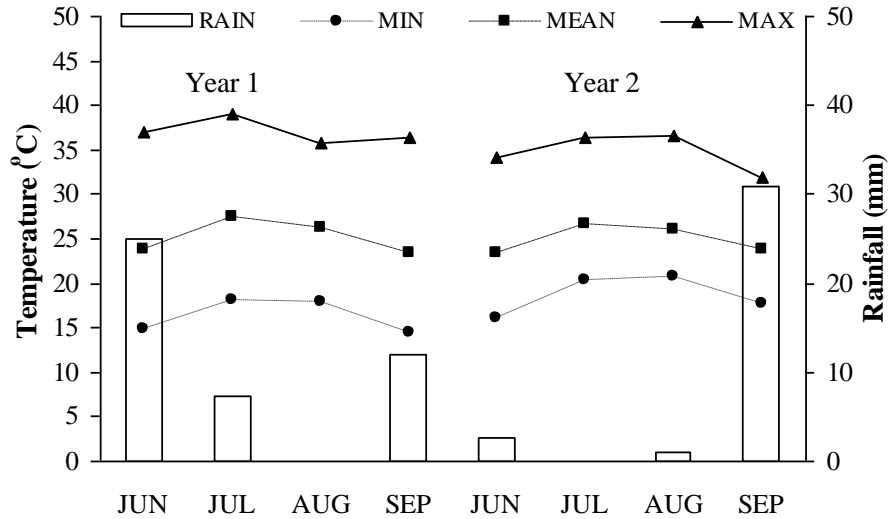


Fig 1. Mean, maximum (Max) and minimum (Min) temperatures, and rainfall during the cropping periods of year 1 and year 2.

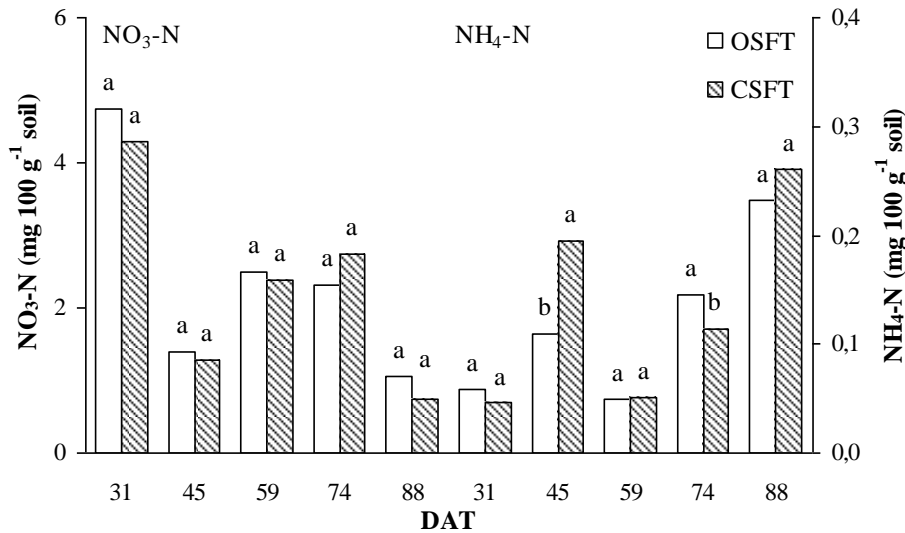


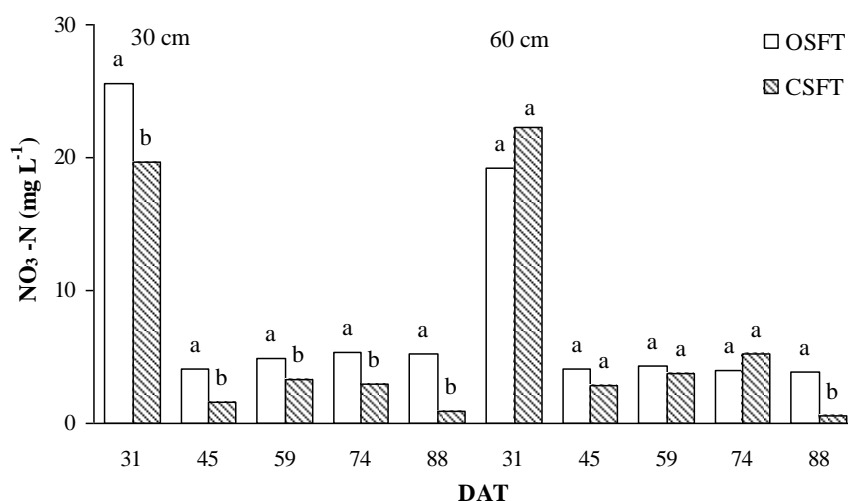
Fig 2. Soil inorganic NO<sub>3</sub>-N and NH<sub>4</sub>-N content in OSFT and CSFT (columns represent the two-year mean values of each fertilization treatment –different letters at each pair of columns indicate a significant difference between fertilization treatments).

More recently, Barbanti et al. (2006) found that the *a* coefficient was 4.41 by submitting their data to a curve fitting with *b* coefficient constrain to -0.5. In the present study, coefficient values were quite similar for both fertilization treatments. Specifically, *a* coefficient was 2.32 in CSFT and 2.47 in OSFT, and *b*=- 0.44 in CSFT and *b*=- 0.41 in OSFT (Fig. 6), indicating that fertilization treatments had no effect on N supply. Barbanti et al. (2006) propose that such low values of the *a* coefficient as the above can be considered as N deficiency. However, no visible N deficiency symptoms were observed, suggesting that the environmental conditions during crop growth are determinant for the use of *a* coefficient as parameter for the estimation of N availability, as previously proposed by Tei et al. (2002). Plant diagnostic methods of N deficiency should be based on the definition of a critical N concentration. To describe the N status of organic and conventional farming systems, we used a range of total plant N and to evaluate crop N status, we employed the N dilution concept proposed by Greenwood et al. (1990) and Lemaire and Gastal (1997). Critical N concentration (N% critical) is defined as the minimum crop N concentration

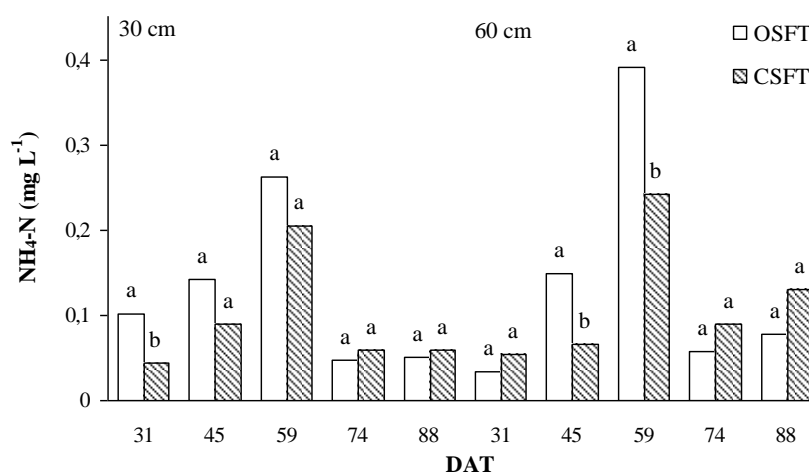
required to achieve maximum crop growth rate (Greenwood et al., 1991), and is analogous to the critical N content (minimum N content necessary to achieve maximum crop growth rate). Plant N concentration decreased during growth cycle and many authors have suggested that this is the result of two processes: (i) self-shading of leaves that induces a non-uniform leaf N content (Hirose and Werger, 1987; Sinclair and Horie, 1989) and (ii) an increase in the proportion of plant structural and storage tissues, that are deprived in N, even when crops grow under non-limiting N supply (Caloin and Yu, 1984; Charles-Edwards et al., 1987). Nevertheless, the decrease in plant N content with time for a given plant genotype largely depends on the environmental conditions that prevail during plant growth (e.g. excessive rainfall) (Alt et al., 2000).

#### Crop N nutrition index

Crop N nutrition index (NNI) was not significantly affected by fertilization treatments in both cropping periods. However, in the first cropping period (year 1) the low value of NNI at



**Fig 3.** Concentration of inorganic NO<sub>3</sub>-N in soil solution at 30 cm depth and 60 cm depth (columns represent the two-year mean values of each fertilization treatment –different letters at each pair of columns indicate a significant difference between fertilization treatments).



**Fig 4.** Concentration of inorganic NH<sub>4</sub>-N in soil solution at 30 cm and at 60cm depth (columns represent the two-year mean values of each fertilization treatment –different letters at each pair of columns indicate a significant difference between fertilization treatments).

45 DAT suggests that plants were grown under limited N availability, while the high values of NNI (>1) at 59 and 74 DAT suggest that plants were grown under excessive levels of soil N (Fig. 7). In the second cropping period (year 2), though, NNI was relatively close to 1 at 45 and 59 DAT, suggesting optimum N availability, but it was lower than 1 at 74 DAT suggesting suboptimal levels of soil N for sorghum growth (Fig. 7). N-limited condition (NNI < 1) seems to be related to the high level of rainfall in June (year 1) and September (year 2). This result suggests that N supply is strongly dependent on rainfall, not only in conventional farming systems, as previously reported by Devienne-Barret et al. (2000) and Barbanti et al. (2006), but also in organic farming systems.

## Material and methods

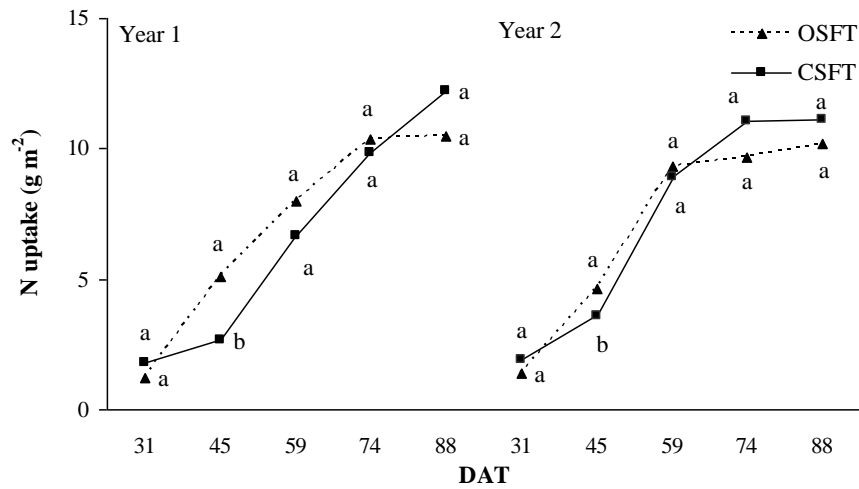
### Plant material

Seeds of sweet sorghum [*Sorghum bicolor* (L.) Moench] cv Keller were sown in Jiffy Pellets containing natural white sphagnum peat on May 15<sup>th</sup> 2004 (year 1) and 2005 (year 2).

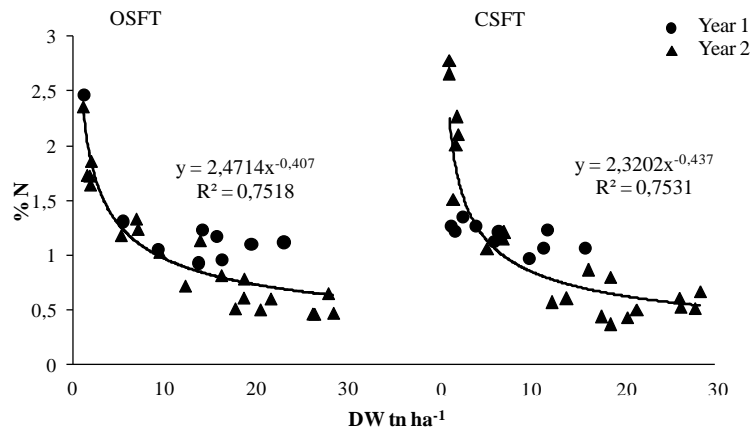
Thirty days after sowing, plants were transplanted to the experimental field. Common vetch (*Vicia sativa* L.) was cultivated in the plots of organic soil fertility treatment (OSFT) before sorghum transplanting.

### Experimental site and treatments

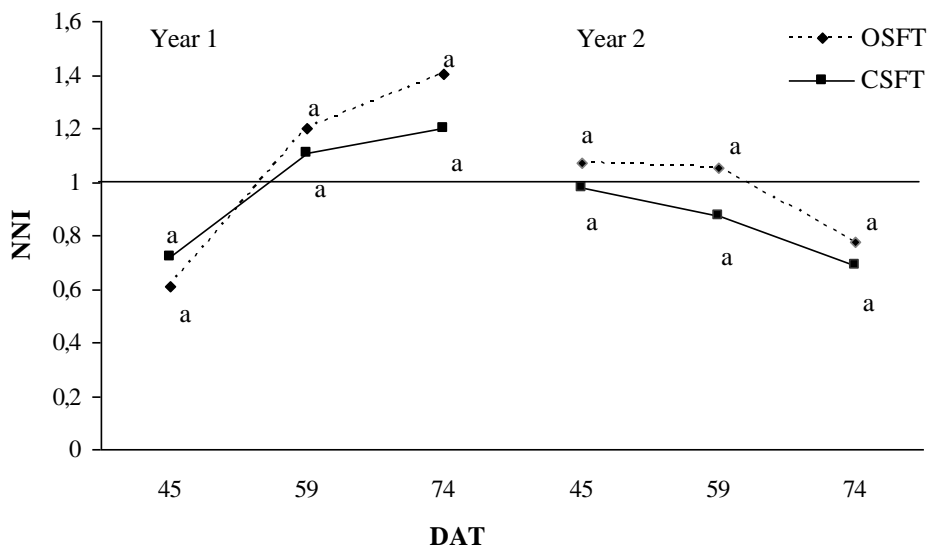
Field experiments were conducted at the experimental station of the University of Patras, Department of Biology, Greece (38°25' N, 21°8' E). Weather data (rainfall, maximum and minimum temperatures) were recorded daily and are reported as mean monthly data for the two cropping periods. Soil contained clay 230 g kg<sup>-1</sup>, sand 480 g kg<sup>-1</sup> and silt 290 g kg<sup>-1</sup>. The chemical characteristics, determined before transplanting, were: pH 7.7-7.8, Olsen-phosphorus 3-5 mg kg<sup>-1</sup> soil, CaCO<sub>3</sub> 4-5%, Ca<sup>2+</sup> 23-24.41 meq 100 g<sup>-1</sup> soil, Mg<sup>2+</sup> 1.13 meq 100 g<sup>-1</sup> soil, K<sup>+</sup> 0.4-0.5 meq 100 g<sup>-1</sup> soil, Na<sup>+</sup> 0.17 meq 100 g<sup>-1</sup> soil and organic matter 1.1%. Each experiment was carried out in a randomized complete block design with three replications. Two different fertilization treatments, conventional soil fertility treatment (CSFT) and organic soil fertility treatment (OSFT), each with three replications, were



**Fig 5.** Plant Nup during cropping season of year 1 and year 2 (different letters at each day of measurement indicate a significant difference between fertilization treatments).



**Fig 6.** Dilution curve of total N concentration at increasing dry weight (DW) of CSFT and OSFT for sweet sorghum as the average of two years.



**Fig 7.** Evolution of the mean NNI for each fertilization treatment during sweet sorghum growth in year 1 and year 2 (values with the same letter at each day of measurement do not differ significantly).

performed. CSFT consisted of 120 kg N ha<sup>-1</sup>, 120 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>, 170 kg K<sub>2</sub>O ha<sup>-1</sup> and 20 kg MgO ha<sup>-1</sup> (Bluestar Extra 12-12-17+2MgO, Karvali, Kavala, Greece), and was incorporated into the upper 20 cm of soil, fifteen days before transplanting. The OSFT comprised a cover crop, white sphagnum peat and K<sub>2</sub>SO<sub>4</sub>. The cover crop was a winter N fixing leguminous plant, common vetch, which was sown at 140 kg ha<sup>-1</sup>, six months before the transplanting of sorghum plants to the field and was incorporated as green manure into the upper 20 cm of soil one month before sorghum plants were transplanted. Additionally, 300 L natural white sphagnum peat (appr. 35% organic matter) per plot (53.9 m<sup>2</sup>) and 170 kg K<sub>2</sub>O ha<sup>-1</sup> (0-0-50, Compo Hellas SA, Amarousion, Greece) were applied. Tillage of CSFT and OSFT plots consisted of plowing and harrowing. Each plot consisted of eleven 7 m long rows (70 cm apart) with 20 cm space between plants (density: 7.14 plants m<sup>-2</sup>). The plots were irrigated using a drip system and were kept free of weeds by hand hoeing when necessary.

### Soil and plant sampling

Soil sampling started fifteen days before sorghum transplanting. Samples were taken from the depth of the plough layer (0-50 cm) using a 2 cm diameter auger. They were air dried, sieved through a 25 mesh sieve, and transported to the laboratory. Soil solution samples were collected at two depths, 30 cm and 60 cm of the plough layer using porous plastic tubes. The tubes were placed in the appropriate depth and the soil solution was collected in the tubes using a vacuum pump. Plant samples (four plants per plot) were collected at 14 days intervals. Dry weight of the aboveground part of the plant was recorded after drying the plant tissues at 72 °C to constant weight.

### Soil and plant N analysis

Soil samples were extracted with KCl according to Keeney and Nelson (1982). NO<sub>3</sub>-N content in both soil extracts (mg 100 g<sup>-1</sup> soil) and soil solutions (mg L<sup>-1</sup>) was determined according to the cadmium reduction method using the NitraVer reagent (Hach Company, Loveland, Colorado USA). The determination of NH<sub>4</sub>-N content in both soil extracts (mg 100 g<sup>-1</sup> soil) and soil solutions (mg L<sup>-1</sup>) was based on the Berthelot reaction according to Houba et al. (1995). N content (%) of the aboveground dry weight biomass (DW) production was determined after persulfate digestion, which converts organic N to NO<sub>3</sub> (Purcell and King, 1996). In brief, samples of 0.05 g were added to 50 mL digestion solution containing 45 g K<sub>2</sub>S<sub>2</sub>O<sub>8</sub> L<sup>-1</sup> and 19 g NaOH L<sup>-1</sup>, and the mixture was placed in an autoclave at 120°C (0.1 MPa) for 1.5 h. After digestion, the mixture was allowed to cool overnight in the autoclave. NO<sub>3</sub> concentration in digested samples was determined by a selective nitrate electrode (Model 51920-00, Hach Company, Loveland, Colorado USA).

### Nitrogen uptake (Nup)

Nitrogen uptake (N g m<sup>-2</sup>) was calculated by multiplying plant density with aboveground DW production and plant tissue N concentration.

### Nitrogen dilution curve

According to Barbanti et al. (2006), a dilution curve of N concentration at increasing DW is outlined, whose shape is represented by a negative power function:

$\%N = a(DW)^b$  (2), where  $a$  and  $b$  parameters are experimentally determined, and  $b < 0$ .

### Nitrogen nutrition index

N status of the plant was evaluated by the nitrogen nutrition index (NNI) (Lemaire et al. 1989):

$$NNI = \frac{N}{N_{crit}} \quad (3),$$

where  $N$  is nitrogen concentration (% DW) of the plant tissue at each growth stage and  $N_{crit}$  ( $\%N_{crit} = a(DW)^b$ ) is the critical N concentration of the plant tissue for maximal growth (morphogenesis) without N storage. When NNI is equal to 1, N nutrition is considered as optimum, while higher values indicate excessive N availability and lower values indicate N deficiency.

### Statistical analysis

Data were subjected to analysis of variance (ANOVA) using the statistical system (SPSS Inc., IL, USA, version 16.0). Mean values were compared using the Least Significant Difference (LSD) test at  $P \leq 0.05$ .

### Conclusions

The findings of this study demonstrate that organic soil fertility management does not have a negative effect on soil N availability and plants Nup efficiency during the growth cycle of sweet sorghum, compared to conventional soil fertility management. Therefore, organic soil fertility management could be considered as an effective alternative cropping system for the cultivation of sweet sorghum in the Mediterranean region.

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