^{15}N-Labelled Fertilizer Recovery by Sweet Sorghum in Mediterranean Climate

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Abstract

A ^{15}N-labelled fertilization trial was carried out on sweet sorghum, grown in semi-arid environments of southern Europe with the aim to monitor the efficiency and effectiveness of the N-fertilisation technique under irrigation and different nitrogen fertilization rates, in factorial combination. A rainfed condition was compared with a full irrigation treatment (100% restoration of total crop water consumption), in a similar way, an unfertilized control was tested with respect to N application rates of 60 and 120 Kg ha^{-1}, respectively. The fertilisation efficiency measured directly through the isotope discrimination technique was on average equal to 15%. The aliquot of nitrogen released by the fertiliser into the soil and not absorbed by the plant becomes part of the different components of the soil nitrogen balance, regardless of its origin.

Key-words: fertilization efficiency; N-fertilizer loss; nitrogen use efficiency, *Sorghum bicolor*.

1. Introduction

Sweet sorghum (*Sorghum bicolor* L. Moench) for biomass and ethanol production was recently fostered by the European agricultural policy to promote non-food crops. The interaction between irrigation and nitrogen fertilization is a crucial aspect of the cropping technique, in order to obtain the most convenient yield as well as an efficient use of nitrogen and water applied together with the minimum risk of environmental impact due to N leaching (Perniola et al., 1999) or volatilisation (Bouwman et al., 1997). Unlabelled N-fertilisation trials provide general indications about the efficiency and effectiveness of the N-fertilisation technique; this kind of information is often merely broad, sometimes misleading. The crop N-fertilizer uptake or the N amount released to the soil as well as that leached to groundwater can be effectively measured through labelled ^{15}N fertiliser only (Carranca et al., 1999). This technique, therefore, allows a more accurate monitoring of the fate of nitrogen derived from fertilizer. Several studies were carried out by means of ^{15}N-labelled fertilization trials, they generally point to a considerable variability in the partition of nitrogen in the soil-plant-atmosphere continuum as a function of the interaction among several factors, the type of fertiliser and the timing of its application, the cultivated species, the climatic environment and the seasonal meteorological time-course, the soil chemical and physical properties are the most relevant features (Recous et al., 1988). To give an idea of this remarkable variability, Machet et al. (1987) report that the crop "real utilization coefficient" (Remy and Viaux, 1982), in other

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words the nitrogen fertilisation efficiency determined at harvest can vary up to the 80% according to different experimental conditions. The limited amount of available information on such topics, particularly with respect to sweet sorghum grown in semi-arid environments of southern Europe, encouraged to plan and start this trial to monitor in this crop with and without irrigation and under different nitrogen fertilizer rates the nitrogen fertilizer recovery through the use of [15]N.

2. Materials and methods

The trial was carried out in a Mediterranean area, at Metaponto (40°00' N, 16°48 'E) in the summer 1999. Metaponto is on the coast of the Ionic sea, in the south-east part of the Basilicata region (South-Italy). Table 1 reports the main soil characteristics of the experimental area. Sweet sorghum (cv. Keller) was sown on 17 May, 1999 with rows 50 cm apart and an average plant density of 11 plants m^{-2} at harvest. Before sowing, 150 Kg ha^{-1} of P_2O_5 and 100 Kg ha^{-1} of K_2O were applied. Other cropping practices included hoeing for weed control and hand harvesting in late October. Two irrigation regimes (irrigation only at sowing, V_0, and re-establishment of 100% of total water consumption during the whole growing cycle, V_{100}) were factorially combined with three nitrogen fertiliser rates (un-fertilised control, N_0, application of 60 and 120 Kg ha^{-1} of nitrogen as ammonium sulphate, 1/3 of which at pre-sowing and 2/3 at the beginning of stem elongation, N_{60} and N_{120}). Crop Evapotranspiration (ETc) was estimated by multiplying reference evapotranspiration (ETo), using Penman-Monteith formula, by the crop coefficients (Kc), the latter determined in the course of a previous trial (Perniola et al., 1993). The crop was drip-irrigated with driplines 1 m apart and on-line drippers of 2 l h^{-1} at 30 cm spacing. In this experiment, labelled ammonium

sulphate was used (^{15}($NH_4)_2SO_4$ 10% enrichment with ^{15}N). In the plots where 60 and 120 Kg ha^{-1} of commercial ammonium sulphate were applied, micro-plots of 4 m^2 were isolated and, keeping the same nitrogen rates as the neighbouring treatments, ^{15}N labelled fertiliser was top-dressed. The micro-plots were accurately isolated prior to the application of unlabelled fertiliser and, in order to ensure uniform distribution of the labelled fertiliser, the latter was dissolved in 20 l of water and applied through hand-operated distribution pump. In each micro-plot, a micro-lysimeter was installed and equipped with a porous cup to collect the soil solution at 90 cm depth. Next to it, a device to capture volatilised ammonia was installed above the soil surface. Volatilisation loss of N-ammonium was detected by capturing ammonia through a static closed system installed at the soil surface and saturated with H_3PO_4 and glycerin. In the lab, a subsequent distillation and titration of the ammonium ion (Marshall and Debell, 1980) allowed the measurement of this component of the N-balance. Frequency of volatilisation measurement was every three days immediately after fertilization and subsequently was weekly, each measurement was the cumulated value of the previous period. At harvest, a representative plant sample was taken from each micro-plot and subsequently subdivided into stalks, leaves and panicles for dry matter determination. Crop N-uptake was calculated by measuring the dry biomass of leaves, stalks and panicles and determining the corresponding Kjeldhal nitrogen content. In the same micro-plots, soil sampling was performed in the 0-30, 30-60, 60-90 cm deep soil layers. Soil and plant samples from the micro-plots, as well as the solutions obtained from the micro-lysimeters and ammonia traps, were analysed for isotope enrichment determination. The isotope ratio was determined through the mass spectrophotometer ANCA-MS by Thermoquest Italia, after burning the sample at 1700 °C in

Table 1. Main soil characteristics at the experimental station.

Sand	Loam	Clay	Organic	Total matter	P_2O_5 Nitrogen	K_2O	pH	Soil bulk density	Field Capacity -0.3 bar	Wilting Point -15 bar
(%)	(%)	(%)	(%)	(g/Kg)	(ppm)	(ppm)		(Kg/dm³)	(% d.w.)	(% d.w.)
2.2	46.7	51.1	1.60	1.2	24.0	356	7.4	0.99	38.7	18.8

the elementary analyser (EA 1110 CHNS-O) and letting gases pass through the mass spectrometer (Finnigan-Mat, Delta plus model). The isotope discrimination method allowed to exactly calculate nitrogen derived from fertilizer, Ndff, as the fraction of nitrogen in the plant, soil, leachate or volatilised sample effectively derived from fertiliser over the total N content of the same sample (Schjoerring et al., 1995); to calculate Ndff the following formula was applied:

$$Ndff = (\%^{15}N_{sample} - \%^{15}N_{back}) / \quad (1)$$
$$(\%\ ^{15}N_{fert} - \%^{15}N_{back})$$

where: $\%^{15}N_{sample}$ = percent ^{15}N enrichment of the sample; $\%^{15}N_{back}$ = natural ^{15}N enrichment in the soil (measured on the N_0 treatment and equal to 0.3663% ^{15}N atoms); $\%^{15}N_{fert}$ = percent ^{15}N enrichment of the fertiliser.

Another useful coefficient is the nitrogen recovery, Nrec, defined as the fraction of nitrogen present in the plant, soil, leachate or recovered volatilised sample with respect to the fertilisation rate (Carranca et al., 1999); the applied formula is:

$$Nrec = (N_{sample} / N_{fert}) \times Ndff \quad (2)$$

where: N_{sample} = total nitrogen content of the sample; N_{fert} = total nitrogen content of the fertiliser.

N-denitrification (N_2, NO and N_2O) is generally considered to be negligible in agricultural systems (Smith et al., 1990), especially when anaerobiosis conditions do not occur, in fact according to our irrigation scheduling (the average irrigation interval was ten days, and with irrigation we restored only the field capacity), and considering rain regimes during the experiment and soil characteristics the denitrification should be really negligible, for this reason these kind of losses was not detected. In this work, the variations in the soil nitrogen reserve were calculated as residue of the N-balance.

The experimental treatments were arranged in the field according to a split-plot design with three replicates, the irrigation regimes in the plots (12 x 6 m) and the nitrogen rates in the sub-plots (12 x 3m). The percent data were submitted to the analysis of variance after angular transformation. The mean discrimination was performed according to the LSD test.

3. Results and discussion

3.1 Climatic pattern and water balance during the experiment

Referring to the whole growing cycle (from May through October) during the trial the average temperature varied from a minimum of 21.6 °C and a maximum of 23.8 °C in 1999, being however close to the long term mean of 22.1 °C; the radiative load showed an average value of 247 W m^{-2}. The overall precipitation recorded was of 187 mm approximately close to the long term mean of the same period (191 mm). The seasonal irrigation volume was of 488 mm, supplied to fully satisfy crop water requirement (ETc) of 587 mm, considering the long extent of the crop cycle and the great crop vegetative vigour, these high and uneven irrigation volumes are well representative of the irrigation requirements of this crop in southern Italy (Perniola et al., 1992). Over the whole growing period, the V_{100} drainage volumes totalled 106 mm.

3.2 Nitrogen isotope discrimination

The analysis of ^{15}N enrichment of plant tissues (Fig. 1) highlighted that in the two compared treatments (N_{60} vs. N_{120}), the relative amount of nitrogen derived from fertiliser was significantly higher in the treatment receiving 120 Kg ha^{-1} as compared with the treatment receiving 60 Kg ha^{-1} N. In particular, out of the total nitrogen present in the plant tissues of treatment N_{120} (96.3 Kg ha^{-1}), 24.5% derived from fertiliser
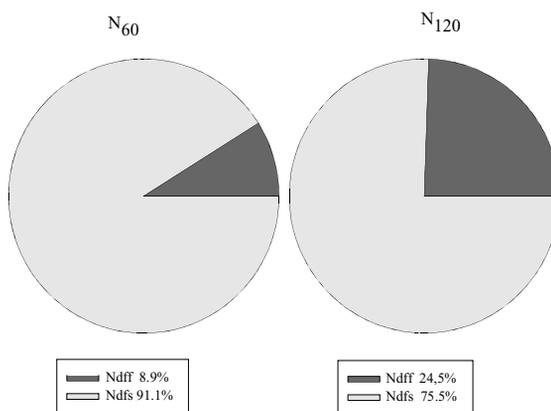

Figure 1. Partitioning of the crop nitrogen content according to the two different fraction: nitrogen derived from fertilizer (Ndff) and nitrogen derived from the soil (Ndfs) on sweet sorghum.

(Ndff) and the remaining part (75.5%) from the soil (Ndfs); whereas, in the N_{60} treatment, only 8.9% of total crop N-uptake (76.9 Kg ha^{-1}) derived from fertiliser (Ndff) and the remaining 91.1% derived from the soil reserves (Ndfs).

A greater fertilisation efficiency was detected in the N_{120} treatment since the fraction of nitrogen present in the plant (% Nrec) was 19.7% of the overall amount supplied with the fertilisation, whereas in treatment N_{60}, the nitrogen present in the plant represented only 11% of the fertiliser amount. From the data analysis regarding the ^{15}N concentration in the soil, it resulted that the applied fertiliser mainly accumulated in the uppermost soil layer, since Nrec varied, as an average of the two treatments, between 44.1 and 9.9% moving from the layer 0-30 to 60-90 cm depth. From the analysis of ^{15}N enrichment of the solution recovered from the micro-lysimeters, referring only to the two samplings performed on June 8, 1999 and July 20, 1999 immediately after fertilisation, it was found that in the upper 30 cm layer, the soil solution of the more fertilised treatment (N_{120}) was found to have 27.8% (% Ndff) and 69.2% (Ndff) of nitrogen derived from fertiliser, respectively. Table 2 reports nitrogen cumulated losses by volatilisation in the form of ammonia at the end of the crop cycle. The statistical analysis showed a significant reduction in losses with decreasing fertilizer rate. The effect of fertilisation was particularly evident in the days immediately after the fertilizer application (data not shown), due to the fact that nitrogen was supplied as ammonium sulphate. As for nitrogen losses by volatilisation in the form of ammonia, referring again only to the days after fertilization, from the analysis of ^{15}N enrichment of

the solution recovered from the ammonia capturing system, it was found that at the first sampling date (1st June 1999) the nitrogen derived from fertiliser (% Ndff) was 1.42% in treatment N_{60} and 2.6% in the treatment N_{120}. Whereas, after the second fertiliser application (6 July 1999), the loss of nitrogen derived from fertiliser was greater: 11.4% in treatment N_{60} and 18.8% in treatment N_{120}, respectively.

In conclusion the experimental trial on sweet sorghum was aimed at studying the environmental aspects as affected by nitrogen fertiliser and irrigation through monitoring some of the main nitrogen balance components, lead to the following concluding remarks. The fertilization efficiency directly measured in the fertilised treatments through the isotope discrimination technique was on average equal to 15%. Other approach to asses fertilizer N recovery by the difference method (determined as the ratio of the increased nitrogen uptake by the fertilised crop as compared with the unfertilised crop over the fertilization rate, Varvel and Peterson, 1991) considerably overestimates fertilization efficiency in sorghum since the calculation is based on an apparent uptake value, i.e. assuming that the plant first takes all the nitrogen applied through the fertilizer (Varvel and Peterson, 1991). Indeed, the plant root system absorbs the nutrients required in the ionic form best suitable for it, whether it derives directly or indirectly from the fertilizer or from the mineralization processes of the soil nitrogen pool. Furthermore, some authors have observed that when nitrogen accumulation (nitric form) inside roots exceeds plant metabolic requirement it is observed a decrease of speed nitrate uptake (back-regulation) (Varanini et al., 2008, Locci et al., 2001). Our results obtained by isotope methods suggest that the aliquot of nitrogen released by the fertiliser to the soil and not removed by the plant becomes part of the different components of the soil nitrogen balance, regardless of its origin.

Table 2. Nitrogen loss by ammonia volatilization. All data are expressed in kg ha^{-1}.

N dose Irrigation	N_{120}	N_{60}	N_0
V_{100}	6.4	5.4	4.5
V_0	5.8	5.0	3.3
		F-test§	LSD
Irrigation		n.s.	-
N fert. rate		**	0.35
Irrig. x N fert.		n.s.	-

** (P < 0.01); n.s. not significant.

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