

Nitrogen fertilisation of durum wheat: a case study in Mediterranean area during transition to conservation agriculture

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Abstract

Nitrogen (N) nutrition plays a key role for high yields and quality in durum wheat (*Triticum turgidum* L. subsp. *durum* (Desf.) Husn); in Mediterranean environments, data regarding N fertilisation management during the transition phase to conservation agriculture (CA) are limited. The aim of this work was to study the effects of N fertiliser forms and rates on yield and some quality traits of durum wheat, during the transition period to CA in Mediterranean areas; moreover, indication on the recommendable N form/rate combinations have been given. Field trials were carried out in south of Italy, during the first two years of transition to CA (from 2010 to 2012) in a durum wheat-based rotation. Following a split-plot design arranged on a randomised complete blocks with three replications, two N forms (main plots) - urea and calcium nitrate - and four N rates (sub-plots) - 50, 100, 150 and 200 kg N ha⁻¹ - plus an un-fertilised Control, were compared. The following parameters were analysed: grain yield, N-input efficiency,

grains protein concentration (GPC), total gluten, gluten fractions and minerals concentration in kernels. Calcium nitrate gave the highest yield (4.48 t ha⁻¹), as predicted by the quadratic model, at 146 kg N ha⁻¹, on average. This was particularly noticeable in 2012, when the distribution of rainfall and temperatures regimes as well as residues' status could have favoured such N-form. These results were confirmed by the observed higher values of all indices describing N-input efficiency. High GPC values (14.8%) were predicted at slightly higher N-rates (173 kg N ha⁻¹, averaging both N forms). In particular, gluten proteins and glutenin/gliadin ratio accrued as the N doses increased, reaching the highest values at 150 kg N ha⁻¹, also positively affecting the quality of durum wheat flour. Iron and zinc concentrations were noticeably increased (38% and 37% on average) by N supply, probably due to the enhanced water use efficiency under CA systems.

Principal component analysis summarised properly the obtained results: analysing the N-rates at 150 kg N ha⁻¹, it was confirmed that yields and quality characteristics of durum wheat were optimised in the wettest year (2011) with calcium nitrate. Moreover, the scarce amount of residues characterising the transition phase to CA, requires N application rates not lower than 150 kg ha⁻¹ in order to ensure stable yields and important quality traits. These N rates should be modulated as the accumulation of crop residues increases over time, thanks to long-term effects of CA on soil chemical, physical and biological properties.

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Introduction

Southern Europe's environment is characterised by cool and wet winters and hot and dry summers with low and erratic rainfall distribution, and by soils with very low organic matter levels and nitrogen (N) content. Nitrogen is subjected to several losses with the main pathways being represented by ammonia volatilisation, denitrification and nitrate leaching (Plaza-Bonil *et al.*, 2014). Moreover, in these environments agricultural systems depend mainly on winter crops; with limited alternatives, farmers rely on short rotations based on durum wheat (*Triticum turgidum* L. subsp. *durum* (Desf.) Husn.), and rarely on sunflower (*Helianthus annuus* L.) and faba bean (*Vicia faba* L.). This cereal crop contributes significantly as a source of carbohydrate and it is a potential contributor of micronutrients and proteins in human diets, which has led to a renewed interest in whole meal durum-based products. Mineral elements have been suggested to have an important role in maintaining good health (Buri *et al.*, 2004), as well as proteins. Proteins concentration in kernels is clearly conditioned by environmental variables such as water availability and temperature during growth, especially through the grain-filling period (Tea *et al.*, 2004), which influence the rate and duration of wheat grain development, protein accumulation and starch deposition (Ottman *et al.*, 2000; Dupont and Altenbach, 2003). However, the most effective

environmental factor on protein accumulation is N availability; recent data on wheat underlined the effects of N also on the modification of wheat gluten protein composition, with respect to single members of gliadin and to the high and low molecular weight glutenins (HMW-GS, LMW-GS) (Hurkman *et al.*, 2013; Wan *et al.*, 2013; Visioli *et al.*, 2016).

Conservation agriculture (CA), among the *greener* solutions currently being discussed (García-Torres *et al.*, 2003; Gilbert, 2012), has the potential of mitigating the negative effects of soil fertility losses and climate changes (Pisante, 2007; Kassam *et al.*, 2009; Stagnari *et al.*, 2010; Pisante, 2013). In general under CA, soil and water conservation are increased, the soil physical and chemical properties as well as its biological activity are improved, and consequently the N losses are reduced (Deng *et al.*, 2006); however, under particular conditions N losses in no-tillage systems could increase compared to tillage-based ones (Mackenzie *et al.*, 1998; Baggs *et al.*, 2003). Moreover, the transition phase to CA is very complex and may influence negatively the farmers; weeds and fertilisation management represent major issues (Knowler and Bradshaw, 2007) and soil physical and biological health takes time to develop. In such conditions, yield of durum wheat could be inconsistent, depending on soil-climatic characteristics and on a number of interacting factors, including residue management, cultural practices and drill performance (Gemtos *et al.*, 1998). Consequently, N fertilisation strategies need to be adequately adjusted, combining rate, timing, splitting, and source of N application, with the aim of optimising yield and its quality (Abedi *et al.*, 2010). Indeed, by adopting CA, thanks to the improved soil surface covering, water capture and retention are enhanced, thus increasing crop growth and N uptake. This may reduce the availability of soil mineral N and may require an increasing in N fertilisation (Morell *et al.*, 2011). Few studies indicate that slightly higher doses of fertiliser are required to achieve significant yields in winter wheat in presence of a layer of surface residues (Gao *et al.*, 2009), especially under long-term periods (>10 years) of CA adoption.

In durum wheat-based agricultural systems of the suited Mediterranean areas, studies investigating the effect of N fertilisation management on yield and quality of this crop, during the transition phase to CA are lacking; in addition, the scientific literature does not provide devised recommendations on the optimum N rates and forms to be applied in these conditions. This dearth of data was the catalyst to this research; the aim was to study the effects of N nutrition on durum wheat yield and some quality traits such as, HMW-GS, LMW-GS and gliadin content, minerals accumulation during the transitional phase from conventional agriculture to CA, in Mediterranean environments; moreover, indication on the suitable combination of N form and rate are given.

Materials and methods

Site description

A two-year field experiment was carried out during 2010-2011 and 2011-2012 growing seasons (referred below as 2011 and 2012, respectively) at the experimental field of the University of Teramo (Mosciano Sant'Angelo, Italy, 42° 42' N, 13° 52' E, 101 m a.s.l.). The climate is typically Mediterranean with a mean of annual rainfall, recorded over a 58-year period, of 732 mm mainly concentrated between October and April. The means of the maximum and minimum temperatures range from 11°C to 29°C and from 2°C to 17°C, respectively. The soil is a typical medium-clay soil with the follows characteristics: 23% sand, 45% silt, 32% clay, 1.36% o.m., pH 8.1, 19% total CaCO₃, 10.6% active CaCO₃, 0.16% total N and 3211 ppm CEC.

Meteorological data were recorded by a meteorological station situ-

ated ~1 km from the experimental field. Minimum, maximum, average temperatures and rainfall referred to the whole crop cycles are reported in Figure 1.

Crop management and experimental design

Conservation agriculture methods were first imposed in 2010. Soil was subjected to no-till management, with repeated reuse of existing beds, which were reformed in the furrows without disturbance of the tops of the beds as needed. The preceding crops were coriander (*Coriandrum sativum* L.) in 2011 and wheat in 2012, which were harvested at the end of July and at the middle of June, respectively. The amount of crop residues (2.9 t ha⁻¹ and 7.0 t ha⁻¹ on average for coriander and wheat, respectively) was estimated by collecting and weighting the leaves and stems on 10 randomly selected sub-units (0.25 m²) in the experimental area. The nitrogen content in vegetative organs was determined with the Kjeldahl method, while the organic carbon (C) content was determined following the Walkley and Black procedure (Walkley and Black, 1934). Wheat straw contained 0.42% N while coriander 1.54% with a C:N ratio of 107.3 and 29.9, respectively. Crop residues were kept on the soil surface and homogeneously distributed in order to obtain a uniform layer of mulch and without interfering with the subsequent sowing operations. Two weeks before sowing, glyphosate (45%) were applied at the rate of 2 L ha⁻¹. Durum wheat (*Triticum turgidum* L. subsp. *durum* (Desf.) Husn., cv *Achille*) was sown on mid-November (8/11/2010 and 17/11/2011) by direct seeding [Gaspardo Direttissima, Gruppo Maschio Gaspardo SpA, Campodarsego (PD), Italy], at a rate of 350 viable seeds m⁻². This sowing density is recommended for durum wheat modern cultivars in Mediterranean environments to obtain a density of about 300-400 ears m⁻². The experimental design consisted in a split-plot arranged on a randomised complete blocks with three replications. Two forms of N (urea and calcium nitrate), represented the main plots, and four levels of N rates (50, 100, 150 and 200 kg N ha⁻¹) the sub-plots; an N-unfer-

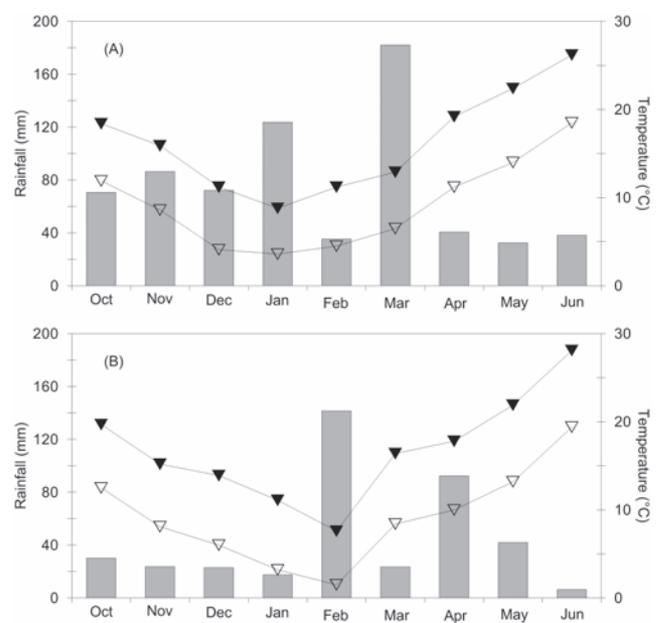


Figure 1. Rainfall (bars), maximum (black symbols) and minimum (empty symbols) temperatures during 2010/2011 (A) and 2011/2012 (B) growing seasons, registered at Teramo (Italy). Rainfall values represent sums, and temperature values means, over one month period.

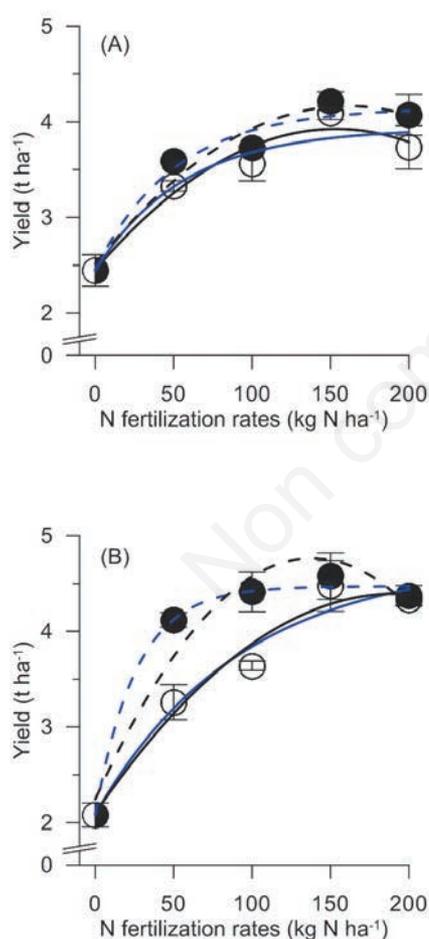
tilised treatment was also included within each main plot as control (CONTROL). The experimental units (sub-plots) were of a 24 m² (3x8 m) with 17 rows spaced 17 cm apart. For all treatments (including CONTROL) the pre-sowing inputs included 96 kg ha⁻¹ of P₂O₅ as single super phosphate (16% P₂O₅); N fertilisation was performed on all N-fertilised treatments (excluding CONTROL) and fractioned as follows: 20% of the expected N dose as pre-sowing fertilisation, while the remaining 80% N was applied as cover dressing in equal amount on two dates: at tillering and at 4th node detectable stages (DC22 and DC34, following the Zadoks Decimal Code; Zadoks *et al.*, 1974). Fertilisers were homogenously and carefully distributed in order to ensure the highest uniformity. At the phenological stage of mid-tillering, post-emergence applications of Atlantis [active ingredients (a.i.) mesosulfuron-methyl, iodosulfuron-methyl-sodium and mefenpir-diethyl; Bayer CropScience Italia, Milan, Italy] at the dose of 0.5 kg ha⁻¹, Biopower (a.i. 3,6-dioxaoctadecylsulphate sodium salt and 3,6-dioxaicosylsulphatesodium salt; Bayer CropScience Italia) at the dose of 1 L ha⁻¹ and Zenith (a.i. fluorasulam and 2,4-D; Dow AgroSciences Italia, Bologna, Italy) at the dose of 0.75 L ha⁻¹ were performed to control both

broadleaf and graminaceous weeds. Fungicide Sphere (trifloxystrobin and cypro-conazole; Bayer CropScience Italia) was applied at the dose of 1 L ha⁻¹ at DC50 stage.

Plant sampling, yield and biomass N determination

At harvest (on June 23 and 20 in 2011 and 2012, respectively), five sub-samples of 1 m linear row of whole plants were randomly collected to determine total dry matter, yield - by weighting the amount of kernels from each plot - and organs nitrogen concentration, determined with the Kjeldahl method. Grains protein concentration (GPC, %) was calculated as grain N concentration multiplied by 5.7 (Sosulski and Imafidon, 1990).

Nitrogen uptake (N_g) was estimated by multiplying grain and straw weight with the respective values of N concentrations for a particular treatment. According to Novoa and Loomis (1981) and Craswell and Godwin (1984), the following parameters were calculated as follows: i) apparent N recovery (ANR, %): as the ratio of (N_g at N_x - N_g at N_0) to applied N at N_x ; ii) physiological efficiency (P_E) (kg kg⁻¹): as the ratio of (grain yield at N_x - grain yield at N_0) to (N_g at N_x - N_g at N_0); iii) agro-



Coefficients	Urea	Calcium Nitrate
<i>Quadratic model</i>		
β_0	2.45±0.132 ***	2.51±0.111 ***
β_1	0.02±0.003 ***	0.02±0.003 ***
β_2	-6.29 e ^{-0.5} ±1.50 e ^{-0.5} **	-6.38 e ^{-0.5} ±1.26 e ^{-0.5} ***
R^2	0.95	0.95
RSS	0.710	0.502
S^2	0.059	0.042
<i>Exponential model</i>		
β_0	3.93±0.166 ***	4.15±0.109 ***
β_1	0.02±0.007 *	0.02±0.005 **
β_2	54.12±20.207 *	45.85±10.709 **
R^2	0.93	0.97
RSS	0.825	0.422
S^2	0.069	0.035

* $P < 0.1$; ** $P < 0.05$; *** $P < 0.01$; **** $P < 0.001$; n.s. = not-significant
 RSS = Residual sum-of-squares
 S^2 = Residual mean square

Coefficients	Urea	Calcium Nitrate
<i>Quadratic model</i>		
β_0	2.10±0.162 ***	2.25±0.194 ***
β_1	0.02±0.004 ***	0.04±0.005 ***
β_2	-6.25 e ^{-0.5} ±1.84e ^{-0.5} **	-1.32 e ^{-0.4} ±2.21 e ^{-0.5} ***
R^2	0.97	0.94
RSS	1.071	1.540
S^2	0.089	0.128
<i>Exponential model</i>		
β_0	4.76±0.395 ***	4.47±0.103 ***
β_1	0.01±0.004 *	0.04±0.011 **
β_2	53.82±17.215 **	16.16±4.829 **
R^2	0.97	0.99
RSS	1.119	0.884
S^2	0.093	0.074

* $P < 0.1$; ** $P < 0.05$; *** $P < 0.01$; **** $P < 0.001$; n.s. = not-significant
 RSS = Residual sum-of-squares
 S^2 = Residual mean square

Figure 2. Durum wheat yield (t ha⁻¹) against nitrogen (N) fertilisation rates (0, 50, 100, 150 and 200 kg N ha⁻¹) in 2011 (A) and 2012 (B). Empty symbols stand for urea, black symbols stand for calcium nitrate. Data are averages ± standard errors, for n=3 independent replicates. On the right, the corresponding parameters of the fitted quadratic (black lines) and exponential (blue lines) models. Solid lines, urea; dashed lines, calcium nitrate.

onomic efficiency (A_E) (kg kg^{-1}): as the ratio of (grain yield at N_x – grain yield at N_0) to N applied at N_x .

Gluten proteins extraction and quantification

Thirty-five g of grains from each treatment were crushed with Knifetec TM 1095 (Foss, Hillerød, Denmark) to obtain a fine powder and used to analyse the composition of gliadin, HMW-GS and LMW-GS fractions. Gluten proteins were extracted from wheat flour by using a modification of the sequential procedure of Singh *et al.* (1999). Fine powder (30 mg) was extracted with 1.5 mL of 55% (v/v) propan-2-ol for 20 min with continuous mixing at 65°C, followed by centrifugation for 5 min at 10,000 rpm. This step was repeated three times and the gliadin component was extracted. Pellet containing the glutenin fraction was resuspended in a solution of 55% (v/v) propan-2-ol, 0.08 M tris(hydroxymethyl)aminomethane hydrochloric acid pH 8.3 and 1% (w/v) dithiothreitol as reducing agent and incubated for 30 min at 60°C with continuous mixing. After centrifugation for 5 min at 14,000 rpm, the supernatant containing both HMW and LMW-GS fractions, was transferred into a new tube. Acetone was then added to reach a final concentration of 40% (v/v) to precipitate HMW-GS. The remaining supernatant was

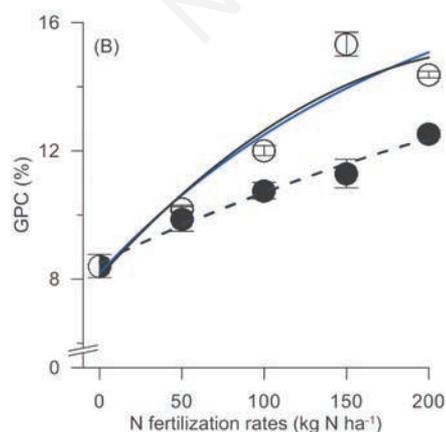
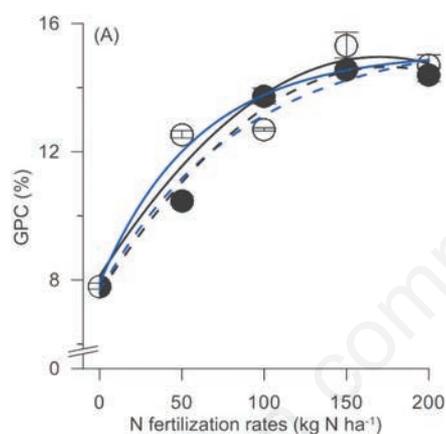
precipitated adding acetone to reach a final concentration of 80% (v/v) and the LMW-GS fraction was recovered. Both protein fractions were dissolved in (50:50 v/v) acetonitrile and H₂O with 0.1% (v/v) trifluoroacetic acid and quantified with the Bradford assay. Three replicates were performed for each sample.

Mineral concentration

Minerals concentrations [calcium (Ca), magnesium (Mg), potassium (K), iron (Fe), manganese (Mn) and zinc (Zn)] of collected grains were determined by atomic absorption spectrometry (Perkin-Elmer A Analyst300, Waltham, MA, USA), according to the Method 968.08 (AOAC, 1995), while phosphorus (P) was determined by the colorimetric method with molybdo-vanadate reagent (Method 965.17) (AOAC, 1995). Mineral concentration was expressed in mg g^{-1} of grains dry matter.

Statistical analysis

Data regarding total gluten protein, glutenins, gliadins and minerals concentration in grains were plotted as a function of N fertilisation rates (kg N ha^{-1}) and the statistical differences between treatments were detected by comparing the means \pm standard errors.



Coefficients	Urea	Calcium Nitrate
<i>Quadratic model</i>		
β_0	8.13±0.478 ***	7.59±0.246 ***
β_1	0.08±0.01 ***	0.08±0.006 ***
β_2	-2.35 e ^{-0.4} ±5.43 e ^{-0.5} ***	-2.32 e ^{-0.4} ±2.80 e ^{-0.5} ***
R^2	0.93	0.98
RSS	9.271	2.461
S^2	0.773	0.205
<i>Exponential model</i>		
β_0	15.11±0.557 ***	15.60±0.678 ***
β_1	0.02±0.004 **	0.01±0.003 ***
β_2	43.90±11.161 **	57.20±11.321 ***
R^2	0.94	0.97
RSS	8.054	4.396
S^2	0.671	0.366

* $P < 0.1$; ** $P < 0.05$; *** $P < 0.01$; **** $P < 0.001$; n.s. = not-significant

RSS = Residual sum-of-squares

S^2 = Residual mean square

Coefficients	Urea	Calcium Nitrate
<i>Quadratic model</i>		
β_0	8.09±0.487 ***	8.53±0.310 ***
β_1	0.06±0.01 ***	0.02±0.007 **
β_2	-1.13 e ^{-0.4} ±5.54 e ^{-0.5} *	-2.31 e ^{-0.5} ±3.52 e ^{-0.5} n.s.
R^2	0.92	0.98
RSS	9.652	3.900
S^2	0.804	0.325
<i>Exponential model</i>		
β_0	18.88±4.432 **	17.51±8.986 *
β_1	0.005±0.003 n.s.	0.003±0.004 n.s.
β_2	109.50±45.30 *	236.60±145.30 n.s.
R^2	0.91	0.98
RSS	10.400	3.875
S^2	0.867	0.323

* $P < 0.1$; ** $P < 0.05$; *** $P < 0.01$; **** $P < 0.001$; n.s. = not-significant

RSS = Residual sum-of-squares

S^2 = Residual mean square

Figure 3. Durum wheat grains protein concentration (GPC) (%) against nitrogen (N) fertilisation rates (0, 50, 100, 150 and 200 kg N ha^{-1}) in 2011 (A) and 2012 (B). Empty symbols stand for urea, black symbols stand for calcium nitrate. Data are averages \pm standard errors, for n=3 independent replicates. On the right, the corresponding parameters of the fitted quadratic (black lines) and exponential (blue lines) models. Solid lines, urea; dashed lines, calcium nitrate.

Data regarding N_g , ANR%, P_E and A_E were subjected to analysis of variance (two-way ANOVA) with N form regarded as the main factor and N rates regarded as secondary factor. Before ANOVA, data were analysed to test the satisfactory of the normality and homoscedasticity assumptions. The significance level was set at $P < 0.05$.

In order to describe wheat yield and GPC in response to N fertiliser, two statistical models (negative exponential and quadratic) were fitted to the recorded data. The exponential model [*i.e.*, the Mitscherlich model (1990)] is defined as follows (Eq. 1):

$$Y = \beta_0 (1 - (\exp(-\beta_1 (X + \beta_2))) \quad (1)$$

where Y is the response variable (grain yield, $t\ ha^{-1}$ or GPC, %), X is the predictor variable (N fertilisation rates, $kg\ N\ ha^{-1}$) and β_0 is the maximum yield, or GPC, when X is not limiting, β_1 is the increase in expected yield, or GPC, per unit X per unit of opportunity for yield, or GPC, to increase and β_2 is the nutrient value of soil in units or equivalents of X .

The quadratic model is defined by Eq. 2:

$$Y = \beta_0 + \beta_1 X + \beta_2 X^2 \quad (2)$$

where Y is the response variable (grain yield, $t\ ha^{-1}$ or GPC, %), X is the predictor variable (N fertilisation rates, $kg\ N\ ha^{-1}$) and β_0 , β_1 and β_2 are the coefficients of intercept, linear and quadratic terms, respectively. For the quadratic model, the predicted maximum yield was calculated by setting the first derivative equal to zero, solving for X , substituting the obtained X into the response equation and solving for Y . For the exponential model, the maximum yield approximately equals the estimated value of the intercept (β_0) of the model.

The principal component analysis (PCA) was applied to interpret and

summarise the correlation among variables [yield, total gluten proteins (TGP), gliadins, HMW-GS, LMW-GS, Ca, P, K, Fe, Mn and Zn] and to evaluate the relation between treatments (N forms and N rates) and variables. In order to simplify the dimensionality of the dataset and to obtain relevant information about the main quality characteristics in relation to yield, only the $150\ kg\ ha^{-1}$ rate was considered, as well as the N-unfertilised CONTROL. Overall, there were six treatments referring to the six year-N-form-N-rate combination: CONTROL_2011, CONTROL_2012, UREA_2011, UREA_2012, NITRATE_2011 and NITRATE_2012. The collected data were visually explored in a two dimensional PCA correlation bi-plot: standardised PC1 and PC2 scores were plotted as symbols, while the correlations between PCs and standardised variables (factor loadings) were plotted as vectors. The PCA was performed on standardised data (averages of $n=3$ independent replicates). All the statistical analyses were performed using the R software (R Foundation for Statistical Computing, Vienna, Austria) (R Development Core Team, 2013).

Meteorological data

The two growing seasons greatly differed in the amount and distribution of rainfall (Figure 1). It was particularly marked in the period ranging from sowing to anthesis: in 2011, the crops received an amount of 507.2 mm of rain, while in 2012 only 314.6 mm. The difference was consistent also from anthesis to the end of grain filling, with amounts ranging from 70.8 mm in 2011 to 47.6 mm in 2012, respectively. The mean air temperature of the whole growing season was higher in 2012 of about $1.5^\circ C$ ($11.7^\circ C$ and $13.1^\circ C$ for 2011 and 2012, respectively).

Table 1. Nitrogen grain uptake ($kg\ ha^{-1}$), apparent nitrogen recovery (%), physiological efficiency ($kg\ kg^{-1}$) and agronomic efficiency ($kg\ kg^{-1}$) of durum wheat as affected by nitrogen (N) fertilisation forms and rates and their combinations, in 2011 and 2012.

Treatment N form	N rate ($kg\ ha^{-1}$)	N uptake ($kg\ ha^{-1}$)	2011			2012			
			ANR%	P_E ($kg\ kg^{-1}$)	A_E ($kg\ kg^{-1}$)	N uptake ($kg\ ha^{-1}$)	ANR%	P_E ($kg\ ha^{-1}$)	A_E ($kg\ kg^{-1}$)
Urea	0	36.0	-	-	-	30.3	-	-	-
	50	71.6	71.1	29.7	21.2	59.9	59.1	37.0	22.0
	100	74.7	38.6	30.7	11.9	75.3	45.0	38.0	17.1
	150	118.7	55.1	22.0	12.1	127.9	65.1	26.3	17.1
	200	97.5	30.7	24.9	7.7	109.7	39.7	30.3	12.0
	Mean of N-form	97.0	48.9	26.8	13.2	93.2	52.2	32.9	17.1
Calcium nitrate	0	36.0	-	-	-	30.3	-	-	-
	50	74.6	77.2	33.8	25.9	69.8	79.0	56.5	44.3
	100	95.6	59.6	24.2	14.4	81.2	50.9	44.4	22.6
	150	108.5	48.3	25.5	12.3	104.8	49.7	38.0	18.8
	200	115.8	39.9	22.2	8.9	107.5	38.6	32.0	12.4
	Mean of N-form	98.6	56.2	26.4	15.4	90.8	54.6	42.7	24.5
Mean of N-rate	0	36.0	-	-	-	30.3	-	-	-
	50	73.1	74.1	31.7	23.5	64.8	69.1	46.8	33.2
	100	85.1	49.1	27.4	13.2	78.2	47.9	41.2	19.8
	150	113.6	51.7	23.7	12.2	116.4	57.4	32.2	18.0
	200	106.7	35.3	23.6	8.3	108.6	39.2	31.1	12.2
F-test	N-form	**	*	n.s.	n.s.	n.s.	n.s.	**	**
	N-rate	**	**	**	**	**	**	**	**
	N-form x N-rate	**	*	n.s.	n.s.	n.s.	*	**	**
LSD _{0.05}	N-form	3.5	4.59	2.69	2.02	6.9	6.25	2.55	2.23
	N-rate	5.5	7.25	4.26	3.19	10.9	9.88	4.04	3.53
	Interaction	7.8	10.25	6.02	4.51	15.4	13.97	5.71	4.99

N-form, nitrogen fertilisation forms; N-rate, N fertilisation rates; N uptake, nitrogen grain uptake; ANR, apparent nitrogen recovery; P_E , physiological efficiency; A_E , agronomic efficiency; LSD, least significant difference at $P < 0.05$; * $P < 0.05$; ** $P < 0.01$; n.s., not-significant.

Results and discussion

Effects of fertilisers on yield, grains protein concentration and nitrogen-input efficiency

The fertiliser N-rate had a significant effect on yield in both growing seasons. Regardless to N-form, yield generally increased as N rates raised up to 150 kg N ha⁻¹ (Figure 2A and B). As already reported by Garrido-Lestache *et al.* (2004), this was particularly noticeable in the wettest year (2011), whilst in drier year (2012) the response was only recorded at rates up to 100 kg N ha⁻¹, especially for calcium nitrate. Moreover, we observed slightly higher yields in 2012 with respect to

2011 as well as a greater effect of calcium nitrate than urea. Year-to-year differences in rainfall from October to May, which includes the autumn and spring periods prior to grain-filling, could have had a significant effect on the observed variations in yields. Indeed, López-Bellido *et al.* (2001) attributed a decrease in wheat yield when rainfall in the September-May period exceeded around 550 mm (as we observed during 2011), probably due to a poor establishment and development of the crop during the vegetative phase, since the excessive winter rainfall leads to waterlogging in typical medium-clay soil. However, the presence of a layer of crop residues could have mitigated this effect, especially at the highest N-rates. With respect to N-form, calcium nitrate allowed to obtain higher yields, particularly in 2012,

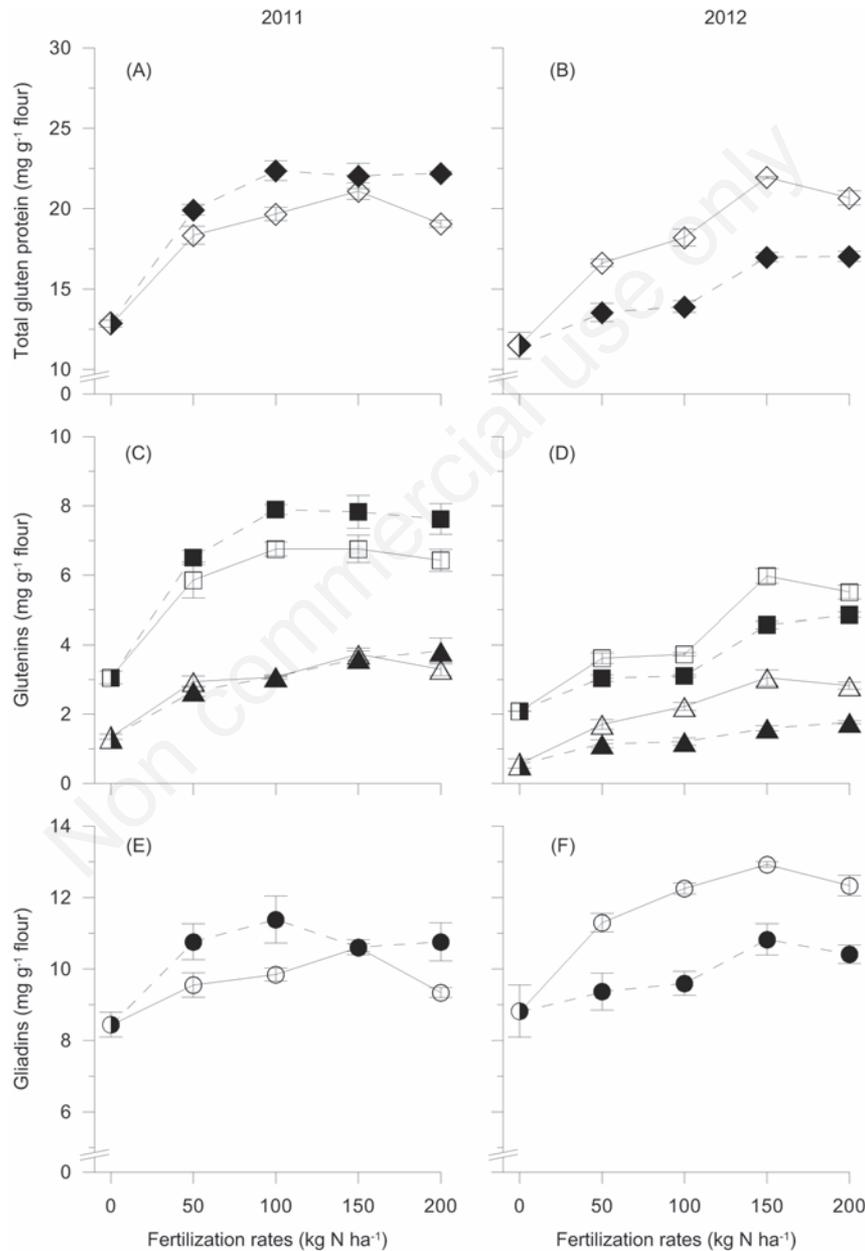


Figure 4. Total gluten proteins (mg g⁻¹ flour), gliadins (mg g⁻¹ flour) and glutenins (mg g⁻¹ flour) in durum wheat grains against nitrogen (N) fertilisation rates (0, 50, 100, 150 and 200 kg N ha⁻¹) in 2011 and 2012. Empty symbols stand for urea, black symbols stand for calcium nitrate (◆, total gluten protein; ●, gliadins fraction; ■, low molecular weight glutenins fraction; ▲, high molecular weight glutenins fraction). Data are averages ± standard errors, for n=3 independent replicates.

when rainfall were mainly located after the second N application split-time and the higher amount of surface residues ensured a better water retention, reducing nitrate leaching. On the other hand, the higher temperatures recorded in 2012 (*i.e.* March) may have induced greater loss of N *via* NH₃ volatilisation (Schoenau and Campbell, 1996) reducing the effectiveness of the less readily available N-form.

In order to synthesise our results, we fitted the exponential and quadratic equations to durum wheat yield data. All the two models significantly explained the relationship between yield and N fertilisation, as indicated by the higher obtained R² values (≥ 0.93) (Figure 2A and B). However, while the exponential model predicts maximum yield when fertiliser application is at infinity (Cerrato and Blackmer, 1990),

the quadratic model makes it possible to estimate the N dose, which correspond to the maximum yield. It diagnosed N rates for maximum yields below 200 kg N ha⁻¹ for each N form-year combination; such rates confirm those of De Sanctis *et al.* (2012) who indicated the dose of 180 kg N ha⁻¹ to obtain a 4 t ha⁻¹ yield in southern Italy, under no tillage. In particular, the model confirmed that the application of calcium nitrate could result in higher yields than urea (2011: 4.17 *vs* 3.93 t ha⁻¹, respectively; 2012: 4.76 *vs* 4.40 t ha⁻¹, respectively), achieved with N fertilisation rates of 161 and 153 kg N ha⁻¹ in 2011 and of 138 and 192 kg N ha⁻¹ in 2012 for calcium nitrate and urea, respectively.

The quadratic and exponential models were also applied to describe the GPC response to N fertilisation rates (Figure 3A and B). In this

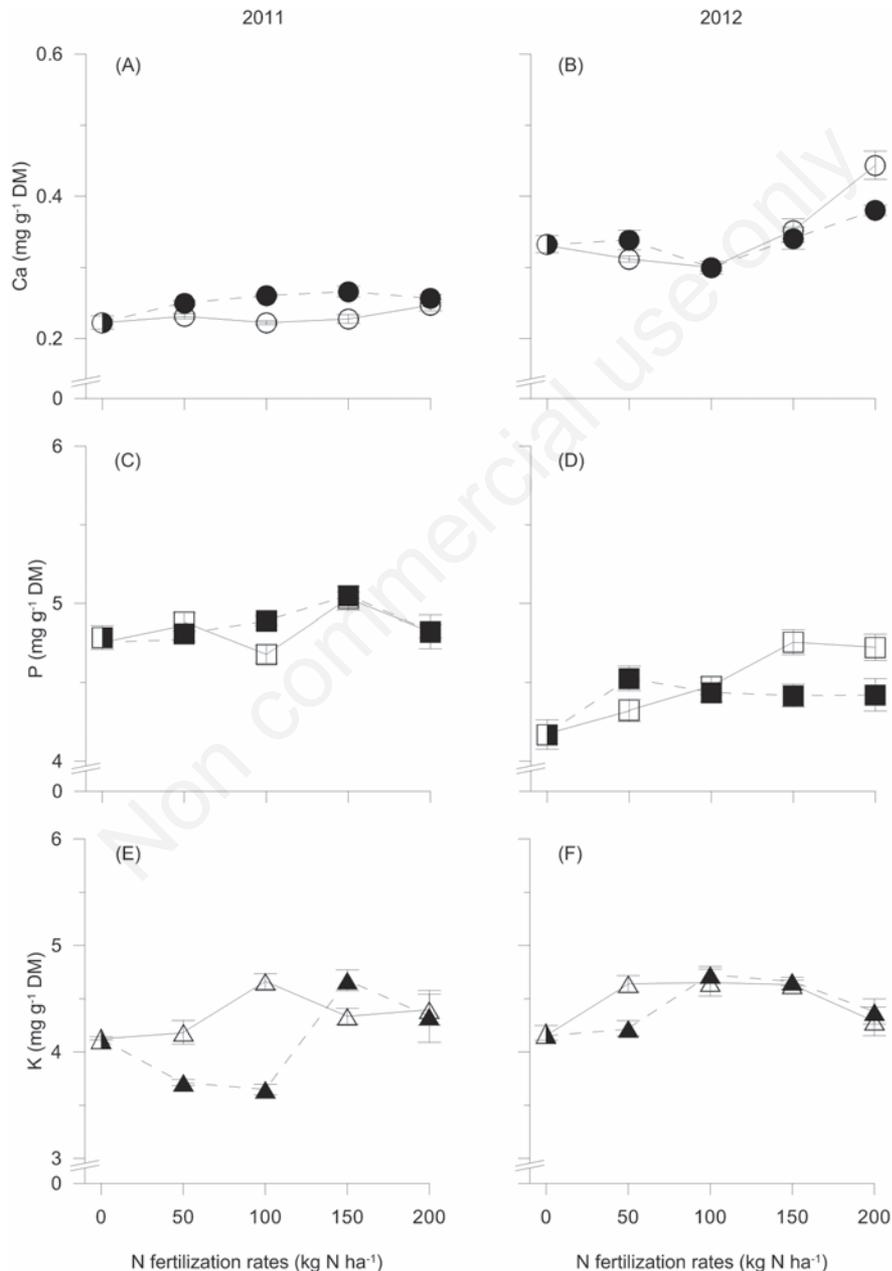


Figure 5. Calcium (Ca) (mg g⁻¹ dry matter, DM), phosphorus (P) (mg g⁻¹ DM) and potassium (K) (mg g⁻¹ DM) concentrations in durum wheat grains against nitrogen (N) fertilisation rates (0, 50, 100, 150 and 200 kg N ha⁻¹) in 2011 and 2012. Empty symbols stand for urea, black symbols stand for calcium nitrate (●, Ca; ■, P; ▲, K). Data are averages ± standard errors, for n=3 independent replicates.

case, the quadratic equation estimated rational N fertilisation rates needed to induce the maximum GPC (14.93% and 14.68% for urea and calcium nitrate, respectively) only in 2011 (see β_2 , Figure 3A) with values from 170.2 to 175.3 kg N ha⁻¹. Such values seem slightly higher than those required to achieve the maximum grain yield, confirming previous findings (Garrido-Lestache *et al.*, 2004). However, similarly to the results of Ryan *et al.* (1997) and Lloveras *et al.* (2001) in Mediterranean conditions, we did not generally observe any additional response of GPC at over 150 kg N ha⁻¹. Normally, yield and grain protein content is negatively correlated principally due to the dilution effect (Triboi and Triboi-Blondel, 2002; Stagnari *et al.*, 2013). Nevertheless, this does not imply that higher grain protein cannot be obtained at

high-yield levels, as we found a simultaneously increase in both yield and GPC (Garrido-Lestache *et al.*, 2004), probably as a result of an efficient N translocation to the developing grains.

This was supported by the values of the parameters estimating N-input efficiency, which are shown in Table 1. Nitrogen uptake (N_g) progressively increased until the doses of 150 and 200 kg N ha⁻¹ for urea and calcium nitrate, respectively. ANR was on average 52.6% and 53.4% in 2011 and 2012, respectively and it was always significantly affected by N-form x N-dose interaction. In general, as the N doses increased the ANR values decreased. This behaviour was not observed in the case of urea applied at 150 kg N ha⁻¹, probably due to the high protein content both in kernels and straw (data not shown), which allowed to cal-

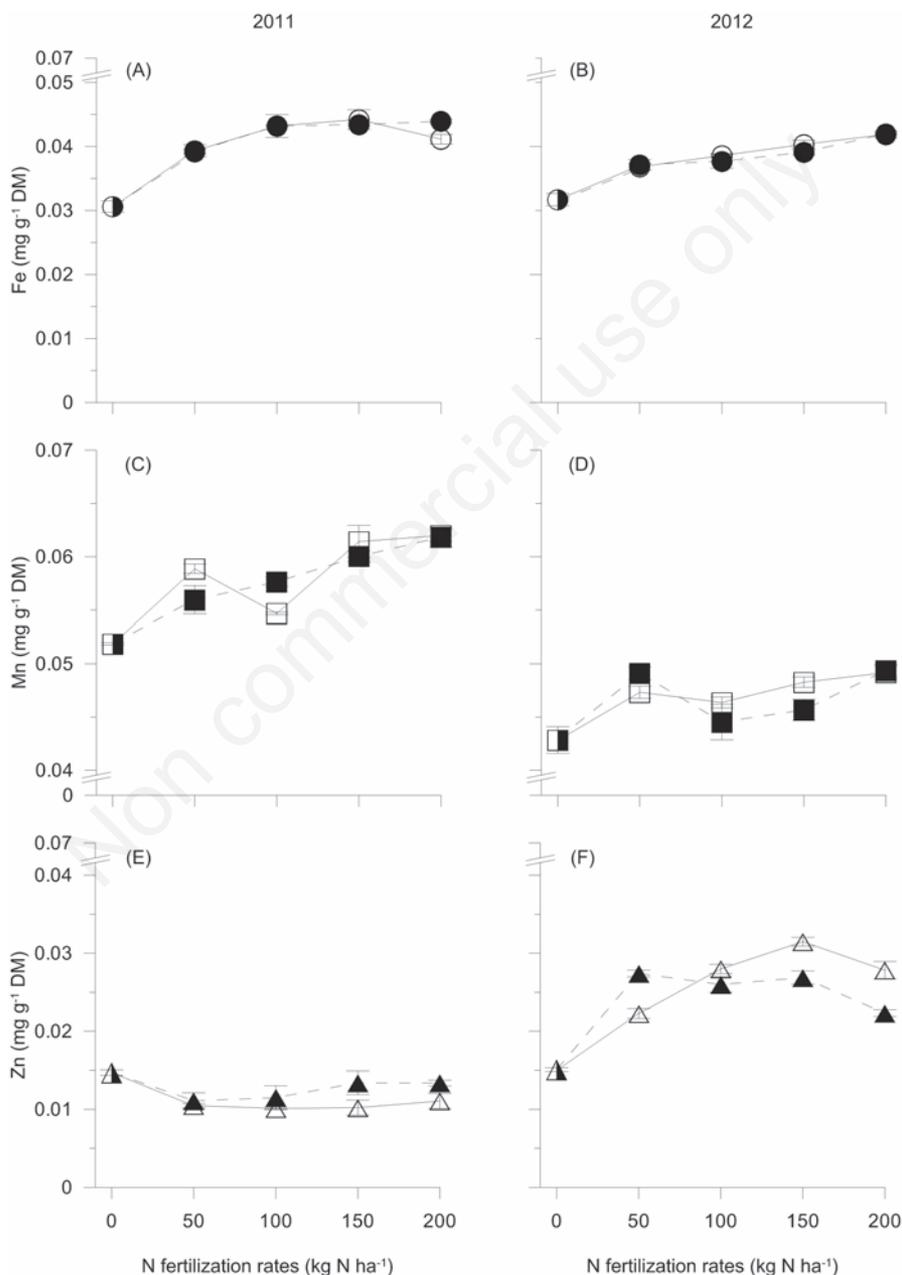


Figure 6. Iron (Fe) (mg g⁻¹ dry matter, DM), manganese (Mn) (mg g⁻¹ DM) and zinc (Zn) (mg g⁻¹ DM) concentrations in durum wheat grains against nitrogen (N) fertilisation rates (0, 50, 100, 150 and 200 kg N ha⁻¹) in 2011 and 2012. Empty symbols stand for urea, black symbols stand for calcium nitrate (●, Fe; ■, Mn; ▲, Zn). Data are averages ± standard errors, for n=3 independent replicates.

culate higher N_g (i.e., in 2012, wheat increased N accumulation of 52.6 kg N ha⁻¹ from 100 to 150 kg N ha⁻¹ treatments). This was an unexpected result, since the rates of increase in yield and nutrient uptake are larger at low levels of N-supply, because the nutrient is the primary factor limiting growth (Dobermann, 2007). Conversely, as nutrient supply increases, incremental yield gains become smaller because of the limiting effects of other yield determinants. Indeed, modern wheat cultivars tend to develop a few early rather than late tillering culms with an extender tiller life-span, which determine an early peak N-demand (i.e., occurring during the early stages of plant development), when N-input is the main source of supply (Foulkes *et al.*, 1998).

As for ANR, in both years the highest physiological efficiency (P_E)

values were obtained with the application of 50 kg N ha⁻¹ (31.7 and 46.8 kg grain per plant's kg N actually taken up averaging over N-form, in 2011 and 2012, respectively). Plants agronomic or N-use efficiency (A_E) progressively lowered with the increasing of N-dose. Averaging over N-form, the values ranged from 23.5 to 8.3 kg grain per kg N-input in 2011 and from 33.2 to 12.2 kg grain per kg N-input in 2012. Calcium nitrate allowed obtaining higher A_E values than urea, confirming previous studies (Guarda *et al.*, 2004; Rahman, *et al.*, 2005). The observed values of indices describing N-input efficiency helped explaining the yield and GPC results, especially with respect to wheat year-to-year variations. When the rainfall were concentrated prior to anthesis (2012), calcium nitrate treatments gave a better N-use (P_E and A_E) favouring

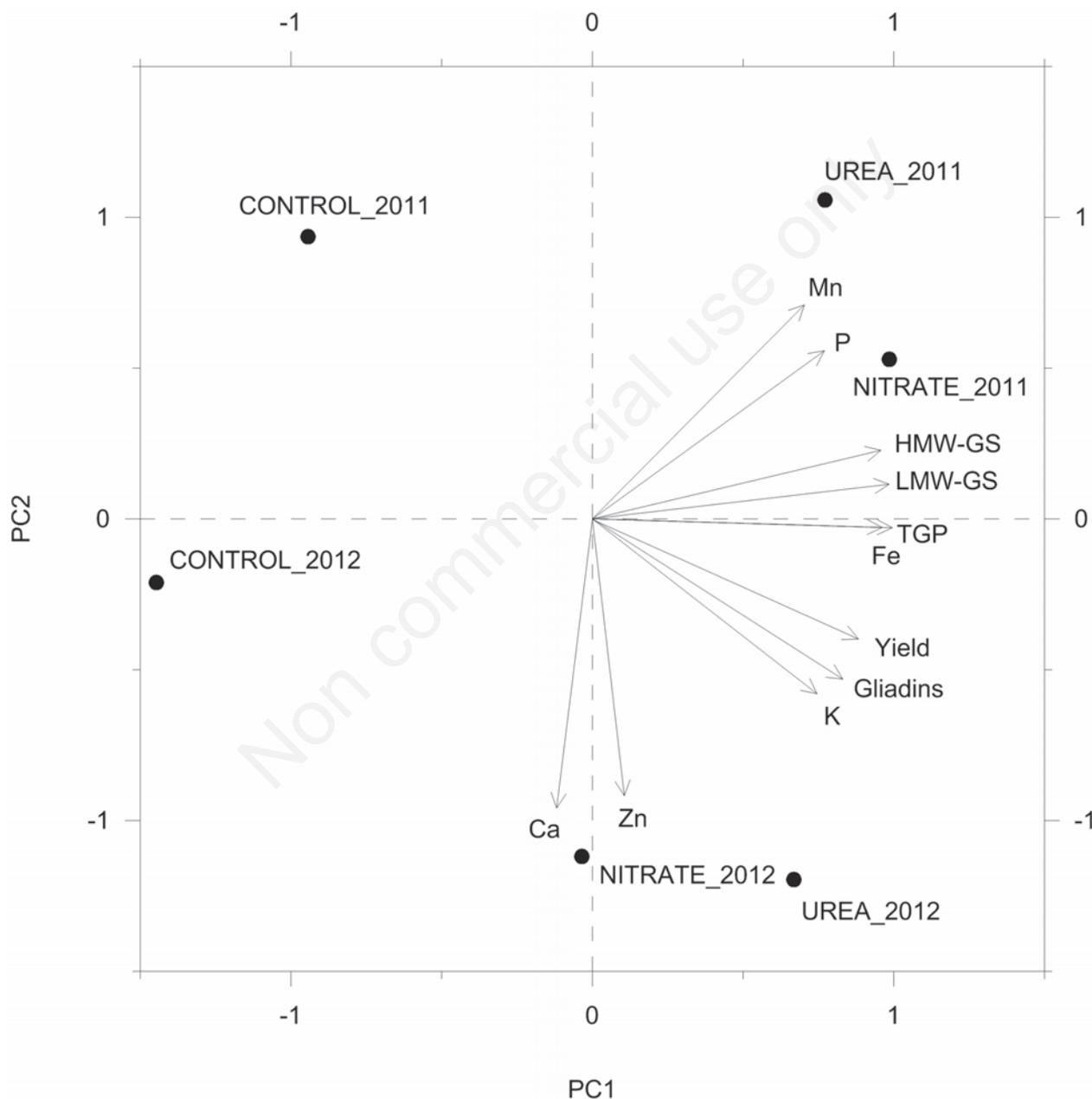


Figure 7. Two dimensional principal component analysis (PCA) correlation bi-plot: symbols stand for the standardised scores on PC1 (x-axis) and PC2 (y-axis) of six treatments [CONTROL_2011: unfertilised plots, year 2011; CONTROL_2012: unfertilised plots, year 2012; UREA_2011: urea at 150 kg nitrogen (N) ha⁻¹, year 2011; UREA_2012: urea at 150 kg N ha⁻¹, year 2012; NITRATE_2011: calcium nitrate at 150 kg N ha⁻¹, year 2011; NITRATE_2012: calcium nitrate at 150 kg N ha⁻¹, year 2012]; vectors represent the correlations between standardised variables [yield; total gluten proteins (TGP); gliadins; high molecular weight glutenins (HMW-GS); low molecular weight glutenins (LMW-GS); Ca; P; K; Fe; Mn; Zn] and PCs, in durum wheat.

higher number of grains per spike (data not shown); when the rainfall were extended through the grain filling period (2011), the efficient use of N inputs did not depend on N-form. However, the mechanisms underlying the effects of rainfall and soil moisture on wheat yield and quality are complex and the same climatic variables do not consistently have the same effects at all site or in all years (Garrido-Lestache *et al.*, 2004), depending also of the pattern on the mineralisation of organic matter and on crop residues composition. In the short term, crop residues can either increase or decrease the N availability, according to their C:N ratio (Schoenau and Campbell, 1996). Besides, the quality of a residue is associated to the time it lasts to protect the soil and maintain its physical properties, which also depends on its distribution and geometric arrangement on soil surface (Ordóñez-Fernández *et al.*, 2007). The higher quantity of autumn-winter rainfall recorded during 2011 could have accelerated coriander residues decomposition (Aulakh *et al.*, 1991), with a reduction in its biomass and, consequently, in its potential of soil's hydraulic properties improvement (Ordóñez-Fernández *et al.*, 2007).

Gluten proteins accumulation

An increase of N concentration in grain would normally result in higher gluten protein accumulation (Schipper, 1991) as indicated by the high significant correlation between total N and gluten proteins content (R^2 range from 0.87 to 0.94, data not shown). Approximately, total gluten proteins increased with N availability, regardless of N form, until the dose of 150 kg N ha⁻¹ (Figure 4A and B); further N availability did not induce any additional increase.

The contribution of different gluten protein fractions to the total gluten protein amount was influenced both by year and N form (Figure 4). In 2011, glutenins and gliadins increased until the dose of 100 kg N ha⁻¹; calcium nitrate performed significantly better in the case of gliadins and LMW-GS. In 2012 glutenins and gliadins increased until the dose of 150 kg N ha⁻¹ with urea inducing significantly higher values of all the gluten fractions, probably due to the higher temperatures during grain-filling - which reduced grain carbohydrate accumulation rather than N accumulation (Garrido-Lestache *et al.*, 2004) - and higher rainfall in May (López-Bellido *et al.*, 2001). Moreover, gliadin content was stable across the year for calcium nitrate while increased for urea in 2012; glutenins significantly lowered by 27 and 49% for urea and calcium nitrate, respectively, in 2012. Since the maximum rate of synthesis of glutenins is reported to occur later than that of gliadins for the majority of durum wheat modern genotypes, the late water stress occurred in 2012 was more detrimental on the synthesis of glutenins than gliadins (Saint Pierre *et al.*, 2008). As a consequence, the GS/gliadins ratio reduced in 2012 by 40% and 47% for urea and calcium nitrate, respectively. The ratio of polymeric *vs* monomeric proteins constitutes a criterion for high quality durum wheat (De Vita *et al.*, 2007) and its high values are generally associated with a decrease in extensibility and with an increase in mixing time (MacRitchie, 1985; Mann *et al.*, 2005). The effect of N dose was significantly more effective on glutenin synthesis, ensuring higher GS/gliadin ratio, matching with recent work which reported differences in the expression of different gluten gene families in response to both N fertilisation (Hurkman *et al.*, 2013) and genotype (Wan *et al.*, 2013).

Minerals

The mineral accumulation in durum wheat grains was quite similar in both years of study, except for Mn and Zn, which were lower and higher in 2012 than in 2011, respectively. The effect of N fertilisation on mineral accumulation in durum wheat grains was not significant or pretty slight in the case of Ca, P, K and Mn (Figures 5 and 6). Ca averaged 0.29 mg g⁻¹, P 4.66 mg g⁻¹, and K 4.48 mg g⁻¹. These results agree

with those of Ficco *et al.* (2009) who found similar mineral concentration in durum wheat modern cultivars. Interestingly, Fe concentrations were increased by N dose of 44% and 32% in 2011 and 2012, respectively; these findings were also observed for Zn in 2012.

Several authors have already reported a negative correlation between grain yield and grain mineral concentration, suggesting a *dilution effect* of the starch on minerals (Monasterio and Graham, 2000). We observed a negligible dilution effect, in accordance with other studies which indicated that micronutrient-rich cultivars can also be higher yielding than less micronutrient-rich cultivars (Graham *et al.*, 2001). In particular, Fe and Zn were among those minerals whose contents were not negatively correlated with yield, and their increase in grains can be combined with improved agronomic traits (Monasterio and Graham, 2000; Graham *et al.*, 2001). Since water availability plays a significant role in mineral mobilisation, water deficit can reduce mineral uptake (Oktem, 2008). Probably, under CA system, the management of crop residues and the minimal soil disturbance alleviated drought stress and enhanced water use efficiency (López-Bellido and López-Bellido, 2001). Thus, with higher water and mineral availabilities (Stagnari and Pisante, 2010), wheat plants experienced higher phloem fluxes associated to mineral transportation. It has also been suggested that some micronutrient cations (Zn, Fe, Cu) tend to be present in higher levels under zero tillage with residue retentions compared with conventional tillage, especially near the soil surface due to the placement of crop residues starting from the first years of no-till (Franzluebbers and Hons, 1996).

Principal component analysis

The results of PCA analysis are shown as a two-dimensional correlation bi-plot (Figure 7); the eigenvalues for PC1 and PC2 are 6.79 and 3.48, respectively, thus capturing 93.4% of the total data variability (Table 2).

Three groups of variables with high positive correlations within each group can be identified. Yield and gliadins fraction in grains constitute the first group, while TGP, LMW-GS, HMW-GS and Fe the second one in which TGP, LMW-GS and HMW-GS are strongly correlated to PC1 (0.99, 0.98 and 0.96, respectively); the third group is represented by some minerals *i.e.*, P and Mn. Ca and Zn positioned differently when compared to the other variables being negatively correlated to PC2 (0.96 and 0.92, respectively).

The effect of the year clearly emerges from the bi-plot analysis: 2011 (upper half) is distinctively separated by PC2 with respect to 2012 (lower half), independently to N form. CONTROL is always clearly inde-

Table 2. Principal component scores of the six year-nitrogen (N) form combinations (treatments) and percentage of variation accounted for each principal component; each N-form was applied at 150 kg N ha⁻¹, an unfertilised CONTROL is included.

Treatments	PC scores	
	PC1	PC2
CONTROL_2011	-2.46	1.75
CONTROL_2012	-3.77	-0.39
UREA_2011	2.01	1.98
UREA_2012	1.74	-2.23
NITRATE_2011	2.57	0.99
NITRATE_2012	-0.09	-2.09
Percentage explained variation	61.75	31.66
Percentage cumulative variation	63.04	94.08

PC, principal component.

pendent from the fertilisation treatments and is characterised by very different PC1 scores (-2.46 and 3.77 for CONTROL_2011 and CONTROL_2012, respectively). Therefore no variable is associated with CONTROL. In 2011 the 150 kg N ha⁻¹ fertilisation rate, regardless of N form, was associated with high TGP, LMW-GS, HMW-GS, P, Fe and Mn concentration (2.01 and 2.57 PC1 scores for UREA_2011 and NITRATE_2011, respectively), while in 2012 it was associated to Ca and Zn content in grains. PCA analysis approach was very effective in discriminating yield and quality characteristics with respect to the combination year-N form.

Conclusions

During the transitional phase to CA in Mediterranean agro-ecosystems, the optimisation of durum wheat yields and grain quality depends strongly on N fertilisation rates and forms as well as on year-to-year weather variations and residues composition. High yields can be combined with optimal accumulation of total gluten and its fractions as well as with optimum GS/gliadin ratio, with the application of N rates ranging from 136 to 156 kg N ha⁻¹ of calcium nitrate (as indicated by the quadratic model). In such environmental conditions, nitrate form allows higher N-input efficiency (*i.e.*, A_E, P_E, ANR%). Besides, to obtain the highest N concentrations (14.7%), higher N fertilisation doses (around 173 kg N ha⁻¹) are needed.

During the transitional phase to CA, the limited amount of crop residues, typical of such dry environments, requires N application rates not lower than 150 kg ha⁻¹; as the accumulation of crop residues increases over time the N fertilisation demand could be likely lowered, as already demonstrated by the available limited data (Rahman *et al.*, 2005), thanks to the recycling of organic C and to long term effects on soil chemical, physical and biological properties.

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