agronomy

Article

Improvements of Durum Wheat Main Crop in Weed Control, Productivity and Grain Quality through the Inclusion of FenuGreek and Clover as Companion Plants: Effect of N FertilizaTion Regime

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Abstract: Assessing the performance of legume species as companion plants is a prerequisite for promoting a low chemical-input durum wheat production system. This study aims to evaluate fenugreek (IC-Fen), clover (IC-Clo) and their mixture (IC-Mix) performances on weed control, productivity, and grain quality of durum wheat main crop under different N fertilization regimes, as compared to durum wheat alone with (SC-H) and without (SC-NH) herbicide. On-field experimentations were carried out in humid and semi-arid conditions. Results showed that legumes offer significant advantages in terms of weed control, soil moisture conservation, productivity, and grain quality for durum wheat cash crops. Results explain that these benefits depend on the legume part and the adopted N fertilization regime. Most significant improvements occurred with the IC-Mix under unfertilized conditions (N0) and relatively low and late N regimes (N1 and N2) where, for example, the partial land equivalent ratio of durum wheat grain yield (PLER) reached 1.25 compared to the SC-NH, with no need to sort the raw grain product (legumes seeds not exceeding 4.3%). Our study illustrates that under low and late N-fertilization condition using promising legumes species combinations result in the improvement of N fertilizer land-use efficiency and hence help to reduce N-fertilization inputs.

Keywords: companion plants; N-fertilization; partial land equivalent ratio (PLER); weed control; grain quality; productivity

1. Introduction

Durum wheat (*Triticum turgidum subsp. durum* (Desf.) Husn) constitutes one of the most pivotal cereal crops for global food security. Worldwide, durum wheat crops cover nearly 30–35 million hectares [1]. In Tunisia, durum wheat represents more than 27% of the total cultivated area [2]. Farmers in these areas confront various natural constraints, including low and erratic rainfall and low fertility of most lands [3]. These constraints are reinforced by a limited land potential for most farmers (about 89% have less than 20 hectares) and the steadily rising chemical input prices, leading to a continuous increase in production costs [2]. Besides, chemical inputs performance has significantly reduced, given the increase in herbicide-resistant weeds [4] and the low N fertilizer efficiency not

exceeding 50% [5]. On the other hand, chemical inputs are also increasingly recognized as majors factors driving global environmental change and food safety hazards [6]. There is a need to develop more sustainable and cost-efficient cereal cropping systems that can be readily adopted by smallholders so as not to impose new burdens on their poor resource.

Mixed crops systems appear as a potential alternative towards more sustainable and efficient production systems. These systems value complementarity and facilitation processes between plants leading to better use of soil resources [7–9]. There are different forms of mixed crops: the intercropping that involves simultaneous cropping of two or more species on the same land [10]; the cover crops that include crops as cover replacing bare fallow, which is grown as green manure prior sowing the main crop [11]; and the intercrops that involve the main cash crop with a cover crop also called companion plants that are sown not to be harvested but to provide agroecological services to the cash crop [12]. For this latter intercropping system, forage legumes appear a suitable candidate as companion plants in cereal crops. Their use as companion plants could contribute to the N requirements of cereal cash crops through their biological N fixation ability and the facilitation processes involving the N transfer from legumes to cereals [13,14]. Hence, this may improve cereal cash crops productivity [15] and well help to reduce N inputs [16]. Legumes as companion plants can also help to limit weed growth through several mechanisms [8]. Some legumes species, like fenugreek (*Trigonella foenum-graecum*) [17] and clover (*Trifolium alexandrinum*) [18] emit allelochemicals that are detrimental for weeds growth [19]. However, those services of legumes as companion plants may depend heavily on the adopted cropping system, including species combinations, and may also vary according to local environmental conditions and soil nutrient availability, mainly N [20,21]. The success of cereal-legume intercrops depends on promoting a niche of complementarity and facilitation between species, which improves the N use efficiency [7,21] and hence helps to reduce N inputs. Therefore, the selection of legume species as companion plants within cereal crops constitute a crucial factor, as they should be less susceptible to N inputs often required for cereal to ensure high yields.

Despite large intercropping literature, the study on companion plants is relatively scarce compared to other intercropping types. Therefore, further research is needed to identify optimal species combinations and N fertilization management to achieve high yields and high N use efficiency simultaneously. Also, to our knowledge, no research studies are available on the mixture of legumes as companion plants within cereal cash crops intended for foods production. The present study aims to evaluate the performance of two legume species (fenugreek and clover) added as companion plants to suppress weeds and improve productivity and grain quality of durum wheat as a cash crop. As well, we aim to evaluate the mixture of these legume species against their added separately as companion plants within durum wheat crop. We hypothesized that the N-fertilization regime could modify the competition between species and could influence the performance of legumes as companion plants within durum wheat cropping systems. Our study, therefore, assesses the effects of N-fertilization regimes (different doses and application times) on the effectiveness of the legumes as companion plants to enhance durum wheat productivity and grain quality.

2. Materials and Methods

2.1. Experimental Sites and Environmental Conditions

Field experiments were conducted from 2015 to 2018 at two sites in the North-west of Tunisia: the Beja El Gnadil site (denoted by BEG) (36°7258′ N, 9°3043′ E) and the Siliana Bourouis site (denoted by SBR) (36°2098′ N, 9°0665′ E) (Figure S1). Agronomic characteristics and soil physicochemical properties are given in the Supplementary Table S1. At both sites, farming practices were conventional based on cereal-legume rotation, with a predominance of cereal crops where the previous crops before the experimental trials was oats (*Avena sativa* L.). Generally, in the north-west of Tunisia, chemical input management in conventional cereal crops relies mostly on the use of herbicides and N-

fertilizers, about 90 to 120 kg ha^{-1} of N-fertilizer for durum wheat [22]. The BEG site has a sub-humid climate while the SBR site has a semi-arid climate. Agricultural production at both sites depends only on natural precipitation. Figure 1 shows data on environmental conditions (monthly precipitation and temperature) for the three experimental seasons. Precipitation varied greatly between both sites but was generally similar between the three experimental seasons. At BEG site, the climate was rainy weather with an average annual precipitation of 621.1 mm, 646.3 mm and 595.3 mm respectively during 2015–2016, 2016–2017 and 2017–2018 seasons. However, at SBR, the climate was semi-arid with an average annual precipitation of 364 mm, 332.2 mm and 355.2 mm respectively during 2015–2016, 2016–2017 and 2017–2018 seasons. The minimum temperature seldom drops below 0 °C during winter, so no frost damage was observed in legumes which are less frost-resistant than cereals (Figure 1).
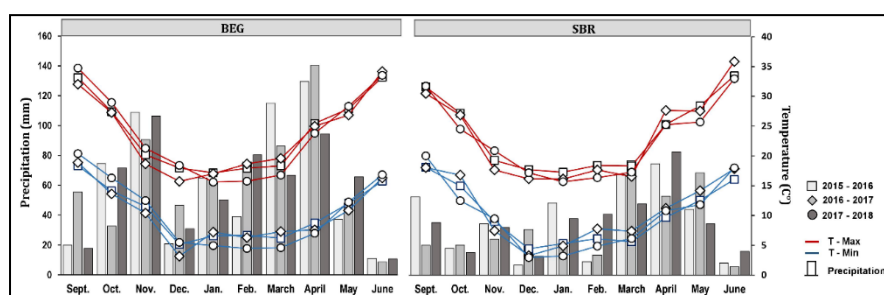

Figure 1. Average monthly precipitation, minimum and maximum temperature at both sites during the three experimental seasons (Data provided by the National institute of meteorology, Tunisia).

2.2. Experimental Design

Durum wheat-legume intercropping patterns were based on the additive principle: durum wheat, (Triticum turgidum subsp. durum (Desf.) Husn) as cash crop was sown at standard sowing density (320 plant m^{-2}) and, legumes were added as companion plants. The legumes compounds were fenugreek (50 plant·m^{-2}) or/and clover (100 plant m^{-2}). Durum wheat variety used was Maali. For legumes, a local cultivar was used for fenugreek (*Trigonella foenum graecum* L). and the variety Masri Baladi for clover (*Trifolium alexandrinum* L.).

The experiment was arranged in split-plot design with the main plot factor randomized according to a RCBD. Nitrogen treatments constituted the main plots and cropping patterns the sub-plots. The sub-plot size was 3.6 m × 5 m.

The following N-treatments were assigned to the main plots:

- N0: Unfertilized treatment.
- N1: Low and late N-fertilization treatment (receiving 30 kg ha^{-1} of N-fertilizer at durum wheat heading stage).
- N2: Moderate N-fertilization beginning at durum wheat stem elongation (receiving two equal N-fertilizer doses of 30 kg ha^{-1}, one at durum wheat stem elongation and the other at heading stage).
- N3: Medium N-fertilization beginning at durum wheat tillering (receiving three equal N-fertilizer doses of 30 kg ha^{-1}, the first at durum wheat tillering, the second at stem elongation and the third at heading).
- N4: High N-fertilization beginning at durum wheat tillering (receiving three equal N-fertilizer doses of 40 kg ha^{-1}, the first at durum wheat tillering, the second at stem elongation and the third at heading).

The following cropping patterns were assigned to the sub-plots:

- SC-H: Durum wheat sole cropping with a conventional weed control using herbicides.
- SC-NH: Durum wheat sole cropping without herbicides application.
- IC-Fen: Intercrops of Durum wheat-Fenugreek.
- IC-Clo: Intercrops of Durum wheat-Clover.

- IC-Mix: Intercrops of Durum wheat-Mixture (fenugreek+clover).

2.3. Sites Management

At both sites the false seedbed technique that consists of preparing a regular seedbed (early) before sowing the actual crops was adopted to better control weeds [23]. Before sowing, the soil was ploughed with disk harrow at a tillage depth of 10 cm, followed by one pass with a rotary disc to prepare the seedbed. All species were sown simultaneously in November with a precision seed drill (Wintersteiger Plotseed, Austria) at a depth of 4 cm. Durum wheat was sown in single rows 0.2 m apart in the sole crop. For intercrops, legumes were sown in the middle of wheat inter-row space. Plants emergence was satisfactory owing to high water availability during the 15 days after sowing (Table S2). To evaluate the effect of the experimental treatments on weed infestation, weeds in all plots were untreated, except for the sole-crop with herbicide (SC-H) plots where the Amilcar OD herbicide was applied at the recommended dose approximately five weeks after sowing (Figure S2). Nitrogen fertilizer was applied, using the Ammonitrate (33.5%), at durum wheat tillering (i), stem elongation (ii) and heading (iii) according to the different N treatments as described above (Figure S2). Durum wheat was harvested at maturity (Zadoks 9-ripening) [24]. Physiological maturity was generally observed in late June at the BEG site and mid-June at the SBR site.

2.4. Sampling, Measurements, and Calculation

2.4.1. Biomass

At durum wheat heading stage (Zadoks 45), weeds biomass was determined by collecting weeds from the central three-meter square in each plot. At durum wheat maturity (Zadoks 80), legumes above-ground biomass was determined by cutting all the plants at ground level from four linear meters in each plot. Then, after drying for 72 h at 75 °C, the weeds and legumes biomass were weighed, and recorded values were converted to $kg\,ha^{-1}$. For straw yield determination, at harvesting, only the central rows of plots were harvested (1.2 m) with a 10 cm cutting bar using experimental combine harvester (Wintersteiger Plot combine, Autriche). Straw was weighed, and values were converted to $t\,ha^{-1}$.

2.4.2. Nodule Weight

At legumes flowering (firstly the fenugreek and then the clover), ten plants from each legume's species were selected randomly from each sub-plot, and the roots were excavated using a spade. The soil was removed carefully from roots to ensure that roots and nodules were as much as possible recovered. Roots were washed carefully with distilled water, and absorbed residual water with absorbent paper, then the nodules were removed quickly. Pink nodules (representing a high efficiency in N fixation) were weighted. Mean values of nodules fresh weight derived from the ten plants were recorded and expressed as mg of nodules per plant.

2.4.3. Net Photosynthetic Rate (*Pn*)

The net photosynthetic rate (*Pn*) was measured for two growing seasons (2016 and 2017) using a portable gas-exchange system (Model Li-Cor 6200, Li-Cor, Lincoln, NE, USA). Measurements for durum wheat and both legumes were performed on sunny days (above 800 $\mu mol\,m^{-2}\,s^{-1}$ PPFD) at durum wheat stem elongation stage (Zadoks 45), between 9.00 a.m. and 1 p.m. (solar time), by holding the chamber perpendicular to the incident light from the sun (flag leaf). The leaves were kept in the chamber until the photosynthesis values were observed as constant as possible, i.e., "steady state" ($\pm$ 2 min). The chambers were open most of the time, exposing the chamber interior to the ambient conditions.

2.4.4. Soil Moisture Analysis

The soil was sampled at legumes flowering for two growing seasons (2016 and 2017). From each plot, four random samples were collected with a soil corer at a depth of 25

cm. Soil moisture was determined gravimetrically by drying fresh soil samples for 48 h at 105 °C according to [25].

2.4.5. Durum Wheat Grain Yield and Quality

After harvesting, in the laboratory, grain samples of each plot were vigorously cleaned. The impurity content (legume seeds percentage) was determined. The moisture content of grain samples was determined using NIR Inframatic 9500 analyzer (Perten Instruments, Sweden) and grain yield in t ha^{-1} was expressed based on 12% moisture content. Thousand kernel weight (TKW) was determined on an analytical balance ($\pm$ 0.1mg) after counting 1000 grains by a seed counter (Numigral II Chopin seed counter, France). Grain N concentration was determined by the Micro-Kjeldahl method. Grain protein content (GPC) was expressed as crude protein by multiplying the value of grain N concentration by 5.7. Ash content was determined according to the AACC method 08-01 [26].

2.4.6. Durum Wheat Intercropping Patterns Efficiency

To evaluate the efficiency of the three intercropping patterns against durum wheat sole crops with no herbicide (SC-NH), the partial land equivalent ratio based on the grain yield (PLER) was calculated. The partial land equivalent ratio is defined as the relative yield of an intercropped species compared to its yield in a sole crop, which can be interpreted, in the present study, as a measure for the contribution of legumes species to the efficiency of land use by the durum wheat crop [10,27]. The PLER was calculated for each N treatment as:

$$PLER = Y_{(IN-L)}/Y_{(SC-NH)} \tag{1}$$

where Y was the grain yield of the intercropping patterns (IN-L) and of the durum wheat sole cropping pattern with no herbicide (SC-NH). PLER values above one, indicate that the intercropping pattern is more productive and more efficient in using N resources than the durum wheat sole cropping pattern (SC-NH) [10].

By analogy with the partial land equivalent ratio, the herbicide response ratio based on the grain yield (HRR) was calculated and compared with the different obtained PLER values. The HRR was calculated as the ratio between the durum wheat sole crops with herbicide (SC-H) and the durum wheat sole crops with no herbicide (SC-NH) regarding the grain yield for each N treatment as:

$$HRR = Y_{(SC-H)}/Y_{(SC-NH)} \tag{2}$$

where Y is the grain yield of the sole crops with herbicide (SC-H) and of the sole crops without herbicide (SC-NH).

2.4.7. Statistical Analyses

All statistical analyses were performed using R [28]. Mixed-effects models were used to analyze the data of each site to produce ANOVA *p*-values for main effects and all interactions using the using the lme function in the nlme package. Three-factor analyses with Season (S), N Treatment (NT), and Cropping Pattern (CP) as fixed effects were carried out. The hierarchical nature of the split plot design was reflected in the random error structures that were specified as S/block/mainplot, where mainplot is an ID for the main plots of a trial [29]. All models were visually checked for homogeneity of variance and normal distribution of residuals using the ggResidpanel package. Only for *Pn* and soil moisture measurements each season under each site were analyzed separately using a split-plot ANOVA model in the R package "Agricolae" [30] for randomized complete block design (RCBD) to assess the effects of NT and CS and their interaction. When the ANOVA indicated significant effects, Tukey's HSD test (α = 0.05) was used to determine significant differences among factor levels. Weed biomass data were log-transformed to meet model residuals requirements, using the ln (x + 1) transformation to account for zeros in the data. The relationships between weeds biomass and legumes biomass in each intercropping

pattern were tested using the Spearman's correlation method and also, the linear regression with block within season as a random effect.

3. Results

3.1. Durum Wheat Grain Yield, PLER, and HRR

At both sites, durum wheat grain yield was highly affected by N treatment (NT) and cropping patterns (CP) ($p < 0.000$). High significant interaction between NT and CP was found at BEG site ($p = 0.004$) whereas not significant at SBR ($p = 0.068$) (Table S3).

According to NT, grain yield was significantly increased in response to the increase of N-input (Figure 2A). Average across cropping patterns, grain yield increased from 3068 (N0) to 4426 kg ha^{-1} (N4) at BEG and from 2057 (N0) to 2470 kg ha^{-1} (N4) at SBR. The response to N-input was shown higher at BEG compared to SBR.
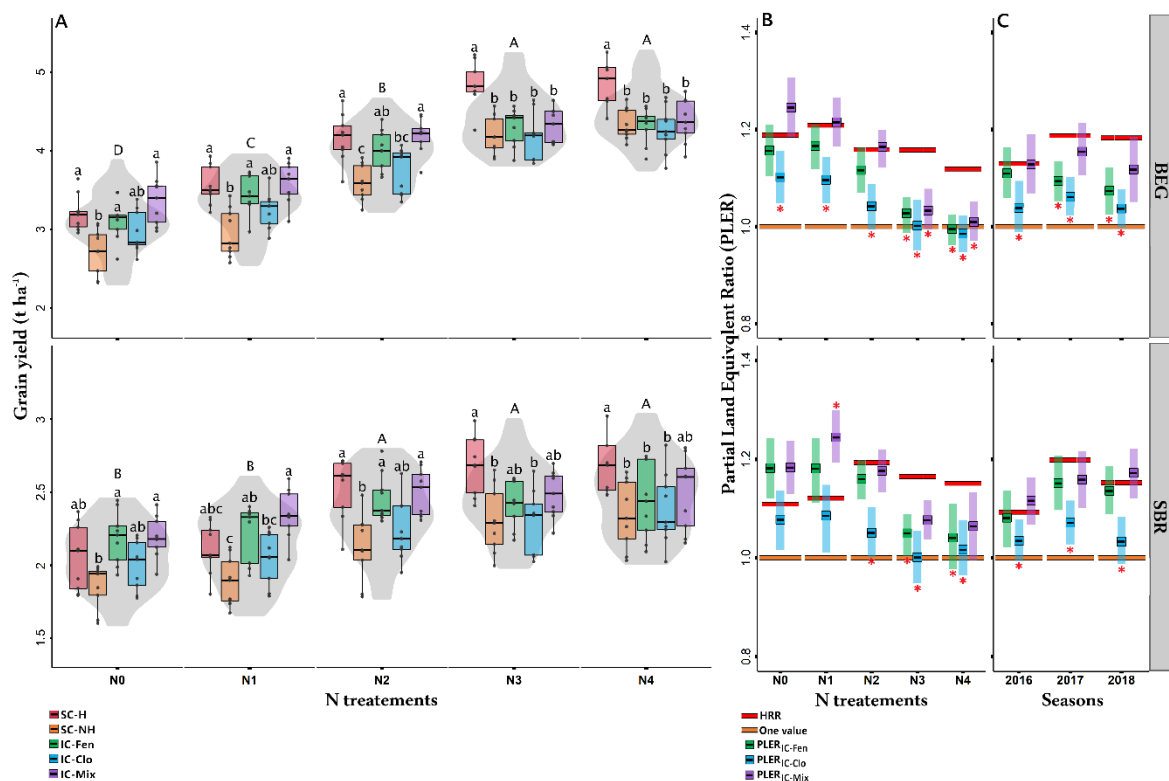

Figure 2. Durum wheat grain yield and PLER at both sites. (**A**) Grain yield affected by N treatment (Violin plot) and cropping pattern within each N treatment (Box plot) averaged over the three seasons; Violin plots headed by a common capital letter are not significantly different at $p < 0.05$ according to Tukey's HSD-test. Within each violin plot, box plots with a common lowercase letter are not significantly different at $p < 0.05$ according to Tukey's HSD-test (for statistical output see Table S3). (**B**): PLER of the three intercropping patterns compared to the herbicide response ratio (HRR) in each N treatment averaged over the three seasons; (**C**): PLER of the three intercropping patterns compared to the herbicide response ratio (HRR) in each season averaged over the five N treatments; In (**B**,**C**) panels, PLER: partial land equivalent ratio-yield in intercrop divided by yield in sole crop with no herbicide; HRR: herbicide response ratio-yield in sole crop with herbicide divided by yield in sole crop with no herbicide; Orange lines indicate a relative yield of one, i.e., with yield equal to that of a sole durum wheat with no herbicide; Red lines indicate the HRR; Tiles represent the means PLER and 95% confidence intervals of each intercropping pattern; Top asterisk (*) indicates a significant increase and bottom asterisk indicates a significant decrease compared to the HRR (Tukey-test, $\alpha = 0.05$).

Within the sole cropping patterns, grain yield was significantly higher in the herbicide-treated pattern (SC-H) compared to the no-treated pattern (SC-NH) under all N treatment, except under N0 and N1 treatments at SBR site (Figure 2A). The herbicide response ratio

(HRR) was between 1.11 and 1.21, that reflected a moderate weed infestation impact (Figure 2B).

The existing of legumes as companion plants showed significant grain yield advantage compared to the SC-NH, depending on the intercropping pattern and the NT (Figure 2A). The highest advantage was obtained with the IC-Mix pattern under N0, N1 and N2 treatments. PLERs in the IC-Mix were 1.25, 1.22 and 1.16 at BEG and were 1.18, 1.24 and 1.18 at SBR, respectively under N0, N1 and N2 treatments (Figure 2B). Under these N treatments, IC-Mix PLERs were globally very close to and even higher than HRRs, where for instance PLER was significantly higher than the HRR under N1at SBR (Figure 2B). Similar effects were obtained with the IC-Fen pattern, but with lower yield advantage compared to the IC-Mix (PLERs in the IC-Fen were between 1.12 and 1.18 under N0, N1 and N2). However, grain yield in the IC-Clo pattern was statistically similar to the SC-NH pattern under all NT. Globally under N3 and N4 treatments, no significant difference between the three intercropping patterns and also the SC-NH pattern with regard of grain yield (Figure 2A). For these N treatments and particularly at BEG, PLERs were between 0.99 and 1.03 which were significantly lower than the HRR (Figure 2B). Yet, at SBR, even though the lowest PLERs were shown with these N treatments, the IC-Mix pattern resulted in PLERs (averaged 1.07) statistically equivalent to the HRR (averaged 1.15) (Figure 2B).

Overall, grain yields of intercropping patterns were largely sustained over the cropping seasons (Figure 2C). The IC-Clo showed the lowest PLERs significantly lower than the HRR and the IC-Mix showed the highest PLERs statistically equivalent to the HRR (Figure 2C). Overall, averaged over seasons, sites and N treatments, grain yield was the lowest in the SC-NH (2.8 t ha^{-1}), very close in the IC-Clo (+4%), intermediate in the IC-Fen (+9%), and the highest in the IC-Mix (+13%) and in the SC-NH (+16%) (Figure 2C).

3.2. Legumes Biomass and Nodulation

Fenugreek biomass and nodules weight were greatly affected by NT ($p < 0.000$) (Table 1). As compared to N0, fenugreek biomass and nodules weight under N1 and N2 showed no significant difference; however, under N3 and N4 significant decreases of both biomass and nodules weight were shown. Average reduction rates under N3 and N4 were 39% and 26% for the biomass and 60% and 50% for the nodules weight, respectively at BEG and SBR sites (Table 1). Fenugreek biomass was, also, affected by the presence of clover in the same row (IC-Fen vs IC-Mix) ($p < 0.000$). As compared to the IC-Fen, fenugreek biomass was 22% and 17% lower in the IC-Mix, respectively at BEG and SBR sites (Table 1). However, fenugreek nodules weight was not affected by the presence of clover in the IC-Mix compared to the IC-Fen (Table 1).

Clover biomass and nodules weight at BEG site were significantly affected by NT and CP and their interaction (Table 1). Within the IC-Clo pattern, clover biomass and nodules weight were significantly reduced under N2, N3 and N4 treatments compared to N0 and N1. Average reduction rate was 53% for the biomass and 72% for the nodule weight (Table 1). However, with the presence of fenugreek in the same raw (IC-Mix), clover biomass and nodules weight was showed significantly reduced only under N3 and N4 treatments compared to N0. On the other hand, under N0 and N1 treatment, clover biomass was showed reduced by the presence of fenugreek (IC-Mix) compared to the IC-Clo (−25%) while the nodules weight was not affected. Interestingly, clover nodules weight was significantly higher with the IC-Mix compared to the IC-Clo in the N2 treatment (+141%) (Table 1). At SBR site clover biomass and nodules weight were significantly affected by NT, while only the nodules weight was significantly affected by CP (Table 1). Overall clover biomass was significantly reduced under N3 and N4 treatment compared to N0 (Averaged −33%). However, clover nodules weight at this site was greatly affected by the presence of fenugreek in the same row (IC-Clo vs. IC-Mix) ($p < 0.000$) (Table 1). Indeed, although the interaction between NT and CP was not significant ($p = 0.091$), clover nodules weight was significantly higher with the IC-Mix compared to the IC-Clo under N0 (+21%), N1 (+20%) and N$_2$ (+54%) treatments.

Table 1. Effects of cropping pattern and N-treatment on legumes biomass and nodule fresh weight at both sites averaged across the three experimental seasons.

		Legumes Biomass (kg ha^{-1})						Nodules Weight (mg Plant^{-1})					
		N0	N1	N2	N3	N4	Mean	N0	N1	N2	N3	N4	Mean
							Fenugreek						
BEG	IC-Fen	976 aA	993 aA	979 aA	630 aB	582 aB	832 a	156 aA	163 aA	143 aA	61 aB	67 aB	118 a
	IC-Mix	768 bA	781 bA	745 bA	472 aB	468 aB	647 b	153 aA	148 aA	140 aA	64 aB	57 aB	112 a
	Mean	872 A	887 A	862 A	551 B	525 B		154 A	155 A	142 A	63 B	62 B	
SBR	IC-Fen	663 aAB	697 aA	629 aAB	521 aB	505 aB	603 a	122 aA	120 aA	112 aA	59 aB	58 aB	94 a
	IC-Mix	574 aA	582 aA	542 aAB	419 aBC	399 aC	503 b	112 aA	117 aA	111 aA	66 aB	53 aB	92 a
	Mean	618 A	639 A	586 A	470 B	452 B		117 A	118 A	111 A	62 B	55 B	
							Clover						
BEG	IC-Clo	646 aA	679 aA	353 aB	267 aB	341 aB	457 a	103 aA	112 aA	32 bB	31 aB	28 aB	61 a
	IC-Mix	492 aA	499 bA	413 aA	232 aB	241 aB	375 b	105 aA	108 aA	78 aB	37 aC	29 aC	71 a
	Mean	569 A	589 A	383 B	250 C	291 C		104 A	110 A	55 B	34 C	28 C	
SBR	IC-Clo	318 aA	334 aA	202 aA	227 aA	229 aA	262 a	68 bA	65 bAB	43 bBC	26 aC	28 aC	46 b
	IC-Mix	307 aA	275 aAB	262 aABC	204 aABC	185 aC	247 a	82 aA	78 aA	67 aB	31 aC	28 aC	57 a
	Mean	312 A	305 A	232 AB	216 C	207 C		75 A	71 A	55 B	29 C	28 C	
							ANOVA						

Source of Variation	df	Fenugreek		Clover		Fenugreek		Clover	
		BEG	SBR	BEG	SBR	BEG	SBR	BEG	SBR
S	2	0.126	0.044 *	0.195	0.662	0.063 °	0.189	0.155	0.483
NT	4	0.000 ***	0.000 ***	0.000 ***	0.004 **	0.000 ***	0.000 ***	0.000 ***	0.000 ***
CP	1	0.000 ***	0.000 ***	0.001 **	0.221	0.080 °	0.206	0.012*	0.000 ***
S × NT	8	0.390	0.783	0.619	0.735	0.676	0.315	0.454	0.781
S × CP	2	0.420	0.423	0.723	0.345	0.246	0.608	0.765	0.741
N × CP	4	0.569	0.796	0.019 *	0.230	0.252	0.277	0.001 **	0.091 °
S × NT × CP	8	0.992	0.977	0.996	0.824	0.953	0.923	0.955	0.999

Within each column, means followed by a common lowercase letter are not significantly different at $p < 0.05$ according to Tukey's HSD-test. Within each line, means followed by a common capital letter are not significantly different at $p < 0.05$ according to Tukey's HSD-test. ANOVA shows *p*-values for the main effects and their interactions; S: Season, NT: N Treatment, and CP: Cropping Pattern. Note: °, *, ** and ***: $p \leq 0.1, 0.05, 0.01$ and 0.001, respectively.

Overall, results showed that fenugreek has produced higher biomass compared to clover. Globally, N fertilization applied three times beginning at durum wheat tillering (N3 and N4), greatly reduced both legumes biomass and nodulation in all intercropping patterns. However, N fertilization beginning at durum wheat stem elongation (N2) negatively affected only clover biomass and clover nodules weight in the C-IN pattern (Table 1). The effect of NT was showed more pronounced at BEG site compared to SBR site.

Although biomass of each legumes species was reduced in the IC-Mix pattern compared to their use separately, the nodules weight was not affected and in contrast it was enhanced particularly for the clover. Overall, total legume biomass (fenugreek biomass+clover biomass) and nodule occurrence are clearly above in the IC-Mix pattern compared to the IC-Fen and IC-Clo patterns.

3.3. Weed Biomass

Results showed that weed biomass was influenced exclusively by the adopted cropping pattern ($p < 0.000$) (Table S3). The SC-NH showed the highest weed biomass averaged 258 and 212 kg ha^{-1} at BEG and SBR, respectively. (Figure 3A). Herbicide application (SC-H) successfully controlled weeds, with an average reduction rate of weed biomass by 78% relative to the SC-NH (Figure 3A).
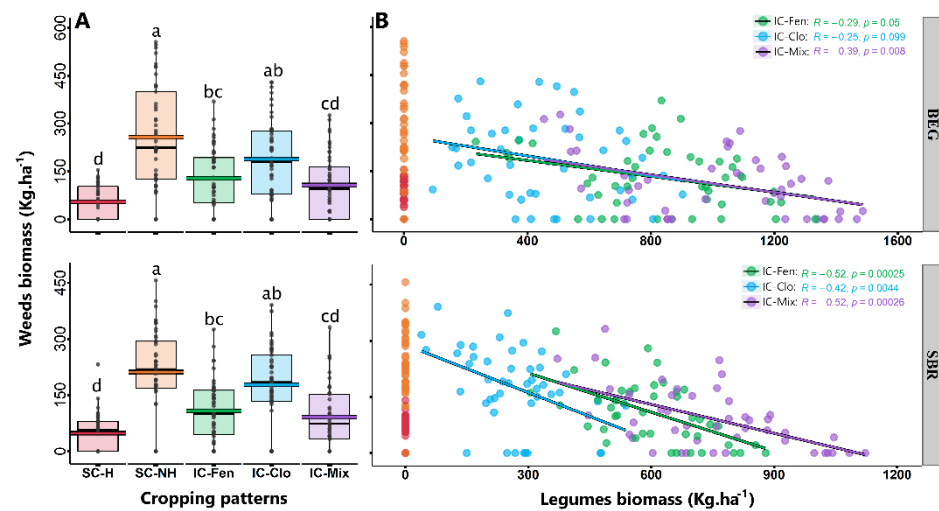

Figure 3. Effect of cropping patterns on weed biomass, averaged over the three seasons and the five N treatments (**A**) and the relationship between weed biomass and legume biomass in each intercropping pattern (**B**) at both sites.(**A**): Box plot indicating the cropping pattern effects on the weed biomass averaged over the three seasons and the five N treatments (Colored lines indicate the mean); Box plots with a common letter are not significantly different at $p < 0.05$ according to Tukey's HSD-test. (**B**): Relationship between weeds biomass and legumes biomass in each intercropping pattern (R-values are the correlation coefficient (Spearman's correlation); colored lines represent linear regression for each intercropping pattern).

Within the intercrop's patterns, the IC-Mix showed the highest weeds suppression performance. Weed biomass in the IC-Mix was significantly reduced by averaged 58% relative to the SC-NH which is statistically equivalent to the herbicide application effect (SC-H). The IC-Fen pattern showed, also, a significant reduction of weed biomass by average 49%. However, in the IC-Clo pattern weed biomass was slightly reduced compared to the SC-NH by 27% at BEG and 17% at SBR (not significant). Our results showed that weed suppression in intercrops was mostly due to fenugreek, along with the increase in the total legume sowing density in the IC-Mix pattern. Indeed, legumes biomass was showed significantly negatively correlated with weeds biomass. The higher the legumes biomass, the fewer weeds could establish (Figure 3B).

3.4. Net Photosynthetic Rate (Pn)

The Net Photosynthetic Rate (*Pn*) was measured at crops flowering stage in 2016 and 2017 seasons. Durum wheat *Pn* at BEG site was greatly affected by NT, CP and NT × CP ($p < 0.000$) (Table S3). Compared with N0, the application of N fertilizer significantly increased *Pn*. Within the sole cropping patterns, the *Pn* values under SC-H in all N treatments were significantly higher than those under SC-NH by 4–11% (Table 2). Within the intercrop's patterns, the *Pn* values under IC-Mix in the N0, N1and N2 treatments were significantly higher than those under SC-NH by 3–9%, which were statistically equivalent to those in SC-H pattern. While the *Pn* values under IC-Fen and IC-Clo only in the N0 and N1 treatments were higher than those under SC-NH by 4–7 and 2–3%, respectively (Table 2). However, in treatments N3 and N4, *Pn* values were largely the same in the intercropping patterns and the SC-NH pattern, which were in all patterns significantly lower than those in the SC-H pattern (Table 2). These results illustrate that only under unfertilized conditions (N0) and with a relatively low and late N-input (N1 and N2), the *Pn* of durum wheat shows an improvement especially with the mixture of fenugreek and clover (IC- Mix) compared to the sole crop. Overall, similar trends were also shown at the SBR site with regard to the effects of CP and NT on *Pn*. Yet, particularly at this site, *Pn* values under IC-Mix were statistically equivalent to those in SC-H pattern in all N treatments (Table 2). The not significant interaction term between NT and CP for *Pn* further showed that CP effects were not NT dependent at this site.

Table 2. Effect of different cropping patterns and N treatments on the net photosynthetic rate (*Pn*, μmol CO_2 m^{-2} s^{-1}) of durum wheat, fenugreek and clover at flowering stage in 2016 and 2017 seasons.

		BEG						SBR					
		N0	N1	N2	N3	N4	Mean	N0	N1	N2	N3	N4	Mean
Durum wheat													
2016	SC-H	15.7 abcD	17.1 abC	18.3 aB	19.1 aA	18.8 aAB	17.8 a	14.2 abC	14.9 abB	15.6 abA	16.1 aA	15.8 aA	15.3 a
	SC-NH	15 cC	16.3cB	17.2 cA	17.2 cA	17.4 bcA	16.6 c	13.9 bC	14.6 bB	14.8 bB	15.4 bA	15.3 cA	14.8 b
	IC-Fen	15.8 abC	16.9 abB	17.7 bA	17.9 bA	18 bA	17.3 b	14.7 aB	15.2 abAB	15.7 aA	15.6 bA	15.7 abcA	15.4 a
	IC-Clo	15.3 bcB	16.8 bA	17.1 cA	17.3 cA	17.3 cA	16.8 c	14.1 abB	15 abA	15.2 abA	15.5 bA	15.4 bcA	15 b
	IC-Mix	16.1 aC	17.3 aB	18.1 abA	17.9 bA	17.9 bcA	17.5 b	14.7 aB	15.4 aA	15.8 aA	15.8 abA	15.8 abA	15.5 a
	Mean	15.6 C	16.9 B	17.7 A	17.9 A	17.9 A		14.3 C	15 B	15.4 A	15.7A	15.6 A	
2017	SC-H	16 bC	17.1 abB	18.1 aA	18.4 aA	18.4 aA	17.6 a	15.5 bcC	16.1 abB	16.8 aA	17.3 aA	17.2 aA	16.6 a
	SC-NH	15.2 cD	16.3 cC	17.2 bcB	17.5 bAB	17.7 bA	16.8 c	15.2 cD	15.7 bCD	16b BC	16.4 bAB	16.5 bA	16 b
	IC-Fen	16.3 abC	17.2 abB	17.4 bcAB	17.6 bA	17.6 bA	17.2 b	15.9 aB	16.4 aAB	16.7 abA	16.9 abA	16.8 abA	16.5 a
	IC-Clo	15.7 bcB	16.8 bA	17 cA	17.4 bA	17.4 bA	16.9 c	15.5 bC	15.9 abBC	16.1b ABC	16.5 abA	16.4 bA	16.1 b
	IC-Mix	16.6 aB	17.3 aA	17.7 abA	17.7 bA	17.6 bA	17.4 b	16.1 aB	16.5 aAB	17 aA	17.1 abA	17ab A	16.7 a
	Mean	16 D	16.9 C	17.5 B	17.7 A	17.7 A		15.6 D	16.1 C	16.5 B	16.8 A	16.8 A	
Fenugreek													
2016	IC-Fen	18 aA	18.3 aA	18 aA	16.5 aB	16.3 aB	17.4 a	15.3 aA	15.3 aA	15.1 aAB	14.9 aB	14.9 aB	15.1 a
	IC-Mix	18.2 aA	18.4 aA	18.5 aA	16.5 aB	16.4 aB	17.6 a	15.2aA	15.2 aA	15 aA	14.9 aA	14.9 aA	15 a
	Mean	18.1 A	18.3 A	18.2 A	16.5 B	16.3 B		15.2A	15.3 A	15 A	14.9 A	14.9 A	

Table 2. *Cont.*

		BEG						SBR					
		N0	N1	N2	N3	N4	Mean	N0	N1	N2	N3	N4	Mean
Durum wheat													
2017	IC-Fen	17.3 aA	17.5 aA	17.7 aA	15.9 aB	15.7 aB	16.8 a	16 aA	16.2 aA	16.2 aA	15.7 aA	15.7aA	16 a
	IC-Mix	17.3 aA	17.3 aA	17.4 aA	16 aB	15.9 aB	16.8 a	15.9 aAB	16.1 aA	16.2 aA	15.8 aAB	15.6 aB	15.9 a
	Mean	17.3 aA	17.4 A	17.6 A	16 B	15.8 B		16A	16.2 A	16.2 A	15.7 A	15.7 A	
Clover													
2016	IC-Clo	16.9 aA	17 aA	15.3 aB	14.1 aC	14a C	15.5 a	14.7 aA	14.8 aA	13.1 bB	13.2 aB	13 aB	13.7 b
	IC-Mix	16.9 aAB	17.1 aA	16.1 aB	14a C	14.1 aC	15.6 a	15 aA	15.2 aA	14.6 aAB	13.4 aC	13.6 aBC	14.3 a
	Mean	16.9 A	17 A	15.7 B	14.1 C	14 C		14.8 A	15 A	13.8 B	13.3 B	13.3 B	
2017	IC-Clo	17 aA	17.2 aA	15.3 bB	14.8 aC	14.8 aC	15.8 b	14.9 aA	15 A	13.5b AB	13.6 aAB	13.1 aB	14 b
	IC-Mix	16.9 aA	17.1 aA	16.3a A	14.8 aB	14.7 aB	16 a	15.3 aA	15.4 A	14.8a AB	13.6 aB	13.7 aB	14.5 a
	Mean	16.9 A	17.1 A	15.8 B	14.8 C	14.8 C		15.1 A	15.2 A	14.2 B	13.6 B	13.4 B	

Within each column, means followed by a common lowercase letter are not significantly different at $p < 0.05$ according to Tukey's HSD-test. Within each line, means followed by a common capital letter are not significantly different at $p < 0.05$ according to Tukey's HSD-test. For statistical details regarding factors and their interaction significance, see Table S4.

With regard to fenugreek, *Pn* was only affected by NT (Table 2 & Table S4). The effect of NT was showed more pronounced at BEG site compared to SBR. Further, at BEG, the *Pn* values under N0, N1 and N2 treatments were maintained similar and were significantly higher than those under N3 and N4 by 9–12%. Likewise, for clover, Pn was greatly affected by NT ($p < 0.000$). However, the effect of NT on *Pn* was showed dependent on CP, since the interaction NT × CP was significant at BEG site for both seasons (Table 2 & Table S4). Indeed, within the IC-Mix pattern the Pn values of clover under N0, N1 and N2 treatments were similar and significantly higher than those under N3 and N4 by 19–23%, while within the IC-Clo the *Pn* values were significantly the highest only under N0 and N1 treatments. Further, with the presence of fenugreek (IC-Mix) the *Pn* values of clover were globally enhanced particularly under N2 treatment (Table 2). This effect was clearly showed at BEG site for 2017 season and at SBR site for both seasons, where Pn values under treatment N2 were significantly higher under IC-Mix than those under IC-Clo. However, under N3 and N4 treatments, the *Pn* values of clover were decreased in both IC-Clo and IC-Mix mostly at BEG site.

3.5. Soil Moisture

At BEG site, soil moisture at legumes flowering was generally homogeneous between all cropping patterns and N treatments over both seasons (Figure 4). However, at SBR site, soil moisture was showed greatly affected by the adopted cropping pattern ($p < 0.001$). The highest soil moisture values were recorded in the IC-Mix and also the IC-Fen. Soil moisture in these intercropping patterns was significantly higher than both SC-NH and SC-H patterns. The improvements of soil moisture in the IC-Fen and IC-Mix patterns were showed maintained in all NT (Figure 4). These results illustrate that the existing legumes as companion plants provide a better canopy that contributes to minimizes soil water evaporation and conserves soil moisture. Indeed, in the SBR site, which is characterized by a semi-arid climate, soil moisture was positively correlated with legumes biomass ($p < 0.001$).
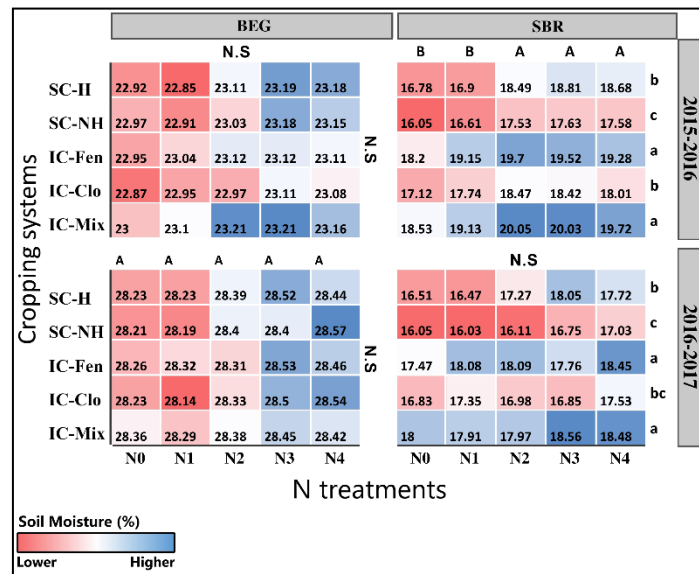
Figure 4. Effects of cropping pattern and N treatment on soil moisture at legumes flowering in 2016 and 2017 seasons, at both sites. Capital letters (A, B) indicate significant differences between N treatments (cropping patterns combined), according to Tukey's HSD-test. Lower-case letters (a, b, c) indicate significant differences between cropping patterns (N treatments combined), according to Tukey's HSD-test. N.S. not significant.

3.6. Grain Product Quality

Overall, durum wheat thousand kernel weight (TKW), grain protein content (GPC), and grain ash content (GAC) were showed improved in the intercropping patterns mainly in the IC-Mix pattern compared to both SC-NH and SC-H (Table 3). The improvement of durum wheat grain quality appears dependent on NT, particularly at BEG. Indeed, only under N0, N1 and N2 treatments there were a significant improvement of TKW, GPC and GAC mainly with the IC-Mix compared to the SC-NH. However, under N3 and N4 treatments, TKW, GPC and GAC were broadly similar whatever the cropping patterns (Table 3).

Table 3. Effects of cropping patten and N treatment on thousand kernel weights (TKW), grain protein content (GPC), grain ash content (GAC) and gross grain product impurity rate of durum wheat at both sites averaged over the three seasons.

	BEG						SBR					
	N0	N1	N2	N3	N4	Mean	N0	N1	N2	N3	N4	Mean
Thousand Kernel Weights (TKW g)												
SC-H	48.9 abB	49.3 abA	49.2 bAB	49.1 aAB	49 aAB	49.1 abc	40.2 bcA	40.4 bcA	40.6 aA	40.5 aA	40.5 aA	40.5 b
SC-NH	48.7 bB	49.1 bAB	49.2 bA	48.8 aAB	48.8 aAB	48.9 c	40.1 cA	40.3 cA	40.4 aA	40.1 aA	40.1 bA	40.2 c
IC-Fen	49 BabC	49.5 abA	49.4 abAB	48.9 aBC	48.8 aC	49.1 ab	40.5 abAB	40.8 abA	40.6 aAB	40.4 aAB	40.3 abB	40.5 ab
IC-Clo	49 abAB	49.4 abA	49.1 bAB	48.9 aAB	48.8 aB	49 bc	40.3 bcA	40.5 bcA	40.4 aA	40.1 aA	40.1 abA	40.3 c
IC-Mix	49.2 aB	49.6 aA	49.7 aA	48.9 aB	48.8 aB	49.2 a	40.7 aA	40.9 aA	40.7 aA	40.5 aA	40.4 abA	40.7 a
Mean	48.9 B	49.4 A	49.3 A	48.9 B	48.8 B		40.4 BC	40.6 A	40.5 AB	40.3 C	40.3 C	

Table 3. *Cont.*

	BEG						SBR					
	N0	N1	N2	N3	N4	Mean	N0	N1	N2	N3	N4	Mean
Grain Protein Content (GPC%)												
SC-H	12.5 cB	13.2 aA	13.5 abA	13.2 aA	13.2 aA	13.1 ab	14.1 cB	14.5 bB	15.2 abA	15.4 aA	15.3 aA	14.9 bc
SC-NH	12.5 cB	12.8 bA	13 cA	13 aA	13.1 aA	12.9 c	14.3 bcC	14.6 bBC	14.7 bABC	15 aAB	15.2 aA	14.8 c
IC-Fen	12.8 abB	13.3 aA	13.4 abcA	13.1 aA	13.1 aA	13.1 ab	14.6 abB	14.9 abAB	15.3 abA	15.2 aA	15.3 aA	15.1 b
IC-Clo	12.7 bcB	13.1 abA	13.1 bcA	13.1 aA	13.1 aA	13 bc	14.5 abcB	14.8 abAB	14.9 bAB	15 aAB	15.2 aA	14.9 bc
IC-Mix	13 aB	13.4 aAB	13.5 aA	13.2 aAB	13.2 aAB	13.3 a	14.8 aB	15.2 aAB	15.6 aA	15.5 aA	15.5 aA	15.3 a
Mean	12.7 C	13.2 AB	13.3 A	13.1 B	13.2 AB		14.5 C	14.8 B	15.1 A	15.3 A	15.3 A	
Grain Ash Content (%)												
SC-H	1.92 aB	1.97 aA	2.01 aA	2.01 aA	2.01 aA	1.98 a	1.98 bcC	2.02 bcBC	2.05 abAB	2.07 aA	2.06 aA	2.04 a
SC-NH	1.88 bB	1.92 bB	1.97 bA	1.95 cA	1.96 bA	1.93 c	1.96 cB	1.99 cAB	2.01 bA	2.02 bA	2.02 bA	2 b
IC-Fen	1.94 aB	1.99 aA	2.01 aA	1.99 abA	1.99 abA	1.99 a	2.01 abB	2.05 abAB	2.06 aA	2.04 abAB	2.05 abAB	2.04 a
IC-Clo	1.93 aA	1.96 abA	1.97 bA	1.96 bcA	1.96 bA	1.96 b	1.99 abcA	2.02 bcA	2.02 bA	2.02 bA	2.02 abA	2.02 b
IC-Mix	1.94 aB	2 aA	2.02 aA	2 aA	2ab A	1.99 a	2.02 aB	2.07 aA	2.07 aA	2.05 abAB	2.05 abAB	2.05 a
Mean	1.92 C	1.97 B	1.99 A	1.98 AB	1.99 A		1.99 B	2.03 A	2.04 A	2.04 A	2.04 A	
Impurity rate (% of fenugreek seeds)												
IC-Fen	2.6	2.1	2	1.2	1.2	1.8	4	3.5	3.3	2.6	2.2	3.1
IC-Mix	3	2.6	2.2	1.4	1.5	2.1	4.3	3.6	2.6	2.4	2.6	3.1
Mean	2.8	2.4	2.1	1.3	1.4		4.1	3.6	3	2.5	2.4	

Capital letters indicate significant differences between N treatments (horizontal comparison) and lower-case significant differences between cropping patterns (vertical comparison) (Tukey-Test, $\alpha = 0.05$). For statistical details regarding factors and their interaction significance, see Table S3.

3.7. Straw Yield

At both sites, durum wheat straw yield significantly varied according to the CP and NT ($p < 0.05$) (Table S3). Straw yield was increased in all intercropping patterns as compared to the SC-NH (Figure 5A). The IC-Mix pattern showed the highest straw yield; which was statistically higher than those in the SC-NH and SC-H (Figure 5A). As compared to SC-NH, straw yield increase rate in the IC-Mix pattern was 13% and 16% respectively at BEG and SBR sites. Overall, regardless of the cropping patterns straw yield was significantly increased when N-fertilization was applied at durum wheat tillering (N3 and N4) or, at the latest, at durum wheat stem elongation (N2) (Figure 5B). Although there was no significant interaction between NT and CP, within the IC-Mix and IC-Fen, the highest additional straw yields relative to the SC-NH pattern were obtained in N0 and N1 treatments (18–27%), followed by N2 treatment (13–16%) over N3 and N4 treatments (4–11%) (Figure 5B).
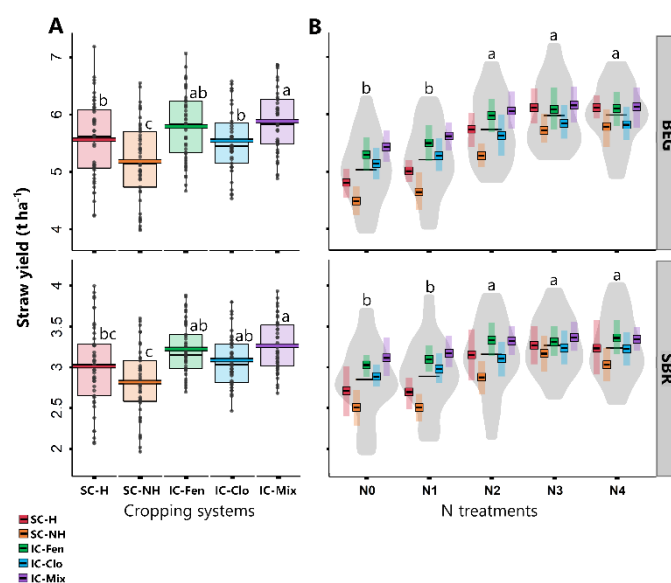
Figure 5. Effects of cropping pattern and N treatment on the straw yield at both sites averaged across the three experimental seasons.(**A**): Box plot indicating the cropping pattern effects on the straw yield averaged over the three seasons and the five N treatments;(**B**): Violin plot indicating the N treatment effects on the straw yield averaged over the three seasons and the five cropping patterns (black lines indicate mean grain yield; Tiles represent the means and 95% confidence intervals according to the cropping pattern in each N treatment); Box plots (**A**) and violin plots (**B**) with a common letter are not significantly different at $p < 0.05$ according to Tukey's HSD-test (for statistical output see Table S3).

4. Discussion

Results of the present study highlight that the performance of legumes to improve durum wheat main crop depend largely on the legume part itself, the adopted N fertilization regime and the interaction between them especially at the humid site (BEG). When added separately as companion plants, i.e., IC-Fen vs IC-Clo, fenugreek performs better than clover in terms of weed suppression and the improvement in durum wheat productivity and grain quality. Still, their mixture in the IC-Mix results in the greatest improvement of durum wheat crop. Compared to durum wheat sole crop with no-herbicide (SC-NH), only under unfertilized conditions (N0) and under relatively low and late N fertilization regimes (N1 and N2), IC-Mix resulted in a clear improvement in productivity (grain and straw yields) and grain quality (TKW, GPC and GAC) of durum wheat, where values were closely similar and even higher than those reported with the use of herbicides (SC-H). However, under the highest N fertilization regimes (N3 and N4), all intercrops had no significant advantages over the SC-NH, especially at the rainy site (BEG). Particularly at this site, durum wheat response to different cropping patterns (CP) was found significantly dependent on the N fertilization treatment (NT) regarding the grain yield, GPC and GAC. Further, the superiority of the conventional durum wheat cropping system, i.e., the sole durum wheat crop herbicide treated (SC-H) under N3 and N4, was clear in terms of grain yield. On the semi-arid site (SBR), even with these N fertilization regimes (N3 and N4), there was a slight improvement in durum wheat grain productivity and quality with the IC-Mix pattern compared to the SC-NH. This could be related to the greater responsiveness of the durum wheat crop to N fertilization at the humid site (BEG) compared to the semi-arid site (SBR) (Figure 1), as crop responses to N fertilization are dependent on soil water availability [31].

In the present study, globally, weeds infestation was not severe. This may be attributed to the sites long-term management for farming production and the adoption of the false seedbed technique [23]. Accordingly, weeds adverse effects regarding durum wheat sole crops productivity and grain quality (SC-NH vs SC-H) were showed globally moderate as compared for instance to other studies [32,33]. Also, this could be, partly, because weeds

were removed at durum wheat heading stage (Zadoks 45), which could have minimized weed competition. Nevertheless, weed suppression under the IC-Mix pattern involving both the fenugreek and the clover as companion plants was very resilient, which reached the herbicide weed control efficiency (in the SC-H). Both legumes, the fenugreek [17] and the clover [18] emit allelochemicals that are detrimental for weeds growth [19]. Our study, therefore, suggests that the adoption of the false seedbed technique combined with the use of fenugreek and clover as companion plants with durum wheat main crop help to suppress weeds. This effect was shown consistent across seasons at both sites. Results show, also, that fenugreek performs better than clover in terms of weed suppression (IC-Fen vs IC-Clo). This may be due to the fast growth features and the high competitive ability of fenugreek compared to clover [34,35] which help to fast-close crop canopy over weeds and thereby limiting early their growth [36].

The benefits of legumes, as companion plants, were shown dependent on their biomass, nodulation, and photosynthetic rate (*Pn*), which were greatly influenced by the adopted N fertilization regime. One of the most important advantages of the cereal-legumes intercrops is the improvement of legumes nodulation and N_2 fixation capacity [37,38]. This is attributed to soil N depletion by cereals, which reduces nitrate inhibition of nodulation and nodule functioning, and also because cereals are more competitive for soil N, forcing legumes to rely on biological N fixation [39]. However, as shown in our study, both legumes biomass and nodulation were significantly reduced under N fertilization regimes beginning at durum wheat tillering stage (N3 and N4). Mostly at BEG (humid), this can be explained by the fact that early and high soil N availability (N3 and N4) leads to a competitive imbalance that favours durum wheat growth. High durum wheat growth at early stage causes shading effects on the under-sown legumes. Hence, resulting in the greatest decrease in their *Pn*. Reduced legumes *Pn* leads to a significant decrease in energy supply to nodules, resulting in reduced nodulation and N_2 fixation [40,41]. Particularly at BEG site, both legumes *Pn* and nodule weight were positively correlated ($p < 0.001$). Similar observations were also reported by [42] under cereal-pea intercropping condition. At SBR (semi-arid), the decrease of legumes nodulation under N3 and N4 treatments may be explained, also, by a nitrate inhibitory effect [43] given that durum wheat N-uptake and thus soil N depletion in the rhizosphere environment may be limited by water-limiting availability. Indeed, under water-limiting conditions such as SBR site, soil water availability has a direct effect on productivity and an indirect effect through its regulatory role in soil N availability [44,45]. Thus, the limited durum wheat biomass establishment (estimated via the straw yield), at SBR compared to BEG, could have minimized light competitiveness between durum wheat and legumes which explain the relatively lower adverse effect of high soil N-availability under N3 and N4 treatment on legumes biomass and *Pn* at SBR compared to BEG site. Our study suggests that competition for light under the humid site and high N availability in the rhizosphere under the semi-arid site are the main factors limiting legume nodulation. The reduced nodulation and N fixing capacity of legumes lead to the lack of N facilitation process, which consists in the transfer of N from legumes to cereals [46,47], hence, the non-benefit of durum wheat from N2 fixation and also the non-advantages of legumes as companion plants as shown in our study in terms of PLER and durum wheat grain quality (GPC, GAC), mostly at the humid site. Our study, therefore, suggests avoiding N-fertilization regimes beginning at tillering stage in cereals-legumes intercrops systems. Of interest to indicate that mainly with the IC-Mix pattern, under a relatively low and late N-fertilization regime (N1 and N2) there was no effect on both legumes' biomass, *Pn* and nodulation compared to the N0 treatment, yet significantly increased overall durum wheat productivity and grain quality. This suggests that the benefits of legumes companion plants for the durum wheat crop increases with the decrease in N availability mostly at the early growth stage.

The effect of N fertilization regime on legumes biomass, *Pn* and nodulation depend, also, on the legume's species. Thus, N fertilization regime beginning at cereal stem elongation (N2) negatively affected only the clover biomass, and nodulation weighs in the IC-Clo

without any significant effect on fenugreek in IC-Fen. Our findings suggest that under conditions of increased soil N availability at durum wheat stem elongation, trait differences among fenugreek and clover influenced their corresponding growth through changes in resource availability. Indeed, clover is known as a low-growing species compared to fenugreek [34,35], so its growth may be more affected by the durum wheat dominance and also by the weeds when N availability increase at durum wheat stem elongation. Durum wheat -and weeds- may on the one hand push legumes to increases its N_2-fixation reliance via competition and depletion for soil N, and on the other hand, reduce growth and N_2 fixation in low-growing legumes like clover via strong competition for light. The same finding concerning clover species was reported by [48], where N-fertilization applied at cereal stem elongation stage significantly decreased both red and white clover biomass compared to the unfertilized treatment under clover-wheat intercrops conditions. When both legumes were added as a mixture (IC-Mix), albeit each legume biomass was relatively reduced compared to its addition alone (IC-Fen and IC-Clo), their nodules weight and *Pn* were maintained unaffected and, instead, improved in particular for clover under N2 treatment. Our results suggest that by mixing these legume species -and thus increasing the diversity of traits-, the interactions between plants into the cropping system i.e., between weeds-legumes (fenugreek+clover)-durum wheat can be modulated under certain condition of soil N availability. Thus, the better weed suppression performance of fenugreek due to its fast growth features could have minimized weed competition early and therefore giving a better growth condition for clover into the IC-Mix pattern. On the other hand, driven by trait differences between fenugreek and clover (i.e., fast growth—low growth and medium root depth—relatively deeper root depth), the frequency of legumes roots and nodules (fenugreek+clover) could be substantially higher in either time and space in the IC-Mix pattern which results in greater bioavailability and -reachability- for durum wheat to benefit from the N_2 fixation through the facilitation process [46,47] and also could further induce nodule function [46,47,49]. Accordingly, this may constitute an additional source of N, which resulted in the improvement of durum wheat *Pn*, productivity (PLER) and grain quality (TKW, GPC) with the IC-Mix under N0, N1 and N2 treatments. Our study, therefore, illustrates that under certain condition of soil N availability using promising legumes species combinations could result in the improvement of N fertilizer land-use efficiency and hence help to reduce N-fertilization inputs for an eco-friendly durum wheat production.

Particularly under water-limiting conditions such as SBR site, our study illustrate that the existing of legumes provides an extra canopy that confers the shading which minimizes soil water evaporation losses. Thus, within the intercrop's patterns, mostly the IC-Mix and also the IC-Fen, there was a significant improvement of soil moisture, which was positively correlated with the legume biomass ($p < 0.001$). This result agrees with previous studies, which demonstrated that intercropping decreased water evaporation and conserved soil moisture [21,50]. Our study provides that under low precipitation conditions, legumes as companion plants improve soil water availability for durum wheat as cash crops mainly at grain filling stage, which results in the increases of the TKW.

The use of legumes, as companion plants, can be considered a powerful strategy to limit pesticide dependency [51], as shown in our study for herbicides but also for insecticides [52], and for disease control [53], which can mitigate food-related chemicals hazards [54]. Thus, using legumes as companion plants could provide incentives to ensure food safety and provide better nutritional values with limited repercussions on the processing and acceptability of cereal-based products. As our study confirms, legumes as companion plants can improve cereal grain weight, protein, and ash content relative to cereals sole crops. Compared to other cereal-legume intercropping systems that require a sorting step of raw cereal products for acceptability when processed for human consumption [55], the use of legumes such as the fenugreek and clover as companion plants helps to avoid such sorting process. On the other hand, having fenugreek seeds in raw grain product at low levels, not exceeding 4.3% as in this study (Table 3), could improve

foods nutritional value and support functional food concept without repercussions on the organoleptic properties [56].

5. Conclusions

In the present study, we evaluated the performance of two legume species (fenugreek, clover and a mixture of them) added as companion plants to enhance durum wheat crop under five N fertilization regimes compared to durum wheat sole crops with and without herbicide. Result reveled that the mixture of fenugreek and clover as companion plants (IC-Mix) may offer significant opportunities for developing sustainable durum wheat production. The mixture of fenugreek and clover as companion plants (combined with better seedbed preparation using for instance the false seedbed technique as the present study) resulted in a better weed suppression performance that reached herbicide efficiency. Mostly under rainy conditions, the highest's performance of the mixture of fenugreek and clover to improve durum wheat productivity and grain quality were found under the unfertilized conditions (N0) or the relatively low and late N-fertilization regimes (N1 and N2), suggesting that N fertilization regime requires attention to ensure the expected benefits of such intercropping systems. Particularly at the semi-arid site (SBR), findings proved that the mixture of fenugreek and clover as companion plants help to preserve soil moisture and hence help to mitigate the water stress. This study proves that the use of legumes, as companion plants, represents an excellent alternative to the conventional cereal cropping system by providing multiple services in line with the sustainability principles.

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