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EVALUATING SPATIAL ARRANGEMENT FOR DURUM WHEAT (*Triticum Durum* Desf.) AND SUBCLOVER (*Trifolium subterraneum* L.) INTERCROPPING SYSTEMS

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KEY WORDS: living mulch, durum wheat yield, subclover reseeding, weed control, nitrogen fertilization, PAR, competitive balance index

HIGHLIGHTS:

- Subclover was tested as living mulch for durum wheat in several spatial arrangements
- Wheat yield was not reduced when the two species were sown in rows 10 cm apart
- Nitrogen fertilization increased wheat yield but decreased subclover performance
- Wheat was dominant, its aggressivity increased with proximity to subclover
- Subclover can improve weed control in intercropped wheat compared to alone wheat

ABSTRACT

Cover crops and mulches can be used for increasing sustainability in winter cereal cropping systems. We performed a 2-year field experiment in Central Italy with the aim of finding a suitable spatial arrangement for durum wheat (*Triticum durum* Desf.) and subclover (*Trifolium subterraneum* L.) as a living mulch system in order to provide a high grain cereal yield and a sufficient subclover reseeding following the wheat harvest. Experimental treatments consisted of: (i) five cropping patterns [wheat and subclover mixed in the same row, with rows 15 cm apart (same row); 2 rows of wheat and 1 row of subclover at a distance of 10 cm between rows (narrow rows); 2 rows of wheat and 1 row of subclover with a distance of 10 cm between the wheat rows and 17.5 cm between the wheat and subclover rows (wide rows); durum wheat sole crop and subclover sole crop, both in rows 15 cm apart]; (ii) two nitrogen fertilization levels (0 and 100 kg ha^{-1} of N); (iii) and two weed management levels (weed-free and weedy). The wheat grain yield was not reduced by the intercropped subclover in narrow rows, while it was around -14% lower in same row and wide rows compared to the pure crop treatment, although the intercropped systems always showed a higher resource use efficiency. When intercropped with subclover, wheat was the competitively superior species and its competitive advantage was greater when it was closer to the legume and/or in presence of nitrogen fertilization. A strong negative relationship between wheat aggressivity and subclover seed production was observed. Following the wheat harvest, the legume reseeding was sufficient to regenerate a cover crop in the autumn of the second year regardless the spatial arrangement, even if the density of the subclover seedlings was almost twice in wide rows compared to same row. Although the intercropped systems were characterized by an increase in plant density compared to the sole crops (100% of wheat + 50% of subclover), the competitive ability of the wheat-subclover system against the weeds was higher than the wheat sole crop only in narrow rows where a significant reduction of both weed density and weed biomass was observed. When the subclover is used as living mulch in durum wheat, a moderate separation between the two species could be a suitable spatial arrangement for obtaining an adequate wheat grain yield, ensuring satisfactory subclover reseeding, controlling the weeds more effectively.

1. INTRODUCTION

Cover crops and mulches can be used for increasing sustainability in modern cropping systems. They have numerous positive effects reducing the need of external agronomical inputs such as fertilizers, herbicides and pesticides (Hauggaard-Nielsen et al., 2001), reducing nitrogen loss and increasing biodiversity (Jackson et al., 2007). A cover crop is usually grown in the period between two main cash crops, occupying the field when it is fallow and replacing the weed flora. If the cover crop is suppressed before sowing the next main crop, the growth cycles of the cover crop and the main crop do not overlap and therefore there is no competition between cover and cash crops. A cover crop can also grow together with a cash crop as a living mulch. In this case the intercropped species may compete for the same resources, resulting in a lower yield of the main crop (Carof et al., 2007). Therefore a suitable living mulch species should compete as little as possible with the main crop while maintaining the benefits. Various strategies have been suggested for reducing the competitive effects of the living mulch against the main crop. These include selection of a suitable living mulch species and/or genotype (Hollander et al., 2007), and agronomic interventions such as the chemical and mechanical suppression of mulch growth (Thorsted et al., 2006; Teasdale, 1996), shifting the relative sowing dates of the intercropped species (Blackshaw et al., 2010) and providing supplemental inputs to compensate for the resources used by living mulch plants (Munoz and Weaver, 1999). In the Mediterranean environment, without irrigation, it is particularly complicated to put a living mulch system into practice due to the shortage of water especially during the summer period when a living mulch can compete severely with the main crop. Several authors have suggested using some types of winter annual legumes as living mulch in winter cereal grain cultivation due to the abundant rainfall throughout the cereal cycle (Caporali and Campiglia, 2001). Subterranean clover (*Trifolium subterraneum* L.) has proved to be a particularly suitable living mulch during the winter cereal crop cycle, an efficient dead mulch after the grain harvesting during the summer period and a cover crop in the following autumn-winter period. (Caporali and Campiglia, 2001). Subterranean clover does not compete strongly with winter cereals for nitrogen due to its nitrogen-fixing capacity, or for light due to its prostrate growth habit and low height compared to tall erect cereals. Furthermore, subclover is produces buried seeds and thus it can regenerate a living cover after autumn rainfall in the second year, even though the adult plants die in late spring. Therefore, this legume offers a much more environmentally-friendly type of management, providing a flexible pattern of cover cropping in the Mediterranean environment. In order to achieve a successful winter cereal/subclover intercropping, two major issues must be addressed: (i) there should be as little difference as possible in cereal yield between cover cropping and conventional (single crop) system; (ii) the intercropped subclover must produce enough seeds to regenerate in the following season providing a suitable cover crop able to supply abundant biomass and cover the ground throughout the following autumn-winter season. These are somewhat conflicting requirements since an increase in the intercropped cereal grain yield could increase the competitive pressure on the subclover, resulting in lower reseeding capacity, while a robust clover growth will reduce wheat growth and yields. A suitable management strategy should be based on the best possible tradeoff between cereal and subclover performance. Little attention has been paid to the spatial arrangements of the intercropped species. In fact, modifying the spacing between the crop rows not only changes the competitiveness of the intercropped species, it can also affect a different weed suppression by the living mulch-main crop system, because different living mulch–main crop spatial arrangements can influence the use of light, nutrients, and water, whose availability is essential for weed germination and growth.

The main objective of this study was to find a suitable spatial arrangement for a subclover living mulch-durum wheat system which can provide a high grain yield and a sufficient subclover reseeding following the wheat harvest. We also investigated how the spatial arrangement can affect the suppressive ability of the intercropped species against the weeds at two nitrogen fertilization levels.

2. MATERIALS AND METHODS

2.1. Exsperimental site and climate

The study was carried out at the experimental farm of the University of Tuscia in Viterbo, Italy (lat. 42°25', long. 12°04', 310 m above sea level), over a two-year period from 2010-2011 to 2011-2012 in two adjacent fields previously kept fallow. This area, located in Central Italy, is characterized by a typical Mediterranean climate with an annual air mean temperature of 14.0 °C, minimum temperature just below 0 °C in the winter and maximum temperatures above 35 °C in the summer. The annual rainfall (800 mm) is mainly concentrated from October to May (based on a 30-year period). The air temperature and rainfall of the study period were collected from an automated meteorological station located 250 m from the experimental site. The soil in the experimental area is volcanic classified as *Typic Xerofluvent* with the following characteristics in the top layer (0-30 cm): 680 g kg^{-1} of dry soil sand, 18 g kg^{-1} of dry soil silt, 14 g kg^{-1} of dry soil clay, bulk density 1.38 g cm^{-3}, pH 6.43 (water, 1:2.5), organic matter 17.1 g kg^{-1} of dry soil (Lotti), total nitrogen 0.97 g kg^{-1} of dry soil (Kjeldahl), and available phosphate 14.38 g kg^{-1} of dry soil.

2.2. Experimental design

The field experiments included five treatments composed of the monoculture of durum wheat (*Triticum durum* Desf., cv. Colosseo) and subclover (*Trifolium subterraneum* L., Campeda) and three different durum wheat/subclover intercropping patterns: (1) wheat and subclover mixed in the same row with a distance of 15 cm between rows (hereafter called same row); (2) 2 rows of wheat and 1 row of subclover with a distance of 10 cm between rows (hereafter called narrow rows); (3) 2 rows of wheat and 1 row of subclover with a distance of 10 cm between the wheat rows and a distance of 17.5 cm between the wheat and subclover rows (hereafter called wide rows); (3). The distance between rows was 15 cm in wheat and subclover monocultures (Fig. 2). Two nitrogen fertilization levels (0 and 100 kg ha^{-1} of N, hereafter called N0 and N100, respectively) and two weed managements (weed-free and weedy, hereafter called WF and We, respectively) were applied for each treatment. The experimental design was a randomized split-split-plot with four replications, with the intercropping patterns and the pure crops as main plots, the nitrogen fertilization levels as the sub-plots, and the weed managements as the sub-sub-plots. The sub-sub-plot size was 12 m^2 (3 m x 4 m).

2.2. Establishment of treatments and crop management

In both years, soil preparation was carried out in September by plowing at a depth of 30 cm followed by two disk harrowings. One hundred kg ha^{-1} of P fertilizer as a triple superphosphate was broadcast and incorporated into the soil at seedbed preparation, while in the nitrogen fertilized plots 100 kg ha^{-1} of N fertilizer was split into two portions, of which 1/3 was applied as calcium nitrate at the tillering stage, and 2/3 as ammonium-nitrate at the stem elongation stage both for pure and intercropped crops. The weeds were removed manually whenever necessary in weed-free plots or left to grow undisturbed throughout the durum wheat cropping season in weedy treatments. Durum wheat and subclover were sown with a research sowing machine on 19 October 2011 and 12 October 2012 according to the intercropping pattern design (Fig. 1). The wheat planting density was 400 seeds m^{-2} in all cases, while the subclover planting density was 600 seeds m^{-2} in pure stand, and 300 seeds m^{-2} in the intercropped treatments. Therefore all intercropped treatments were characterized by an increase in plant density compared to the sole crops in a semi-additive series (Kelty and Cameron, 1995). The durum wheat grain was harvested on 4 July 2012 and 9 July 2013.

2.3. Measurements and analysis

Durum wheat, subclover and weed aboveground biomass were harvested separately at wheat physiological maturity. The wheat plants were cut at ground level from 10 one-meter-long adjacent rows in a central area of each plot, and the following characteristics were measured overall: plant height (with awns excluded), number of fertile spikes, kernels per spike, thousand grain weigh (TGW). The straw was separated from the grains and both the fractions were dried at 70 °C until constant weight. The wheat harvest index was calculated as the ratio between grain weight and the total aboveground biomass. The harvested grains were used for evaluating the following wheat grain quality characteristics: the test weight (TW), which reflects the density and the volume occupied by the grains and expressed as kg hL^{-1}, was measured with a Shopper chondrometer equipped with a 1 L container; the percentage of vitreous kernels, which is an indicator of milling and cooking quality, was determined according to the method given by ISO (1987) by examining the cross-section of 100 cut kernels. Subclover and weed aboveground biomass, in 4 randomly-placed 90 x 90 quadrats near the center of each plot was cut at the ground level. The weeds were counted, the subclover pods were harvested and the number of seeds contained in each pod determined. Subclover seedlings, in 4 randomly-placed 90 x 90 quadrats placed near the middle of each plot, were measured on 10 October 2012 and 21 October 2013 to evaluate the re-seeding capacity of subclover.

The photosynthetically active radiation flux (PAR, μmol m^{-2} s^{-1}) was measured at ground level with a linear ceptometer (SS1-UM-2.0, DELTE-T Devices LDT, Cambridge, England) placed vertically ten times in the centre of the wheat paired rows of each weed free sub-plot every thirty days starting from the wheat tillering stage up to wheat physiological maturity in 2012. A SPAD-502 (Minolta, Osaka, Japan) and a Dualex were used to obtain estimates of chlorophyll content of the wheat leaves (hereafter called SPAD readings) and the polyphenolic content (Phen.) of the wheat leaves, respectively. SPAD readings and Phen. were measured at wheat anthesis on the same day on fully developed laminae of the flag leaf. Dualex readings were taken from both adaxial and abaxial sides of the flag leaf, while SPAD readings were only taken on the adaxial side of the leaf (Tremblay et al., 2010). Both SPAD readings and Dualex measurements were taken ten times, one reading per plant in approximately the same leaf position, in each replication in each wheat weed free sub-plot and averaged for each treatment (Tremblay et al., 2010). The SPAD reading /Phen. ratio was calculated and used as an indicator of leaf nitrogen content at wheat canopy level (Cartelat et al., 2004).

2.4. Definition and calculation of competition indices

To assess the competitive ability of durum wheat intercropped with subclover and the competitive ability of the crop system (sole crop or intercropped system) against the weeds the following indices were used:

1) The relative biomass total (RBT calculated only for weed free crops):

$$RBT = (Ds/D)+(Sd/S) = RBd+RBs$$

Where Ds is the aboveground biomass per unit area of the durum wheat intercropped with the subclover; Sd is the aboveground biomass of the subclover intercropped with the durum wheat; D is the aboveground biomass of the durum wheat in pure stand; and S is the aboveground biomass of the subclover in pure stand, respectively.

RBd and RBs are the relative biomass of durum wheat and subclover, respectively. RBT describes how much biomass the mixture produces in comparison with pure stands. RBT greater than 1 indicates increased biomass production in mixtures (Snaydon, 1991).

2) Aggressivity (A) calculated only for weed free crops:

$$Ad = \frac{1}{2} [(Ds/D) – (Sd/S)]$$

Where Ad is the aggressivity of the durum wheat against the subclover in the intercropped systems and the subscripts as the same meaning as above. Ad is a measure which evaluates how aggressively the durum wheat behaves when intercropped with subclover. It can range from + 1 to -1. Ad higher, or lower, or equal than 0 indicates that the durum wheat is dominant, dominated, or equally dominant compared to the subclover (McGilchrist & Trenbath, 1971).

3) The competitive balance index (Cb):

$$Cb = \ln [(SYSw/SYS)/(Wsys/W)]$$

Where SYSw is the aboveground biomass per unit area of the crop system in weedy conditions (intercropped durum wheat plus subclover, or durum wheat pure stand, or subclover pure stand, respectively); Wsys is the aboveground biomass per unit area of the weeds in the crop system; SYS is the aboveground biomass per unit area of the crop system in weed free conditions; and W is the aboveground biomass per unit area of the weeds in pure stand, respectively.

Cb is a measure which quantifies the competitive ability of the crop system (durum wheat and subclover in pure stand or intercropping) against the weeds in crop-weed mixture. If Cb is higher, equal or lower than 0, the crop system is more, equally or less competitive compared to the weed (Wilson, 1988).

2.5. Statistical analysis

The data on durum wheat, subclover, weeds and competition indices were analyzed with analyses of variance (ANOVA) using JMP statistical software package, version 4.0 (SAS Institute, Cary, NC), with the year as a random effect. Percentage data were *arcsin* transformed, and weed density square root transformed (x + 0.05) before analysis to homogenize the variance (Gomez and Gomez, 1984). The data reported in the tables were back transformed. A split-split-split-plot experimental design with four replications was used for the durum wheat and subclover variables, where the year was considered the main factor, planting pattern as the split factor, nitrogen fertilization as the split-split factor, the weed management as the split-split-split factor. A split-split-plot experimental design with four replications was used for the weed variables, indices of competition, SPAD and Dualex readings (Chl., Phen, and Chl/Phen.), where the year was considered the main factor, planting pattern the split factor, nitrogen fertilization as the split-split factor. The main effect and interaction means were compared using Fisher's protected LSD test at 5 % of probability level ($P < 0.05$). Linear regressions were performed for selected durum wheat or subclover variables and competition indices.

3. RESULTS

3.1. Weather conditions, wheat and subclover phenological stages

Rainfall, and maximum and minimum temperatures differed in the two experimental periods (Table 1). The 2011/2012 season was generally dryer, and 2012/2013 wetter than the historical means (Table 1). Rainfall from wheat seeding to wheat physiological maturity (October-June) was 259.1 mm in 2011/12 and 699.2 mm in 2012/2013, and rainfall was particularly abundant in spring 2013 compared to spring 2012 (253.0 vs. 121.0 mm). Air temperatures were similar to the long-term average in both cropping cycles, except in March, May and June 2012, when the maximum temperatures were considerably higher than the same months in 2013. In both cropping seasons, the minimum air temperatures seldom dropped below 0 °C in winter, so no frost damage was observed in subclover, which is less frost resistant than wheat. Subclover flowered earlier in spring 2012 than spring 2013 (second 10-days period of March and first in April, respectively), and the wheat heading-anthesis stage was also earlier (first 10-days period of May in 2012 and second 10-days period of May in 2013). The subclover began to senesce and die in the second 10-days period in May 2012 and in the first in June 2013. The physiological maturity of the wheat was observed around middle June in 2012 and at the end of June in 2013. The different intercropping patterns and pure crops did not generally have any effect on the phenological stages of the wheat and subclover. Following the wheat harvest, the re-establishment of the subclover began in the beginning of the autumn since it was favoured by the abundant rainfall and the mild air temperatures observed in September and October 2012 and 2013.

3.2. Durum wheat yield and yield components

There were significant effects of year, cropping pattern, nitrogen fertilization and weed control on the yield and yield characteristics of wheat (Table 2). The plants were taller in 2012/2013 compared to 2011/2012 (66.2 *vs* 64.3 cm), both in wide and narrow rows intercropping and for sole crop compared to same row intercropping (on average 66.0 *vs* 63.0 cm), in N100 compared to N0 (67.7 *vs* 62.8 cm), and in weed free compared to weedy conditions (66.6 *vs* 63.9 cm). Wheat grain yield differed significantly between the years (3.2 and 4.4 t ha^{-1} in 2011/2012 and 2012/2013, respectively), the fertilization treatments (2.99 and 4.59 t ha^{-1} in N0 and N100, respectively), the weed treatments (4.13 and 3.45 t ha^{-1} in weed free and weedy conditions, respectively), while smaller differences were observed among the cropping partners. Sole wheat and narrow rows intercropping yielded on average 11% more than wide rows and same row. A similar effect of the year, nitrogen fertilization and weed control was observed on straw, number of spikes, and kernels per spike (Table 2). The cropping pattern strongly influenced the number of fertile spikes, which was higher in sole wheat crop followed by narrow rows, same row and wide rows intercropping (322, 289, 263 and 227 number m^{-2}, respectively). There were also significant differences in the number of kernels per spike among the cropping patterns ($P \leq 0.01$), which showed the highest value in wide rows intercropping and the lowest value in same row intercropping (31.6 vs. 27.0 n. $spike^{-1}$, respectively). TGW, TW, and vitreousness were higher in 2011/2012 compared to 2012/2013, in N100 compared to N0 and in weed free compared to weedy treatments, while wide rows intercropping showed the highest values of TGW and TW and sole wheat showed the lowest values of vitreousness (Table 2).

3.3. Subclover yield, yield components and reseeding capacity

The subclover performance generally decreased when it was closer to the wheat, and in presence of nitrogen fertilization or weeds, and there were significant cropping pattern x nitrogen fertilization and cropping pattern x weed management interactions (Table 3). The aboveground biomass of subclover ranged from 69 to 409 g m^{-2} of DM. Subclover was higher in sole subclover followed by wide rows, narrow rows and same row intercropping, and in N0 compared to N100 except in sole subclover and same row intercropping, which showed similar values with and without nitrogen fertilization. The subclover aboveground biomass was also higher in weed-free than in weedy conditions (on average 246 g m^{-2} and 159 g m^{-2} of DM, respectively), except in the same row intercropping which showed comparable values. The heads and the seeds of subclover ranged from from 136 to 1468 n. m^{-2} and from 378 to 3954 n. m^{-2}, respectively. They showed similar trends of those observed for the aboveground biomass, although they tended to be similar in wide rows and narrow rows intercropping (Table 4). As expected, the reseeding capacity of subclover was related to the seed production, it was the highest in sole subclover, followed by wide rows, narrow rows, and same row intercropping (on average 2046, 694, 546, and 323 n. of seedlings m^{-2}, respectively). The subclover generally regenerated better in absence of weeds and nitrogen fertilization except in same row intercropping where no differences were observed with and without nitrogen fertilization level (Table 3).

3.4. Interception of PAR, wheat leaf chlorophyll and polyphenolics content (Phen)

The fraction of intercepted PAR (fiPAR) increased from wheat tillering stage (January) to wheat heading (end of April), after which it began to decline, but the trend was slightly different among the planting patterns (Fig. 2). Until wheat heading, higher values of fiPAR were generally observed in the same row intercropping, followed by narrow rows intercropping, sole wheat, and wide rows intercropping. Subsequently, throughout the wheat grain filling stage, the fiPAR values continued to be higher in same row, slightly lower in narrow rows and wide rows intercropping, while in the lowest fiPAR values were found for wheat pure stand. Nitrogen fertilization increased fiPAR values, which tended to show lower values for the cropping patterns compared to the N-unfertilized crops (Fig. 2).

There was a large variation in SPAD readings, Phen. content, and the ratio SPAD readings/Phen. values of wheat flag leaf among the treatments (Table 4). The SPAD readings were higher in 2013 than 2012 (42.8 vs. 39.9, respectively) and for N100 than N0 (47.3 vs. 35.2, respectively), while it was higher in wide rows, intermediate in narrow rows, lower in same row intercropped and sole wheat (on average 44.3, 42.5, and 39.2, respectively). The phen. content was not influenced by the year or cropping pattern (mean 3.16), while it was higher in N0 than N100 (3.31 *vs* 3.01, respectively). The SPAD readings/Phen. ratio showed similar trends to that observed for SPAD readings, except for cropping patterns where it was higher in wide and narrow rows intercropping than in same row intercropping and sole wheat (on average 13.82 vs. 12.64, respectively). There was a positive relationship between the SPAD readings/Phen. ratio and the vitreousness of wheat grain in both years (R^2 = 0.57 and 0.71 in 2012 and 2013, respectively).

3.5. Weed density and weed biomass

Weed density and weed aboveground biomass at wheat harvesting were significant for year x nitrogen fertilization ($P \leq 0.05$) and cropping pattern x nitrogen fertilization ($P \leq 0.05$) interactions (Table 5). Higher weed density and weed aboveground biomass were observed in 2011/2012 compared to 2012/2013 in N-unfertilized treatments (77.4 *vs* 60.5 plants m^{-2}, respectively). The weed density was generally higher in N0 than in N100 (on average 52.8 vs. 42.7 plants m^{-2}, respectively), except in wide rows and same row intercropping which showed similar values with and without nitrogen fertilization (Table 5). Among the cropping patterns, the subclover sole crop always showed the highest weed density (on average 92.7 plants m^{-2}), while in intercropping the weed density was generally highest in wide rows intercropping, followed by same row and narrow rows intercropping (on average 52.3, 30.2 and 22.0 plants m^{-2}, respectively). The wheat sole crop showed a similar weed infestation to wide row intercropping in N0 and to same row intercropping in N100.

Large variation in weed density was associated with large variations in weed aboveground biomass which ranged from m 22.89 to 209.11 g m^{-2} of DM in narrow row intercropping N0 and sole subclover N100, respectively. Weed aboveground biomass was higher in N100 than N0 (on average 107.1 vs. 58.4 g m^{-2} of DM, respectively), except in wide rows intercropping and narrow rows intercropping which showed similar values at both nitrogen levels. Aboveground weed biomass was generally higher in subclover sole crop, intermediate in wheat sole crop, wide rows and same row intercropping, and lower in narrow rows intercropping (Table 5).

3.6. Indices for evaluating durum wheat – subclover and weed competition

The RBw index was significant for intercropping patterns, while RBs, RBT and Ad indices were significant for intercropping patterns x nitrogen fertilization (Table 6). RBw was highest in narrow rows intercropping, intermediate in same row intercropping, and lowest in wide rows intercropping at both nitrogen levels (0.96, 0.89, 0.85, respectively). RBs ranged from 0.16 to 0.61decreasing when subclover and wheat were grown closer and in the presence of nitrogen fertilization except for same row intercropping which showed similar values (Table 6). RBT was always higher than one showing its maximum value in unfertilized wide rows intercropping (1.51). RBT tended to be higher in N0 than N100 (on average 1.32 vs. 1.15, respectively), and in wide rows compared to same row intercropping (on average 1.38 vs. 1.04, respectively). Wheat was more aggressive than subclover (Ad ranged from 0.14 to 0.36). However, the nitrogen fertilization and the proximity of wheat and subclover generally increased Ad, which was always higher in same row than in wide rows intercropping (on average 0.36 vs. 0.18, respectively). Significant linear negative relationship were found between Ad and subclover seed production (R^2 = 0.81 in 2011/2012 and

0.64 in 2012/2013, respectively, Fig. 3), Ad and the seedling population of reseeded subclover (R^2 = 0.77 in 2011/2012 and 0.67 in 2012/2013, respectively, Fig. 3).

At wheat physiological maturity, the competitive balance index (Cb) showed a cropping pattern x nitrogen fertilization interaction effect (Fig. 4). All cropping systems were more competitive than the weeds even though when the Cb values were extremely variable ranging from 0.44 in subclover sole crop N100 to 2.60 in narrow rows intercropping N0. Among the cropping patterns, Cb was higher in narrow rows intercropping, followed by same row intercropping, sole wheat, wide rows intercropping, and sole subclover (on average 2.32, 1,85, 1,38, 1,13 and 0.48, respectively). Cb was also higher in N0 than in N100 except in wide rows intercropping and sole subclover where it was similar at both nitrogen levels.

4. DISCUSSION

Weather conditions in 2012/2013 were generally more favourable for obtaining high durum wheat grain yields and for producing subclover biomass and seed production compared to 2011/2012. The abundant rainfall throughout 2012/2013 resulted in satisfactory wheat and subclover establishment in autumn and wet conditions during anthesis and grain filling period (April-June) also contributed. However, durum wheat-subclover intercropping performed better than sole crops in both years, although the grain yield responses of both species were significantly affected by the cropping pattern. The total productivity of the systems, evaluated as relative biomass total (RBT), was always higher than 1, indicating a higher total production per unit area (Agegnehu et al., 2006; Akter et al., 2004) and consequently better land-use efficiency in intercropping systems than sole crops (Thorsted et al., 2006). RBT was generally higher in wide rows and narrow rows intercropping than in same row intercropping ranging from 1.51 to 1.19, while RBT was only slightly higher than 1 in same row intercropping. Therefore, resource use complementarity between wheat and subclover decreased as the proximity of the two species increased. In ecological terms, an increase of intimacy between the two species corresponded to an increase of the niche overlap. As expected wheat was the dominant species in the mixtures, and it is clear that wheat competed successfully with subclover probably due to the short canopy height of the legume (ranging from 12 to 17 cm, data not shown) compared to the tall canopy of the wheat (ranging from 63 to 68 cm).

Since the main objective of this study was to obtain a similar cereal yield in intercrop and sole crop systems only the narrow row intercropping showed a wheat grain yield which was not significantly different from wheat sole crop. Wide rows and same row intercropping reduced wheat grain yield by approximately 14 % compared to wheat pure crop. By analyzing the wheat yield components, we found that wide rows intercropping showed the lowest number of fertile spikes per unit of area, while it produced the highest number of unfertile culms (data not shown). Considering that the SPAD readings and SPAD readings/Phen. ratio values of wheat flag leaves in wide rows intercropping were similar to narrow rows intercropping, and higher than in same row intercropping and sole wheat, intercropping the cereal does not appear to have suffered in wide rows during the last part of the reproductive period. This hypothesis is consistent with the high number of kernels per spike, test weight (TW), thousand grain weigh (TGW), and vitreousness observed in white wide rows intercropping at harvesting. Although the same wheat seeding rate was used for all cropping patterns, the spatial arrangement in wide rows determined a closer proximity of the wheat plants in the row compared to other sowing geometries (1.1 vs 1.7 cm between the wheat plants on the row, respectively) which may have determined a high intraspecific competition of wheat particularly during the early growth stage (Thorsted et al., 2006). When the wheat was intercropped with subclover in the same row, similar values of SPAD readings and SPAD readings/Phen. ratio to wheat sole crop were observed. Since nitrogen is one of the main factors influencing the formation of chlorophyll (Lehr et al., 1962), these results indicated that there were no nitrogen nutrition advantages between the wheat mixed with subclover and the wheat sole crop. Although there is evidence that some legumes can transfer a part of the fixed nitrogen to the neighbouring grasses when they are in mixture (Bergkvist et al., 2011), subclover does not seem to be capable of supplying nitrogen to the wheat, at least prior of wheat flag leaf emergence as stated by Munoz and Weaver (1999), who observed that in a mixture of subclover and ryegrass (*Lolium multiflorum* Lam.), the grass did not receive nitrogen from the living clover. Moreover, wheat intercropped with subclover on the same row produced very few fertile spikes and kernels per spike indicating a high level of competition between the two species. Since the wheat was not shaded by the subclover, the competitive effect was not due to a reduction in light. The legume probably had a negative effect on the cereal plants because of underground interactions such as competition for resources or allelopathy (Enache and Ilnicki, 1990). Allelochemicals would move from the subclover into the soil and then into the wheat root system by diffusion, they create a radius effect causing higher concentrations near their root proximity. Therefore, when clover and wheat are mixed together in the same row, the allelopathic effects of subclover should be stronger compared to those in narrow and wide rows intercropping, where the subclover plants were 10.0 and 17.5 cm from the wheat plants respectively. Similar negative effects on yield were observed for tall main crops such as corn (Abdin et a., 1998) and cabbage (Brandsaeter et al., 1998) when intercropped with subclover. However, even if the subclover living mulch caused a reduction in wheat grain yield, it always increased grain vitreousness compared to sole wheat. Vitreousness is an indicator of the milling and cooking qualities of durum wheat grain. Low values generally indicate that the wheat was stressed throughout the grain filling period, usually due to nitrogen shortage. In this study, the senescence of the subclover begun 3-4 weeks before the wheat grain reached physiological maturity. Therefore, the wheat could have taken advantage of the decreased interspecific competition and the nitrogen released by the mineralization process of the dead subclover in the last part of the grain filling period.

The performance of subclover intercropped with durum wheat depended mainly on its spatial arrangement (Thorsted et al., 2006) and nitrogen fertilization level (Nassiri and Elgersma, 2002). As expected, subclover was the subordinate species in the durum wheat-subclover intercropping and it became weaker with increasing proximity to wheat. Therefore the wheat proved to be more aggressive in same row intercropping, and showed its lowest values in wide rows intercropping. The subclover was completely shaded by the taller canopy of the wheat in same row intercropping starting from the end of the wheat tillering stage, while subclover received direct sunlight part of the day in narrow row and even more in wide rows intercropping throughout the cropping period as wheat canopy closure was never observed (data not shown). Although subclover is a sciophilous species, the gradual increase in shade due to its proximity to the wheat could have led to the progressive reduction of the aboveground biomass and of the heads and seeds in the subclover living mulch compared to the sole subclover (Queen et al., 2009). A clear effect of the competition between wheat and subclover was the negative relationship between the wheat aggressivity index and the subclover seed production. The intercropped legume was able to produce seeds in all spatial arrangement conditions, however, the number of regenerated seedlings in autumn of the second year was sufficient for regenerating a new ground cover (Evers et al., 1988), even if the seedling density was almost twice as high in wide row compared to same row intercropping (on average 1023 vs 502 seedlings m^{-2}).

As expected, wheat benefited from the nitrogen fertilization, resulting in an increase in yield and yield characteristics, while subclover was generally depressed, reducing its aboveground biomass and seed production (Ghaley et al., 2005). A greater availability of soil nitrogen determined a higher wheat height and aboveground biomass, which resulting in a more severe shading of subclover. Higher levels of nitrogen may also have negatively influenced the N_2-fixation of subclover, causing a further reduction of subclover growth and seed production (Munoz and Weaver, 1999). The subclover was particularly depressed by the nitrogen fertilization in wide rows and narrow rows intercropping, probably due to the increase in shade compared to the unfertilized crop. In same row intercropping, where the legume was already completely shaded without nitrogen fertilization, the administration of nitrogen did not cause a significant worsening of the subclover performance. Therefore, the greater availability of soil nitrogen determined a higher level of cereal aggressivity in wide row and narrow row intercropping, while in same row intercropping, wheat aggressivity remained high yet stable. A greater subclover depression, due to the nitrogen fertilization, determined lower values of RBs and RBT, confirming that the highest efficiency of resource use in legume-cereal intercropping is achieved when no external nitrogen is supplied (Hauggaard-Nielsen and Jensen, 2001).

A living mulch, such as subclover, which is strongly dominated by the main crop can hardly be a good competitor against the weeds. In fact, subclover proved to be inefficient in suppressing weeds both in sole crop and when used as living mulch. Although all intercropped systems were characterized by an increase in crop plant density compared to the sole crops, the competitive ability of the wheat-subclover mixture against weeds was higher than wheat sole crop only in narrow row intercropping regardless at both nitrogen levels. In practical terms, a significant reduction of both weed density and weed aboveground biomass was only achieved by combining a high crop system density (durum wheat + subclover) with a suitable spatial arrangement between living mulch and the main crop. Considering that the weeds are generally present in the inter-row space, a narrow spacing between the rows could enable wheat and subclover plants to be more efficient in subtracting limited resources from the weeds thus reducing weed germination and growth (Corre-Hellou et al., 2011). The narrow row spatial arrangement was more efficient in intercepting photosynthetic active radiation (fiPAR) especially throughout the reproductive wheat and subclover period compared to wide and same row intercropping. Subclover placed in strips between the wheat paired rows could result in a reduction of weed infestation due to a greater niche overlap between the legume and the weeds.

5. CONCLUSION

Although the subterranean clover is a suitable cover crop for the Mediterranean environment, the results of this study indicate that when it is used as living mulch in durum wheat, it requires an appropriate spatial arrangement of the two species to maintain a high cereal grain yield and provide a better weed control. The best way for obtaining a similar cereal yield in both intercropped and pure wheat and for control the weeds more efficiently is to separate the two species by sowing the wheat in rows at a distance of 10 cm from the subclover rows.

When the legume was mixed with the cereal in the same row, causing maximum interference between wheat and subclover, the cereal exerted its highest level of aggressivity towards the legume yet showed a reduction of grain yield compared to the sole crop. An excessive separation between wheat and subclover, as occurred when the legume was sown in rows 17.5 cm apart from the wheat rows, determined a high weed infestation and a reduction of cereal grain yield, while nitrogen fertilization always determined an increase in durum wheat aggressivity and grain yield, which in turn caused a reduction of subclover aboveground biomass and seed production. Considering that the legume produced enough seeds to regenerate a cover crop in the autumn of the second year regardless the spatial arrangement, a moderate separation between subclover and wheat could be considered a suitable spatial arrangement in order to obtain an adequate wheat grain yield and ensure a satisfactory subclover reseeding. However, the identification of additional seeding techniques such as placing the living mulch in strips instead of rows, could provide a more efficient weed control which is still one of the main problems when using living mulch systems with small grain cereals in the Mediterranean areas.

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Table 1. Weather data (monthly average of the daily minimum and maximum temperatures, and monthly total amount of rainfall) over the 2-year study period (2011/2012 and 2012/2013) and long-term mean values for the experimental site (30-year mean values).

Month	Temperatures (°C) Mean max			Mean min			Rainfall (mm)		
	2011/2012	2012/2013	30-year	2011/2012	2012/2013	30-year	2011/2012	2012/2013	30-year
September	28.3	24.9	25.7	16.0	15.3	13.7	72.8	65.7	73.1
October	21.0	21.5	20.9	10.3	12.1	10.7	45.5	140.0	85.0
November	16.5	16.3	14.9	7.0	8.7	6.2	17.2	189.4	110.5
December	12.6	10.6	11.0	4.7	2.5	2.9	37.1	69.3	79.9
January	11.3	10.2	10.5	2.1	3.0	1.8	18.0	128.8	57.2
February	8.2	9.6	11.5	-0.2	1.4	1.6	30.3	55.9	52.2
March	18.0	13.1	14.6	5.8	5.3	3.8	4.1	91.9	57.4
April	17.5	18.8	17.6	7.5	8.7	6.4	56.6	50.7	65.7
May	21.9	19.9	22.6	9.8	10.2	10.2	53.6	81.0	60.5
June	29.5	26.3	26.9	15.3	13.3	13.4	6.7	29.4	42.7
July	31.7	32.6	30.5	17.9	17.4	16.4	4.2	34.2	32.3

Table 2. The main effect of year, planting pattern, nitrogen and weed management on the yield and yield characteristics of wheat. Values belonging to the same variable and treatment without common letters are statistically different according to LSD (0.05).

	Height (cm)		Yield (t ha^{-1} of DM)		Straw (t ha^{-1} of DM)		Fertile spike (n. m^{-2})		Kernel (n. spike^{-1})		TGW (g)		TW (kg hl^{-1})		Vitreousness (%)	
2011/2012	64.3	b	3.20	b	6.23	b	253	b	24.1	b	49.3	a	81.4	a	90.7	a
2012/2013	66.2	a	4.38	a	7.37	a	297	a	33.7	a	45.8	b	80.6	b	86.4	b
Wide rows intercropping	67.4	a	3.51	b	6.25	b	227	d	31.6	a	48.8	a	81.7	a	90.6	a
Narrow rows intercropping	64.6	ab	3.91	a	7.08	a	289	b	28.8	b	46.9	b	80.9	b	89.1	a
Same row intercropping	63.0	b	3.59	b	6.65	b	263	c	27.0	c	47.5	ab	80.9	b	89.0	a
Sole wheat	66.1	a	4.14	a	7.22	a	322	a	28.2	bc	47.0	b	80.3	b	85.6	b
0 kg N ha^{-1}	62.8	b	2.99	b	5.67	b	248	b	25.4	b	46.2	b	79.9	b	82.9	b
100 kg N ha^{-1}	67.7	a	4.59	a	7.93	a	302	a	32.4	a	48.9	a	82.1	a	94.3	a
Weed free	66.6	a	4.13	a	7.36	a	290	a	30.2	a	47.9	a	81.3	a	90.6	a
Weedy	63.9	b	3.45	b	6.24	b	260	b	27.6	b	47.3	a	80.6	b	86.6	b

Table 3. Aboveground biomass, heads, seeds of subclover at wheat physiological maturity, and number of regenerated seedlings of subclover in autumn after wheat harvesting for the spacing treatments and the fertilization levels. Values belonging to the same characteristic and treatment without common letters are statistically different according to LSD (0.05), in columns for planting pattern (lower case letters) and in rows for weed management and nitrogen fertilization (upper case letters).

	Aboveground biomass ($g\ m^{-2}$ of DM)				Heads ($n.\ m^{-2}$)				Seeds ($n.\ m^{-2}$)				Regeneration ($n.$ seedlings m^{-2})			
	0 kg N ha^{-1}		100 kg N ha^{-1}		0 kg N ha^{-1}		100 kg N ha^{-1}		0 kg N ha^{-1}		100 kg N ha^{-1}		0 kg N ha^{-1}		100 kg N ha^{-1}	
Wide row intercropping	234	bA	127	bB	408	bA	287	bB	1228	bA	819	bB	847	bA	541	bB
Narrow row intercropping	172	cA	110	bcB	338	bA	193	bcB	991	bA	612	bcB	689	cA	402	cB
Same row intercropping	86	dA	69	cA	200	cA	159	cA	585	cA	436	cA	355	dA	291	dA
Sole subclover	409	aA	411	aA	1185	aA	1280	aA	3222	aA	3382	aA	2103	aA	1988	aA
	Weed free		Weedy		Weed free		Weedy		Weed free		Weedy		Weed free		Weedy	
Wide row intercropping	220	bA	141	bB	348	bA	286	bA	1282	bA	766	bB	874	bA	514	bB
Narrow row intercropping	164	cA	118	bB	296	bcA	235	bA	933	cA	619	bA	631	cA	460	bB
Same row intercropping	92	dA	64	cA	223	cA	136	cB	643	dA	378	cB	393	dA	254	dB
Sole subclover	507	aA	313	aB	1468	aA	968	aB	3954	aA	2649	aB	2401	aA	1690	aB

Table 4. The effects of year, planting pattern, and nitrogen on SPAD, Dualex readings (Phen.), and the SPAD/Phen ratio of wheat flag leaf in the weed free plots. Values belonging to the same characteristic and treatment without common letters are statistically different according to LSD (0.05).

	SPAD readings		Phen.		SPAD/Phen.	
2011/2012	39.9	b	3.16	a	12.80	b
2012/2013	42.8	a	3.16	a	13.64	a
Wide row intercropping	44.3	a	3.21	a	13.98	a
Narrow row intercropping	42.5	b	3.14	a	13.65	a
Same row intercropping	39.2	c	3.14	a	12.63	b
Sole wheat	39.2	c	3.14	a	12.64	b
0 kg N ha^{-1}	35.2	b	3.31	a	10.67	b
100 kg N ha^{-1}	47.3	a	3.01	b	15.78	a

Table 5. Weed density and aboveground biomass for the planting pattern, the nitrogen fertilization level, and the year. Values belonging to the same characteristic and treatment without common letters are statistically different according to LDS (0.05), in columns for planting pattern and year (lower case letters) and rows for nitrogen management (upper case letters).

	Weed density (n. plants m^{-2})				Weed aboveground biomass (g m^{-2} of DM)			
	N_0		N_{100}		N_0		N_{100}	
Wide row intercropping	53.2	bA	53.4	bA	76.8	bA	98.8	cA
Narrow row intercropping	27.7	cA	16.2	dB	22.9	dA	39.1	eA
Same row intercropping	29.5	cA	30.9	cA	34.8	cdB	68.7	dA
Sole wheat	50.2	bA	31.0	cB	43.5	cB	119.8	bA
Sole subclover	103.4	aA	81.9	aB	113.8	aB	209.1	aA
2011/2012	61.3	aA	46.8	aB	63.2	aB	116.1	aA
2012/2013	44.3	bA	38.6	aA	53.5	aB	98.0	bA

Table 6. Relative biomass of subclover (RBs), relative biomass total (RBT), and wheat aggressivity (Ad) at wheat physiological maturity for the planting pattern and fertilization level treatments. Values belonging to the same characteristic and treatment without common letters are statistically different according to LSD (0.05), in columns for planting pattern (lower case letters) and in rows for nitrogen fertilization (upper case letters).

	Relative Biomass wheat (RBd)	Relative Biomass subclover (RBs)		Relative Biomass Total (RBT = RBd + RBs)		Aggressivity of wheat (Ad)	
		0 kg N ha^{-1}	100 kg N ha^{-1}	0 kg N ha^{-1}	100 kg N ha^{-1}	0 kg N ha^{-1}	100 kg N ha^{-1}
Narrow row intercropping	0.96 a	0.41 bA	0.25 bB	1.40 bA	1.19 aB	0.29 bB	0.34 aA
Same row intercropping	0.89 b	0.19 cA	0.16 cA	1.06 cA	1.01 bA	0.36 aA	0.35 aA
Wide row intercropping	0.85 c	0.61 aA	0.36 aB	1.51 aA	1.25 aB	0.14 cB	0.22 bA

Figure 1. Layout of wheat-subclover intercropping systems and monocultures. W = durum wheat; S = Subclover.

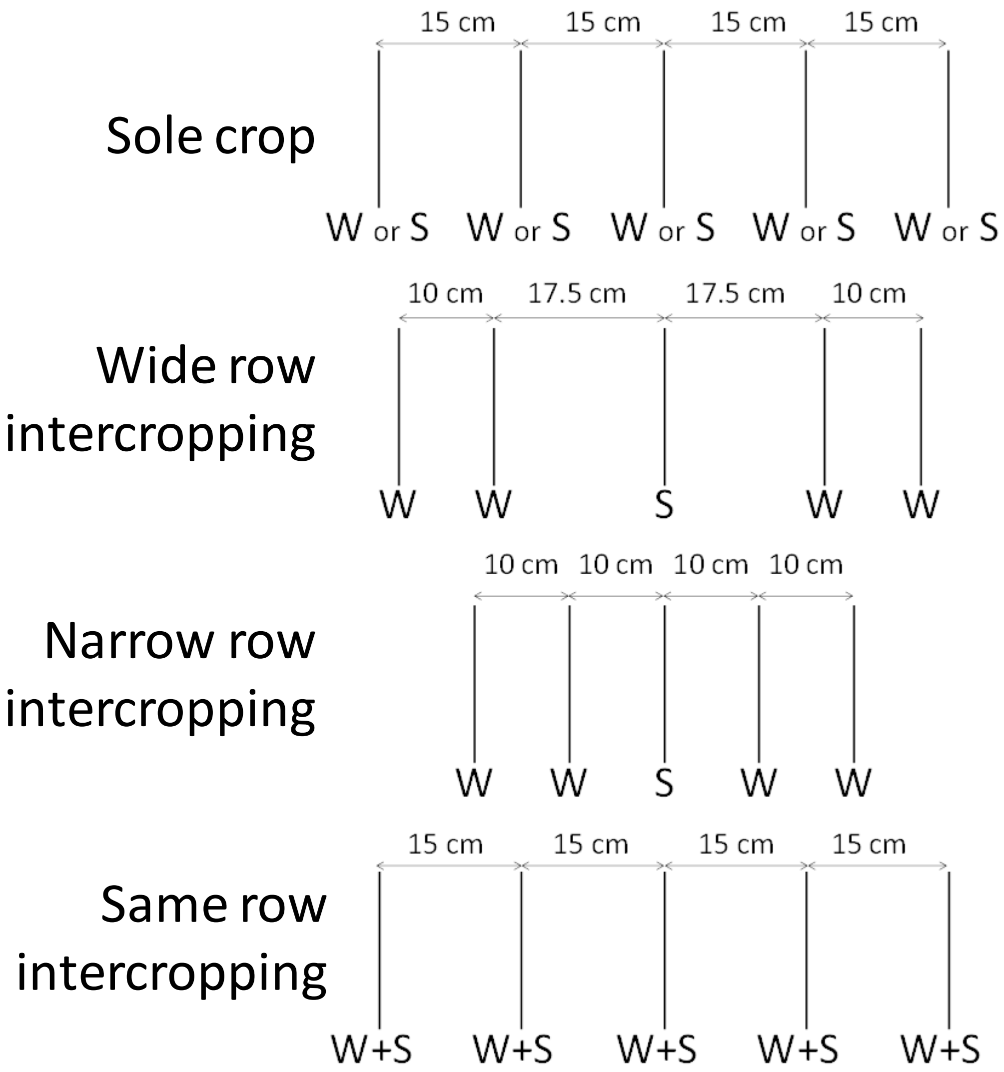

Figure 2. The effect of planting pattern on the dynamic changes in fraction of intercepted photosynthetic active radiation (PAR) in N0 (A) and N100 (B) nitrogen fertilization level throughout the 2012 growing season. Error bars represent ± standard error from mean (n. = 40).

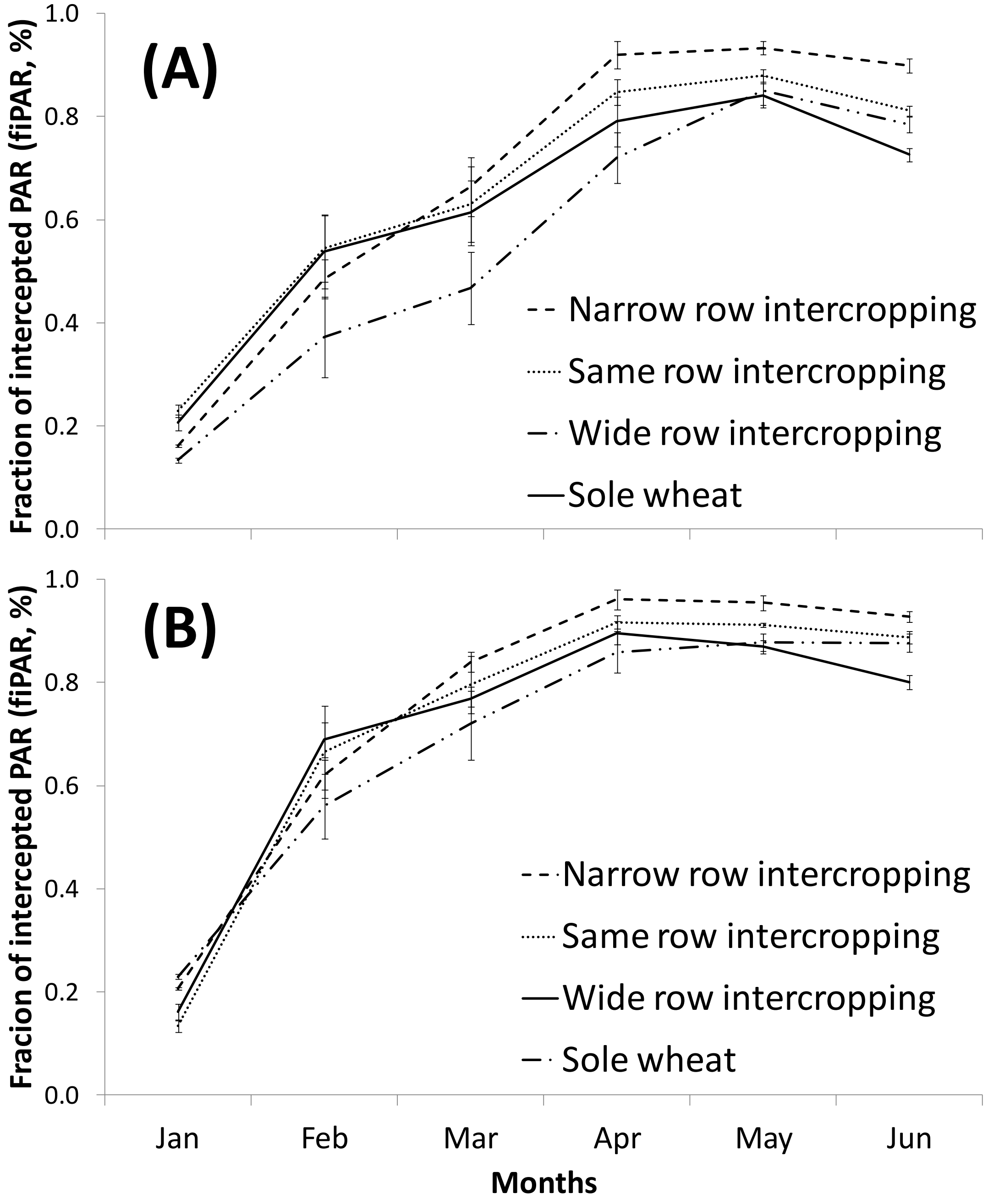

Figure 3. Relationships between the subclover characteristics (number of seeds, and number of seedlings) and the aggressivity index of durum wheat (Ad) in weed free conditions averaged over the cropping pattern and nitrogen fertilization treatments. Data correspond to the 2012 (A) and 2013 (B) growing seasons and the significance level is (***), (*) significant at P < 0.001 and P < 0.05 level, respectively.

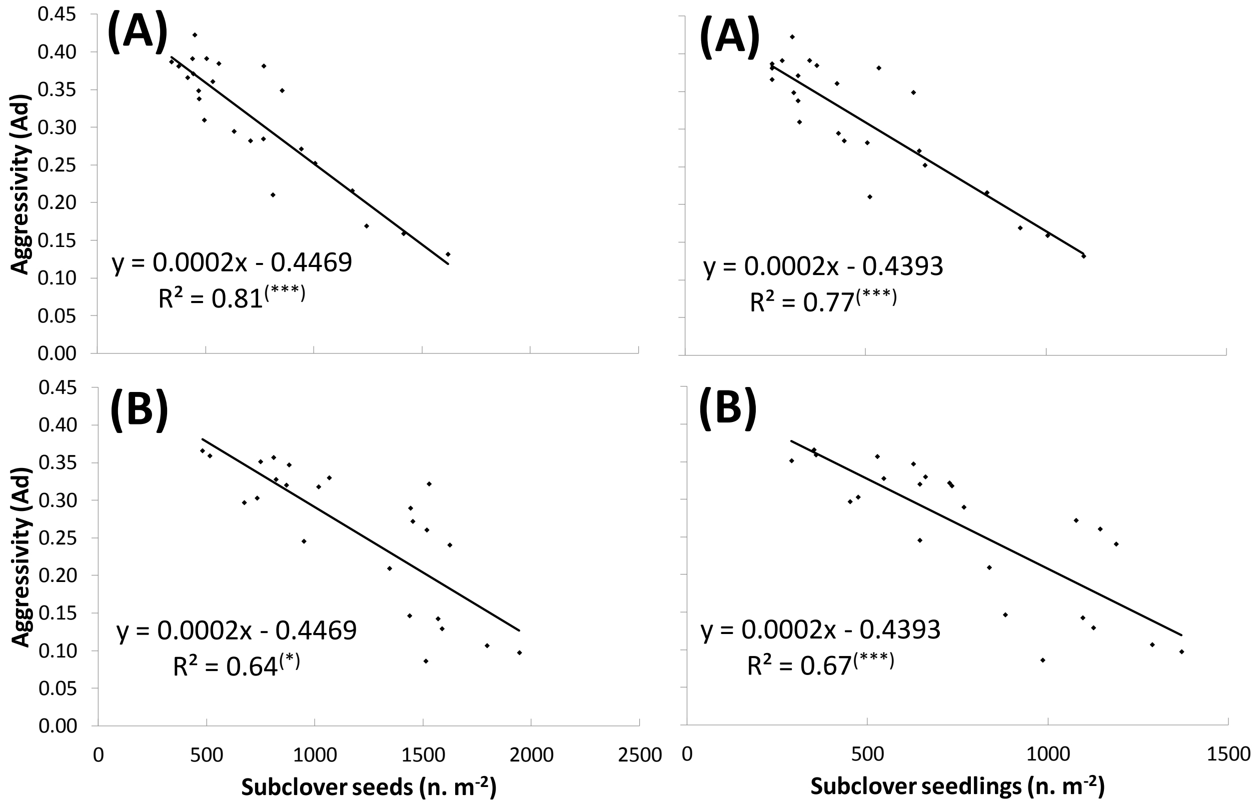

Figure 4. Competitive balance (Cb index) at wheat physiological maturity for the planting pattern and the nitrogen fertilization level treatments. Values belonging to the same characteristic followed by the same letter are not significantly different according to LSD (0.05).