WHEAT PERFORMANCE WITH SUBCLOVER LIVING MULCH IN DIFFERENT AGRO-ENVIRONMENTAL CONDITIONS DEPENDS ON CROP MANAGEMENT

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HIGHLIGHTS

- Grain yield of wheat was mostly reduced when intercropped with subclover
- Subclover as living mulch in wheat performed better in low input systems
- Wheat intercropped with subclover needs appropriate crop management

ABSTRACT:

Intercropping has been proposed as a useful strategy for reducing external inputs in cereal-based cropping systems, while maintaining adequate levels of crop yield. Intercropping of wheat and subclover (*Trifolium subterraneum* L.), implemented as living mulch, is recommended, but there is limited experimental proof for its suitability in different environments. The main objective of this study was to provide an overview and evaluation of wheat-subclover intercropping under different agro-environmental conditions. Coordinated field experiments were conducted over a two-year period in six sites located in four agro-environmental zones: Atlantic North (Neu-Eichenberg, Germany), Continental (Freising, Germany – Tänikon, Switzerland), Mediterranean North (Viterbo, Italy), Mediterranean South (Sidi Alla Tazi and Sidi El Aidi, Morocco). Wheat–subclover intercropping was compared with a pure wheat. Additionally, the other treatments adopted in specific sites were: soil tillage (conventional and minimum tillage); nitrogen fertilization (high and low level); compost application (with and without), cropping system (conventional and organic). The measurements recorded were: soil coverage, wheat and subclover phenological stages, wheat grain yield and yield components, subclover and weed biomass. The data of each site were analyzed separately and were also used for a meta-analysis in order to obtain an overview of how pedo-climatic conditions affect the interactions of subclover living mulch with wheat and weeds. Overall, wheat-subclover intercropping reduced weed infestation at most sites (from 22 to 75%). Intercropping also resulted in grain yield losses (from 1 to 18%) compared to pure wheat. In agro-environmental zones where subclover growth was limited by cold temperatures (Atlantic North) or dry conditions (Mediterranean South), hardly any grain yield reduction of intercropped wheat was observed. In contrast, intercropping significantly reduced wheat grain yield at the sites where subclover developed properly (Mediterranean North) probably because of the competition between the species. Subclover biomass and wheat grain yield were also negatively correlated and yield reductions were generally due to a reduced number of fertile spikes. The yield gap between intercropped and pure wheat was reduced when: (i) wheat seed density was similar in intercropped and pure wheat; (ii) there was a proper spatial arrangement of subclover and wheat; (iii) the amount of added mineral nitrogen fertilizer was reduced, while compost application did not influence the cropping systems. The use of subclover living mulch in wheat appears to be most suitable for low input systems. Future research should focus on the development of appropriate crop management practices for intercropping in order to avoid wheat yield loss.

KEY WORDS: Cereal – legume intercropping; Wheat grain yield; Weeds; Cropping system; Nitrogen fertilization.

INTRODUCTION

The simplification of cropping systems over the last decades has been accompanied worldwide by an increased use of chemical fertilizers, pesticides, and heavier mechanization. These practices have produced higher crop yields, yet they have simultaneously contributed to an increase of environmental issues, such as soil erosion and water contamination (Nazari et al., 2015). Consequently, it is necessary to design innovative cropping systems for greater nutrient use efficiency and resource conservation, which are more sustainable from an environmental point of view. Importantly, agro-ecosystems should be more diversified by increasing the number of species grown and using more leguminous crops (Bedoussac and Justes, 2011) or including cover crops (Wittwer et al., 2017). The adoption of intercropping could be a useful strategy for reducing external inputs while maintaining adequate levels of crop yield (Tilman et al., 2002).

Intercropping is defined as the co-cultivation of two or more species in the same space and for a significant part of their growing season, without necessarily being sown and harvested together. An established cover crop intercropped and grown simultaneously with an annual cash crop is known as living mulch (Hartwig and Ammon, 2002). The benefits of using living mulch as cropping systems include a reduction of water runoff and soil erosion, and the suppression of weed germination and weed establishment through competition for limited resources and/or the production of allelochemicals (Costanzo and Barberi, 2014). However, competition with the main crop for limited resources such as water, light and nutrients should be minimal (Hiltbrunner et al., 2007). This can be achieved when the intercropped species occupy different niches in time and space (Malézieux et al., 2009) using complementary resources (Bedoussac and Justes, 2010). Using a legume living mulch in an intercropped cereal system increases biodiversity and could reduce the need of external inputs such as nitrogen (N) fertilizers, herbicides and pesticides, thus improving the sustainability of the cropping system (Bedoussac and Justes, 2010).

Subclover (*Trifolium subterraneum* L.) is an annual legume with prostrate and non-rooting stems that adapts well to mild winters during which its vegetative growth and part of the reproductive phenophase occurs. Seeds buried naturally in the soil in the spring remain dormant until high summer temperatures drop and rainfall or irrigation occurs in the autumn. Since the plants die due to the increase in temperatures in late spring, it is not necessary to suppress subclover mechanically or chemically. Therefore, due to its particular life-cycle subclover seems to meet the requirements of a successful living mulch during the cereal crop cycle and an efficient dead mulch after wheat grain harvest during the summer period (Campiglia et al. 2014). Furthermore, its natural re-establishment ensures a new legume cover crop free of charge in the following period which can be used as cover crop or dead mulch for cultivating vegetable crops, such as tomato, pepper and eggplant under no-tillage conditions (Campiglia et al., 2014 and 2010). Therefore, using subclover as living mulch in wheat appears to be a suitable option for reducing the external inputs and costs of agricultural production. Moreover, living mulches are compatible with both organic and conservation agricultural systems (Canali et al., 2017). However, as yet few studies have evaluated the effects of subclover–wheat intercropping systems. Hence, it is necessary to determine if the wheat-subclover intercropping system works in selected agro-environmental conditions to provide benefits, before it can be recommended as a agricultural practice. We hypothesized that using subclover as living mulch in a wheat–subclover intercropping systems is feasible in several agro-environmental conditions and could be an environment-friendly management practice that has beneficial effects on the agro-ecosystem. The main objective of this study was to provide an overview and evaluation of subclover used as living mulch in wheat under a wide range of pedo-climatic conditions spanning from the Atlantic North to the Mediterranean South. A total of 14 coordinated two-year field trials in seven sites were evaluated. The specific aims of the study were: (i) to assess subclover living mulch performance; (ii) to evaluate the effects of subclover living mulch on yield and yield components of wheat; (iii) to determine the ability of wheat-subclover intercropping to suppress weeds.

MATERIALS AND METHODS

Site characteristics, crop management and experimental design

Wheat–subclover intercropping experiments were carried out over two growing seasons across a vast area (from 51°22’ to 33°07’ N and from 6°22’ to 12°04’ E) in seven sites located in four agro-environmental zones as defined by Jongman et al. (2006): Atlantic North (Neu-Eichenberg, Germany), Continental [Freising, Germany (2 sites) and Tänikon, Switzerland)], Mediterranean North (Viterbo, Italy), Mediterranean South (Sidi Alla Tazi and Sidi El Aidi, Morocco) (Fig. 1, Table 1). At each site, the experiment was performed twice, in 2012/13 and in 2013/14, resulting in a total of 14 site-year combinations. Wheat cultivars were selected to match the agro-environmental conditions of each site, while the subclover cultivar (Campeda) was the same in all sites. The cultivar Campeda belongs to subsp. *subterraneum* of subterranean clover native of Sardinia island in Mediterranean basin (Piano et al., 1997) and released as cultivars in Australia (Nichols et al., 2009). According to Nichols et al. (2013), Campeda cultivar shows a growth habit semi-erect and limited height. This cultivar is characterized of medium flowering class and a high level of residual hardseededness (about 30%), which contribute to its good persistence even in agro-environmental conditions featuring moderately severe spring-summer stress. Campeda forms very thick and dense swards, also owing to its outstanding seed yield. Agronomic practices and plant protection measures were carried out at appropriate times at the given sites following regional recommendations.

At Neu-Eichenberg, the field experiments were carried out at the organic experimental farm of Kassel University (hereafter called KU), in two adjacent fields previously cropped with grass-clover for two years. The soil in the experimental area is classified as *Haplic Luvisol* (Soil Survey Staff, 2009). Experimental factors were (i) tillage: either prior to winter wheat sowing inversion tillage with a moldboard plough at a depth of 25 cm, followed by seed bed preparation with disk harrow (hereafter called conventional tillage) or non-inversion tillage using a chisel plough at a depth of 10 cm, followed by seed bed preparation with a disk harrow in the first year, while in the second year the seed bed preparation was performed by direct drilling of wheat and subclover living mulch after undercutting the grass-clover pre-crop once (hereafter called minimum tillage); (ii) cropping system: pure winter wheat (*Triticum aestivum* L., cv. Achat) and wheat-subclover intercropping;

(iii) organic fertilization: application of yard waste compost at a rate of 5 t dry matter ha^{-1} (hereafter called WC+) and no application of yard waste compost (hereafter called WC-). The distance between the wheat rows was 30 cm in both pure and intercropped treatments. Wheat plant density was 350 seeds m^{-2} in both cropping systems, while subclover was sown at a density of 300 seeds m^{-2}. The weeds were left to grow undisturbed except in the first experimental year where they were controlled via hoeing and harrowing in spring. The experimental design was a randomized split-split-plot with four replicates, with soil tillage as main plot, cropping system as sub-plot and organic fertilization as sub-sub-plot. The sub-sub-plot size was 90 m^2 (6 m x 15 m).

At Freising, the field experiments were carried out on two experimental farms of the Technical University of Munich (hereafter called TUM) that are located 4 km apart: the experimental farm of Viehhausen, which was managed organically (hereafter called TUMorg) and the experimental farm of Dürnast, managed conventionally (hereafter called TUMconv). The preceeding crops were soybean and faba bean in the two experiments at TUMorg, and maize in the experiments carried out at TUMconv. The soil in both experimental areas is classified as *Cambisol* (Soil Survey Staff, 2009). The experimental factors at TUMconv were (i) cropping system: pure winter wheat (*Triticum aestivum* L., cv. Achat) and wheat-subclover intercropping; (ii) nitrogen fertilization: 100 kg N ha^{-1} (hereafter called N+) and 50 kg N ha^{-1} (hereafter called N-). The N+ corresponds to the farmer's normal practice, the N- fertilization level tested in this experiment represent a plausible rate that could be adopted under intercropping conditions. At TUMorg, the experimental factors were the same two cropping systems as in TUMconv with no fertilizer treatments. In both sites, the distance between the wheat rows was 12.5 cm in pure wheat, while the intercropping pattern was 2 rows of wheat with a distance of 12.5 cm and a 25 cm strip of subclover between the paired wheat rows. Wheat plant density was 450 and 300 seeds m^{-2} in pure wheat and intercropped wheat, respectively, while subclover was sown at a density of 300 seeds m^{-2}. The weeds were left to grow undisturbed throughout both wheat cropping seasons. At TUMconv, the experimental design was a randomized split-plot with four replicates, with the cropping system as main plot and the nitrogen fertilization level as the sub-plot, while at TUMorg, the experiment was monofactorial, with a simple randomized block design. In both sites the smallest plot size was 20 m^2 (10 m x 2 m).

At Tänikon, the field experiments were carried out at the experimental farm of Agroscope (hereafter called AGS) in two fields previously cropped with forage pea. The soil in the experimental area is classified as *Hapludalf* (Soil Survey Staff, 2009). The experimental factors were (i) cropping system: pure winter wheat (*Triticum aestivum* L., cv. CH Claro) and wheat-subclover intercropping; and (ii) nitrogen fertilization level: 140 kg N ha^{-1} (hereafter called N+) and 70 kg N ha^{-1} (hereafter called N-]. The N+ corresponds to the farmer's normal practice, the N- fertilization level tested in this experiment represent a plausible rate that could be adopted under intercropping conditions. The sowing patterns and densities were as at TUM described above. The weeds were controlled with herbicide in the pure wheat treatment in spring, whereas no weed control measures were performed in the intercropped treatment. The experimental design was a randomized split-plot with four replicates, with the cropping system as main plot and the nitrogen fertilization as the sub-plot. The smallest plot size was 48 m^2 (6 m x 8 m).

At Viterbo, the field experiments were carried out at the experimental farm of Tuscia University (hereafter called UNITUS) in two fields previously kept fallow. The soil in the experimental area is classified as *Typic Xerofluvent* (Soil Survey Staff, 2009). The experimental factors were (i) cropping system: pure winter wheat (*Triticum durum* Desf., cv. Colosseo) and wheat-subclover intercropping; and (ii) nitrogen fertilization level: 100 kg N ha^{-1} (hereafter called N+) and 50 kg N ha^{-1} (hereafter called N-). The N+ corresponds to the farmer's normal practice, the N- fertilization level tested in this experiment represent a plausible rate that could be adopted under intercropping conditions. The sowing patterns and densities were as at TUM described above. The weeds were left to grow undisturbed throughout both wheat cropping seasons. The experimental design was a randomized split-plot with four replicates, with the cropping system as main plot and the nitrogen fertilization level as the sub-plot. The smallest plot size was 48 m^2 (12 m x 4 m).

At Sidi Allal Tazi and Sidi El Aidi, the study was carried out at the experimental stations of the National Institute for Agricultural Research (INRA) of Morocco by INRA and ICARDA, respectively, with identical design. The soil in the experimental areas is classified as *Vertic Calcixeroll* (Soil Survey Staff, 2009). The experimental factors were (i) cropping system: pure wheat (*Triticum aestivum* L., cv. Kharouba) and wheat-subclover intercropping; and (ii) nitrogen fertilization level: 100 kg N ha^{-1} (hereafter called N+) and 50 kg N ha^{-1} (hereafter called N-). The N+ corresponds to the farmer's normal practice, the N- fertilization level tested in this experiment represent a plausible rate that could be adopted under intercropping conditions. The distance between wheat rows was 15 cm in the pure and intercropped system, and subclover was broadcasted in the intercropped system. In both systems, the seeding rate of bread wheat was 400 seeds m^{-2} and subclover was sown at the density of 300 seeds m^{-2}. The weeds were left to grow undisturbed throughout both wheat cropping seasons. At the ICARDA site, at the beginning of the second growing season, the field was irrigated in order to assure uniform seed germination under dry conditions. The experimental design was a randomized split-plot with four replicates, with the cropping system as main plot and the nitrogen fertilization level as the sub-plot. The smallest plot size was 31.5 m^2 (7 m x 4.5 m).

Measurements and analysis

For each site and in each year, the phenological stages of both wheat and subclover were recorded. In all field experiments, ground coverage of wheat, subclover and weeds grown in each plot was visually estimated at the beginning of wheat stem elongation (Brandsaeter and Netland, 1999). Subclover and weed aboveground biomass were collected at subclover flowering by hand-clipping the plants within a 1 m x 1 m quadrat placed randomly at the centre of each plot at the soil surface, while wheat was harvested at physiological maturity. The wheat plants were cut at ground level and plant height, number of fertile spikes, kernels per spike, thousand grain weight (TGW) were recorded. The wheat straw was separated from the wheat grains and both fractions as well as subclover and weed aboveground biomass were oven dried until constant weight in order to determine the dry matter content (DM). The subclover seedlings were measured in the autumn after harvesting the wheat in order to evaluate the reseeding capacity of subclover.

Statistical analysis

For each site, the data on wheat grain yield and yield components were analyzed with analyses of variance (ANOVA) using JMP statistical software package version 4.0 (SAS, 1996), considering the year as a repeated measure across time in all sites (Cody and Smith, 1997). At TUMconv, AGS, UNITUS, INRA and ICARDA a split-plot experimental design with four replicates was used for the wheat variables, where the cropping system was considered the main factor, the nitrogen fertilization as split factor and the year as repeated measure. At KU a split-split-plot experimental design with four replicates was adopted for analysing the wheat characteristics, where the soil tillage was considered as the main factor, the cropping system as split factor, the yard waste compost application as the split-split factor, and the year as repeated measure. At TUMorg a one factorial analysis with four replications was performed for evaluating wheat yield and yield components. Percentage data of soil cover were arcsine transformed before analysis in order to homogenize the variance (Gomez and Gomez, 1984). The data shown in the results were back-transformed. The effect means were compared with Fisher's protected LSD ($P < 0.05$).

Wheat grain yield, wheat yield components and weed aboveground biomass data of intercropped wheat and pure wheat treatments were extracted from the selected field experiments as response variables. The natural log of the response ratio (ln R) was used as a measure of effect size (Hedges et al., 1999): $\ln R = \ln (X_{IW}/X_{PW})$, where X_{IW} and X_{PW} are the measured values of the response variable under intercropped and pure wheat cropping systems, respectively. Meta-analysis was performed using a non-parametric weighting function. JMP software was used to calculate mean effect sizes and to generate bias-corrected 95% confidence intervals (CIs) for each mean effect size using a bootstrapping procedure (Huang et al., 2015). In order to ease interpretation, the effect size was expressed as the percentage change, which was estimated by (R – 1) x 100%. A negative percentage change indicates a decrease in the response variable under intercropped plots compared with pure plots, while a positive value indicates an increase. The mean percentage change was considered to be significantly different from zero if the 95% CI did not overlap with zero (Hedges et al., 1999). Linear regressions were performed for selected variables.

RESULTS

Development of wheat and subclover

In all sites, no significant differences were observed in the emergence of wheat between pure and intercropped treatments in the two experimental years (data not shown). However, wheat established one to three weeks earlier than subclover, even if both species were sown simultaneously (Fig. 2). At the beginning of wheat stem elongation, the cereal crop showed a higher soil coverage compared to subclover (on average 50 *vs*. 9 % of soil coverage, respectively, Table 2). However at this stage, total soil coverage in the wheat–subclover intercrops was higher than pure wheat in all sites [on average 57 (48 % wheat + 9 % subclover) *vs*. 49 %, respectively], with the exception of ICARDA (Table 2). At UNITUS, TUM and AGS, subclover soil coverage was much higher than that observed in the other sites, while wheat soil coverage intercropped with subclover was lower compared to the pure wheat crop (Table 2). Where it survived, subclover flowered after wheat in the northern sites, while it flowered before wheat in the Mediterranean North and Mediterranean South sites (Fig. 2). Seed ripening of subclover preceded the wheat in the southern agro-environmental zones (UNITUS, INRA and ICARDA) and was simultaneous with wheat in Switzerland (Fig. 2).

Subclover biomass production and its characteristics

At subclover flowering, the highest amount of subclover aboveground biomass was observed at UNITUS (on average over the years 229 g m^{-2} of DM, Fig. 3), while at AGS and TUM, although the subclover grew regularly in the early phenological stages, the amount of subclover aboveground biomass at flowering stage was low (64 and 53 g DM m^{-2}, respectively, Fig. 3). At INRA and ICARDA, the growth of the subclover living mulch was very poor (23 and 2 g DM m^{-2}, respectively, Fig. 3), while at KU no aboveground biomass was observed after winter. Consequently the subclover seed production was variable among the agro-environmental zones. It was insignificant at KU, scarce at AGS, TUM, ICARDA and INRA, while it was plentiful a

UNITUS where the subclover was able to regenerate abundantly in the following autumn (on average 506 subclover seedlings m^{-2}).

Wheat grain yield and yield components

There were few interactions among the main effects regarding the wheat grain yield and yield components, therefore the analysis was focused on the main effects and two-way interactions observed in the ANOVA (Table 3). Grain yields of pure wheat ranged from 5.5 t ha^{-1} at KU to 3.2 t ha^{-1} at ICARDA, with a mean of 4.4 t ha^{-1}, while in intercropped wheat it varied from 5.4 t ha^{-1} at KU to 3.2 t ha^{-1} at ICARDA, with an average of 4.1 t ha^{-1} (Fig. 4). At UNITUS, TUM and AGS, cereal grain yield was significantly reduced compared to pure wheat (on average -16, -10, and -18 %, respectively), while at KU, INRA and ICARDA there were no effects of the cropping system (Fig. 4). However, considering the agro-environmental zones, in Mediterranean North and Continental zones, the grain yield of intercropped wheat was significantly reduced compared to pure wheat (Fig. 5a). This decrease was generally due to fewer fertile spikes (Fig. 6), even if at UNITUS it was also due to the lower kernel number per spike (Fig. 4). There was a negative association between the aboveground biomass production of the subclover and the change in wheat grain yield (Fig. 7a).

Wheat grain yield was also significantly affected by N fertilization and experimental year at all sites except for ICARDA site, where fertilization was not significant (Table 3). Among the experimental sites, N fertilization proved to have positive effects on wheat grain yield and yield components, and it only interacted with year at INRA (Table 3), while no significant changes in cereal production were observed following the compost application at KU (Table 3). As expected, applying N fertilization to all conventional sites after wheat tillering (Fig. 2) favored the growth of the cereal both in pure and in intercropping systems. However, the effects were more consistent in pure wheat than in the intercropped wheat (Fig. 5b).

Effects of subclover living mulch on weeds

At wheat stem elongation, weed soil coverage in wheat pure crops was generally higher in organic compared with conventional cropping systems at wheat stem elongation (on average 16.4 *vs.* 11.2 % of soil coverage, Table 2). However, the weed soil coverage in the intercropped wheat was generally lower than in the pure wheat crop (on average 8 *vs.* 13 % of soil coverage, respectively). The results on change in weed biomass between the intercropped and pure wheat at subclover flowering varied significantly among the various agro-environmental zones (Fig. 8a). In the Mediterranean North, the subclover strongly reduced the weed aboveground biomass by approximately 53% in intercropped wheat compared to pure wheat where no weed control was performed (data not shown). There was a significant negative relationship between the change in weed aboveground biomass comparing intercropped to pure wheat treatments and the subclover aboveground biomass (Fig. 7b). Moreover, a great reduction of weed aboveground biomass was achieved with low N input (Fig. 8b), as subclover biomass was negatively affected by higher N inputs (Fig 3).

DISCUSSION

Development of wheat and subclover

In all agro-environmental zones the intercropped wheat emerged before subclover and its early growth was faster than the living mulch (data not shown). The slow initial development of the legume compared to the cereal could be partly due to the establishment of costly nodulation in terms of energy and nutrient requirements (Leung and Bonomley, 1994). Although subclover emerged regularly after sowing in autumn in all sites, it did not survive the winter season in the Atlantic North (KU), therefore no seeds and little biomass were produced. In Atlantic North conditions, winter survival of subclover is severely threatened due to the low winter temperatures (Brandsæter et al., 2002). However, in cold environments like Atlantic North, the poor winter survival of subclover could be improved by anticipating the sowing time of the legume and sowing the wheat into the clover via strip-tillage. In fact, according to the findings of Brandsaeter et al. (2008), early sowing could positively affect the winter survival rates of subclover. Moreover, subclover genotypes more cold-tolerant than the cv. Campeda, which was adopted in this study, should be evaluated, even if there is little information about cold tolerance of different subclover cultivars (Teixeira et al., 2015; OSCAR, 2016). At the beginning of wheat stem elongation, the cereal was much more efficient than subclover in terms of light absorption, due to its earlier growth and greater leaf development throughout the winter period when temperatures were probably too low for subclover development. However, total soil coverage in the wheat-subclover intercrops was always higher than pure wheat in all sites. The higher total soil coverage in the intercropped crops as a whole (wheat + subclover) compared to pure wheat is desirable as it reduces the risk of soil erosion (Lithourgidis, 2011), it can reduce weed cover (Thorsted et al., 2006), and it increases light interception and therefore biomass accumulation (Agegnehu et al., 2008). In particularly, at UNITUS, TUM and AGS, soil coverage of the subclover was much higher than that observed in the other sites, while wheat soil coverage when intercropped with subclover was lower compared to the pure wheat crop (Table 2). At these sites, wheat density was reduced by 1/3 in the intercropping treatments. The subclover sowing arrangement was also very close to the wheat plants and this could have led to increased competition between both species starting from early growth stages (Thorsted et al., 2006).

Subclover biomass production and its characteristics

The subclover biomass production was very variable depending on different agro-environmental zones. At subclover flowering, the highest amount of subclover aboveground biomass was observed at UNITUS proving that this legume adapts well to the climatic conditions of the Mediterranean North zone. Under these conditions, the subclover was able to produce a high amount of aboveground biomass and mature seeds, which regenerated abundantly in the following autumn. In the Continental zone (AGS and TUM) the legume grew regularly in the early phenological stages, but at flowering the amount of aboveground biomass production was low (Fig. 3) probably due to the cold temperatures at these sites during winter when air temperatures dropped below 0 °C several times in January and February (Fig 1). Consequently, at TUM and AGS subclover reseeding was very poor the following autumn (less than 20 seedlings m^{-2}) suggesting that few subclover plants survived after winter and were able to produce mature seeds. However, in these sites the subclover provided more soil coverage than the omitted wheat row did in the controls and apparently competed with the wheat. In fact, the wheat grain yield was significantly lower in intercropped wheat than in pure stands at AGS, TUM and especially at UNITUS. In these sites, competition and lower wheat seeding rates (-33% compared to pure wheat) probably contributed to this outcome. The sowing density of intercropped species was achieved through a substitution series (Kelty and Cameron, 1995) and the wheat seed density was probably too low to ensure a satisfactory density of plants and consequently of fertile spikes. In fact, the decrease in grain yield, in intercropped wheat compared to the pure wheat, was mainly due to fewer fertile spikes per surface area, which proved to be the grain yield component most affected by changes in stand density in wheat according to Blaser et al. (2006). Furthermore, the low plant density of the wheat probably reduced its competitive ability, especially at UNITUS which showed the highest wheat yield gap in intercropped wheat compared to pure wheat, as well as the highest aboveground biomass of subclover (Fig. 3). By adopting an additive design, same wheat seed density used in both pure and intercropped wheat (Kelty and Cameron, 1995), as in the case at the INRA and ICARDA sites, it may be possible to reduce or eliminate the grain yield gap in intercropped wheat compared to pure wheat. This would ensure a reliable wheat grain yield under pedo-climatic conditions where the survival and establishment of subclover is uncertain, such as those observed at KU and ICARDA. In general, increasing wheat seed density improves the competitive ability of the cereal, as well as the number of wheat plants and fertile spikes (Weiner et al. 2010). Therefore, it is reasonable to assume that similar effects could be obtained when the wheat is cultivated with clover living mulch (Hiltbrunner et al., 2007b). This was confirmed for the TUM site and to some degree at UNITUS by several experiments, where maintaining narrow rows of wheat usually resulted in the best grain yields when intercropped with subclover (OSCAR, 2016).

Wheat grain yield and yield components

As expected, there were no effects of the cropping system at KU as (i) the wheat seed rate was similar in both cropping systems, (ii) the amount of available N was probably too low to meet wheat requirements, (iii) the subclover failed, which meant that there was no competition between the intercropped species. Likewise no yield differences were observed at INRA and ICARDA where the same seeding rates had been applied in both systems and the clover performed poorly. Therefore, it seems that appropriate densities must be determined in accordance with local climatic conditions in order to ensure adequate growth of both intercropped species.

Generally, nitrogen fertilization had e positive effects on wheat grain yield and yield components, even if the effects was more consistent in pure wheat than in the intercropped wheat. In fact, the potential of intercropping as a means of increasing the contribution of nitrogen obtained from biological fixation seems to decrease with increases in the nitrogen fertilization level (Andersen et al., 2005).This is supported by the fact that the subclover tended to be negatively affected by the application of high mineral nitrogen doses (Fig. 3) and produced few seeds (data not shown), thus decreasing its potential to deliver desired ecosystem services. Similar results were obtained in previous studies, which investigated the effect of inorganic nitrogen availability on the behavior of various winter cereal–legume intercropping systems (Gaudin et al., 2014). Thus, low input systems seem to benefit more when subclover is used as living mulch in wheat in terms of subclover biomass accumulation (Fig. 3). Moreover, Wittwer et al. (2017) showed that the benefits from cover crops, as ecological management practice, are best acknowledged when management intensity is reduced. Unlike the nitrogen fertilization, no significant changes in wheat grain yield were observed following the compost application at KU (Table 3). This could be due to the low availability of mineral nitrogen considering that the yard waste compost applied to the wheat was 90 and 75 kg ha^{-1} of N with C:N ratios of 16 and 26, in 2012 and 2013, respectively. Probably only a part of these amounts was available during the wheat cultivation period due to the slow release of mineral nitrogen from yard waste compost, especially in cold climates like that of the Atlantic North (Laber, 2002).

Effects of subclover living mulch on weeds

The presence of subclover always reduced weed infestation in intercropped wheat compared to wheat pure crop, even if the weed soil coverage in wheat pure crops was higher in organic compared with conventional cropping systems. This is in agreement with several studies that reported a higher presence of weeds in organic than in conventional cropping systems (Campiglia et al., 2015; Halde et al., 2015). However, the reduction of weed soil coverage observed at all sites indicates that subclover can contribute to weed management in intercropping systems, although Campiglia et al. (2014) found that subclover was not a good competitor against weeds due to its low growth rate. Nevertheless, the results on change in weed biomass between the intercropped and pure wheat varied significantly among the various agro-environmental zones (Fig. 8a), thus reflecting site-specific subclover performances. The potential suppressive effect on weeds was particularly evident and could be demonstrated in the Mediterranean North, where subclover reduced weed aboveground biomass by approximately 53% in intercropped wheat compared to pure wheat where no weed control was performed (data not shown). In this environment, the subclover could establish and grow properly and produced a high aboveground biomass. Contrastingly, although weed cover was reduced at wheat stem elongation, weed biomass was much higher in the intercropped treatments at AGS where herbicides were applied to control the weeds in the pure wheat. Thus, weed competition by subclover was insufficient and may not be enough to fully replace herbicide applications.

However, some weed reduction was even observed in environments where there was a shortage of subclover at flowering with a significant effect at INRA (Table 2). Perhaps in these agro-environments, the subclover had a negative effect on weed establishment in autumn and after it withered, the aboveground biomass, which remained on the soil surface, may have acted as organic dead mulch. Under these conditions, the cereal-legume association may fill more than one ecological niche and may therefore contribute to suppressing a greater number of weed species with different ecological requirements (Anil et al., 1998). This can be considered an important aspect associated with using subclover as living mulch in wheat in environments where the subclover can only survive for part of the cropping cycle and therefore cannot self-reseed. Indeed, it is evident that low nitrogen input reduces weed biomass better than high nitrogen input, as subclover biomass is increased and less nutrient are available for weeds to grow (Blackshaw, 2004). As already highlighted for wheat grain yield production and subclover biomass accumulation, intercropping wheat and subclover seems to be a suitable strategy in low input systems for controlling weeds as well.

CONCLUSION

Overall, the research presented in this manuscript based on field experiments in different agro-environmental conditions suggests that subclover used as living mulch in wheat can have positive effects on wheat based cropping system performance. However, although the presence of subclover generally reduced weed infestation, wheat was grown at a lower density than in the pure stand when intercropped with subclover and yielded on average 6 % less than pure wheat, even if there were significant differences depending on the environment and crop management system. It seems that reducing the sowing density of wheat may not be the best option when it is intercropped with subclover. In most experiments, the grain yield reduction was determined by a low number of fertile spikes and occasionally by a reduction of the thousand grain weight. However, it was evident that there was a negative relationship between subclover development and grain yield production in intercropped wheat. In fact, when the subclover living mulch grew in favorable climatic conditions, as in Mediterranean North (UNITUS), it caused the highest grain yield losses of up to 18% compared to pure wheat. However, using appropriate management practices can reduce or eliminate the yield gap between intercropped and pure wheat. In particular, when subclover is used as living mulch in wheat our findings suggest that it is preferable: (i) to adopt similar seed density in intercropped and in pure wheat to avoid a reduction of fertile spikes; (ii) to find an appropriate spatial arrangement of the legume and cereal to reduce possible competitive effects between the two species; (iii) to reduce the amount of nitrogen fertilizer administered. Therefore, using subclover as living mulch in wheat is a feasible practice that is principally suitable for low input cropping systems, although appropriate crop management practices should be developed in accordance with local environmental conditions in order to avoid wheat yield loss.

ACKNOWLEDGMENT

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REFERENCES

Agegnehu, G., Ghizaw, A., Sinebo, W., 2008. Yield potential and land-use efficiency of wheat and faba bean mixed intercropping. Agron. Sustain. Dev. 28, 257–263.

Andersen, M.K., Hauggaard-Nielsen, H., Ambus, P., Jensen, E.S., 2005. Biomass production, symbiotic nitrogen fixation and inorganic N use in dual and tri-component annual intercrops. Plant Soil 266, 273–287.

Anil, L., Park, J., Phipps, R.H., Miller, F.A., 1998. Temperate intercropping of cereals for forage: a review of the potential for growth and utilization with particular reference to the UK. Grass Forage Sci. 53, 301–317.

Bedoussac, L., Justes, E., 2010. Dynamic analysis of competition and complementarity for light and N use to understand the yield and the protein content of a durum wheat-winter pea intercrop.

Plant Soil 330, 37–54.

Bedoussac, L., Justes, E., 2011. A comparison of commonly used indices for evaluating species interactions and intercrop efficiency: Application to durum wheat–winter pea intercrops. Field Crop Res. 124, 25–36.

Blackshaw, R.E., 2004. Application method of nitrogen fertilizer affects weed growth and competition with winter wheat. Weed Biol. Manag. 4, 103–113.

Blaser, B.C., Gibson, L.R., Singer, J.W., Jannink, J.-L., 2006. Optimizing Seeding Rates for Winter Cereal Grains and Frost-Seeded Red Clover Intercrops. Agron. J. 98, 1041-1049.

Brandsæter, L.O., Heggen, H., Riley, H., Stubhaug, E., Henriksen, T.M., 2008. Winter survival, biomass accumulation and N mineralization of winter annual and biennial legumes sown at various times of year in Northern Temperate Regions. Eur. J. Agron. 28, 437–448.

Brandsaeter, L.O., Netland, J., 1999. Winter annual legumes for use as cover crops in row crops in Northern regions: I. Field experiment. Crop Sci. 39, 1369–1379.

Brandsæter, L.O., Olsmo, A., Tronsmo, A.M., Fykse, H., 2002. Freezing resistance of winter annual and biennial legumes at different developmental stages. Crop Sci. 42, 437–443.

Campiglia, E., Mancinelli, R., De Stefanis, E., Pucciarmati, S., Radicetti, E., 2015. The long-term effects of conventional and organic cropping systems, tillage managements and weather conditions on yield and grain quality of durum wheat (*Triticum durum* Desf.) in the Mediterranean environment of Central Italy. Field Crop Res. 176, 34–44.

Campiglia, E., Mancinelli, R., Radicetti, E., Baresel, J.P., 2014. Evaluating spatial arrangement for durum wheat (*Triticum durum* Desf.) and subclover (*Trifolium subterraneum* L.) intercropping systems. Field Crop Res. 169, 49–57.

Campiglia, E., Mancinelli, R., Radicetti, E., Caporali, F., 2010. Effect of cover crops and mulches on weed control and nitrogen fertilization in tomato (*Lycopersicon esculentum* Mill.). Crop Prot. 29, 354–363.

Canali, S., Ortolani, L., Campanelli, G., Robacer, M., von Fragstein, P., D'Oppido, D., Kristensten, H.L., 2016. Yield, product quality and energy use in organic vegetable living mulch cropping systems: researchevidence and farmers' perception. Ren. Agric. Food Syst., In Press.

Costanzo, A., Barberi, P., 2014. Functional agrobiodiversity and agroecosystem services in sustainable wheat production. A review. Agronomy for Sustainable Development, 34, 1 – 20.

Gaudin, A.C.M., Janovicek, K., Martin, R.C., Deen, W., 2014. Approaches to optimizing nitrogen fertilization in a winter wheat–red clover (*Trifolium pratense* L.) relay cropping system. Field Crop Res. 155, 192–201.

Gomez, K.A., Gomez, A.A., 1984. Statistical procedures for Agricultural Research, second ed. John Wiley & Sons, New York, USA.

Halde, C., Bamford, K.C., Entz, M.H., 2015. Crop agronomic performance under a six-year continuous organic no-till system and other tilled and conventionally-managed systems in the northern Great Plains of Canada. Agric. Ecosyst. Environ. 213, 121–130.

Hartwig, N.L., Ammon, H.U., 2002. Cover crops and living mulches. Weed Sci. 50, 688-699.

Hedges, L.V., Gurevitch, J., Curtis, P.S., 1999. The meta-analysis of response ratios in experimental ecology. Ecology 80, 1150–1156

Hiltbrunner, J., Liedgens, M., Bloch, L., Stamp, P., Streit, B., 2007b. Legume cover crops as living mulches for winter wheat: Components of biomass and the control of weeds. Eur. J. Agron. 26, 21–29.

Hiltbrunner, J., Streit, B., Liedgens, M., 2007a. Are seeding densities an opportunity to increase grain yield of winter wheat in a living mulch of white clover? Field Crop Res. 102, 163–171.

Huang, M., Zhou, X., Cao, F., Xia, B., Zou, Y., 2015. No-tillage effect on rice yield in China: A meta-analysis. Field Crop Res. 183, 126–137.

Jongman, R.H.G., Bunce, R.G.H., Metzger, M.J., Mücher, C.A., Howard, D.C., & Mateus, V.L. (2006). Objectives and applications of a statistical environmental stratification of Europe. Landscape Ecol. 21, 409–419.

Kelty, M.J., Cameron, I.R., 1995. Plot design for the analysis of species interactions in mixed stands. Common Forest. Rev. 74, 322–332.

Laber, H., 2002. Kalkulation der N-Düngung im ökologischen Gemüsebau. Sächsische Landesanstalt für Landwirtschaft. Available at: http://orgprints.org/865/ [Accessed August 30, 2016].

Leung, K., Bonomley, P.J., 1994. Growth and nodulation characteristics of subclover (*Trifolium subterraneum* L.) and Rhizobium leguminosarum BV. Trifolii at different soil water potentials. Soil Biol. Biochem. 26, 805–812.

Lithourgidis, A., Dordas, C.A., Damalas, C.A., Vlachostergios, D.N., 2011. Annual intercrops: an alternative pathway for sustainable agriculture. Aust. J. Crop Sci. 5, 396–410.

Malézieux, E., Crozat, Y., Dupraz, C., Laurans, M., Makowski, D., Ozier-Lafontaine, H., Rapidel, B., de Tourdonnet, S., Valantin-Morison, M., 2009. Mixing plant species in cropping systems: concepts, tools and models. A review. Agron. Sustain. Dev. 29, 43–62.

Nazari, S., Rad, G.P., Sedighi, H., Azadi, H., 2015. Vulnerability of wheat farmers: Toward a conceptual framework. Ecol. Indic. 52, 517–532.

Nichols, P.G.H., Cocks, P.S., Francis, C.M., 2009. Evolution over 16 years in a bulk-hybrid population of subterranean clover (*Trifolium subterraneum* L.) at two contrasting sites in south-western Australia. Euphytica 169, 31-48.

Nichols, P.G.H., Foster, K.J., Piano, E., Pecetti, L., Kaur, P., Ghamkhar, K., Collins, W.J., 2013. Genetic improvement of subterranean clover (*Trifolium subterraneum* L.). 1. Germoplasm, traits and future prospects. Crop & Pasture Sci. 64, 312-346.

OSCAR. 2016. Optimising Subsidiary Crop Applications in Rotations, Final report. 39 p. available at: http://cordis.europa.eu/docs/results/289/289277/final1-final-report-complete.pdf

Piano, E., Pecetti, L., Carroni, A.M., 1997. Campeda, Limbara, Losa and Antas: the first italian cultivar of subterranean clover (In Italian). Sementi elette, 43: 27-32.

SAS, 1996. SAS User's Guide: Statistics. SAS Institute, Cary, NC.

Soil Survey Staff, 2009. Soil Survey Geographic (SSURGO) Database for [U.S.] [WWW Document]. United States Dep. Agric. accessed [05/13/2009]. URL http://soildatamart.nrcs.usda.gov.

Teixeira, C.S.P., Lucas, D., Moot, D.J., 2015. Optimization of subterranean clover for dryland pastures in New Zealand. Lincoln Universiy, project n. 408090, p. 63. http://www.lincoln.ac.nz/pagefiles/26159/2015-11-30_sub_clover_review_reduced.pdf (accessed 21.12.2016).

Thorsted, M.D., Olesen, J.E., Weiner, J., 2006. Width of clover strips and wheat rows influence grain yield in winter wheat/white clover intercropping. F. Crop. Res. 95, 280–290.

Tilman, D., Cassman, K.G.K., Matson, P.A., Naylor, R., Polasky, S., 2002. Agricultural sustainability and intensive production practices. Nature 418, 671–677.

Weiner, J., Andersen, S.B., Wille, W.K.-M., Griepentrog, H.W., Olsen, J.M., 2010. Evolutionary Agroecology: the potential for cooperative, high density, weed-suppressing cereals. Evol. Appl. 3, 473–479.

Wittwer, R.A., Dorn, B., Jossi, W., van der Heijden, M.G.A., 2017. Cover crops support ecological intensification of arable cropping systems. Scientific Reports (in press).

Tables and figures

Table 1. Site details for the joint field trials carried out during 2012 – 2014 in Germany, Swiss, Italy, and Morocco representing Central north European temperate, Central south European temperate, Mediterranean Europe, Mediterranean Africa. AI = Aridity Index (De Martonne, 1926).

Agro-environmental zone	Atlantic North	----------------- Continental ----------------			Med. North	--------- Med.South -------	
Country	Germany	Germany		Switserland	Italy	Morocco	
Site	Neu-Eichenberg	------------ Freising---------------		Tänikon	Viterbo	Sidi Alla Tazi	Sidi El Aidi
Acronym	KU	TUMconv	TUMorg	AGS	UNITUS	INRA	ICARDA
Location	51°22’ N 9°54’ E 237 m a.s.l.	48°23’ N 11°41’ E 640 m a.s.l.	48°58’ N 11°57’ E 445 m a.s.l.	47°29’ N 8°54’ E 537 m a.s.l.	42°25’ N 12°04’ E 310 m a.s.l.	33°31’ N 6°22’ E 11 m a.s.l.	33°07’ N 7°37’ E 240 m a.s.l.
Clay (< 2 µm, g kg^{-1} of dry soil)	133	665	630	210	190	208	510
Silt (2-60 µm, g kg^{-1} of dry soil)	834	205	212	350	215	360	280
Sand (60-2000 µm, g kg^{-1} of dry soil)	33	130	158	440	595	146	210
Soil pH	6.2	6.5	6.5	7.3	6.7	7.11	8.3
Soil organic matter (mg C kg^{-1})	2.0	13.7	16.8	20.8	13.1	15.5	15.6
Soil nitrogen (mg N kg^{-1})	1.7	1.5	1.5	1.8	1.7	1.2	1.2
Average annual Temperature (°C)	8.9	7.5	7.8	8.7	14.1	20.0	19.2
Annual rainfall (mm)	698	800	786	1184	760	260	180
AI and classification	36.9 Wet	45.7 Wet	44.2 Wet	63.3 Very wet	31.5 Mildly wet	8.7 Semi dry	6.2 Semi dry

Table 2. Soil coverage of wheat in both pure and intercropped stands, subclover in the intercropped stands and weeds, in both pure and intercropped stands, measured at the beginning of wheat stem elongation. Values belonging to the same variable followed by the same letter in rows for cropping systems (upper case letter) and in columns for site (lower case letter) are not significantly different (LSD P<0.05).

	Soil coverage component (%) at stem elongation of wheat				
	Wheat		Subclover	Weeds	
Site	Pure	Intercropped		Pure	Intercropped
KU	48.6 cA	47.8 bcA	3.2 c	16.1 aA	12.9 aA
AGS	53.8 bA	50.2 abB	14.0 b	11.4 bcA	6.4 bB
TUMorg	49.1 cA	42.6 cB	9.0 bc	16.6 aA	10.5 aB
TUMconv	56.2 aA	53.3 aB	9.1 bc	10.6 bcA	5.8 bcB
UNITUS	58.4 aA	49.5 bB	20.2 a	9.7 cA	2.4 cB
INRA	50.4 bcA	49.2 bA	5.1 c	13.1 bA	6.7 bB
ICARDA	47.7 cA	45.8 cA	0.6 c	13.3 bA	9.9 aA

Table 3. F-value of analysis of variance of wheat yield and yield components at the different sites. Straw (g DM m^{-2}), grain yield (g DM m^{-2}), fertile spikes m^{-2}, kernels spike^{-1} (*, **, ***, significant at $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$, respectively). *D.f.* = Degree of freedom.

	d.f.	Straw	Yield	Spike	Kernel	TGW
TUMconv						
Cropping System (CS)	1	0.2136***	0.0466***	0.0003***	0.0012***	0.9492***
Fertilization (F)	1	0.0052***	0.0031***	0.6140***	0.8014***	0.8823***
CS x F	1	0.1737***	0.0070***	0.3376***	0.3496***	0.7717***
Year (Y)	1	0.0028***	0.0002***	0.3101***	0.5274***	0.9272***
No significant interactions						
TUMorg						
Cropping System (CS)	1	0.9398***	0.0435***	0.0205***	0.0481***	0.9543***
Year (Y)	1	0.0214***	0.0068***	0.3708***	0.0496***	0.0490***
CS x Y	1	0.3271***	0.6261***	0.6073***	0.7639***	0.9434***
KU						
Cropping System (CS)	1	0.4439***	0.7484***	0.8206***	0.6222***	0.8360***
Soil tillage (ST)	2	0.0012***	0.0001***	0.0001***	0.6245***	0.0010***
CS x ST	2	0.9586***	0.4974***	0.8627***	0.9867***	0.9620***
Compost Fert. (F)	1	0.0973***	0.0852***	0.1827***	0.4729**	0.1426***
CS x F	1	0.5084***	0.8431***	0.6160***	0.4599**	0.5346***
ST x F	2	0.0491***	0.2843***	0.3968***	0.8474***	0.0664***
CS x ST x F	2	0.1295***	0.7729***	0.4103***	0.6959***	0.1983***
Year (Y)	1	0.0475***	0.0308***	0.0064***	0.0047***	0.0001***
Y x CS	1	0.0010***	0.8108***	0.4316***	0.5843***	0.5956***
Y x ST	2	0.3912***	0.0060***	0.3676***	0.2243***	0.0001***
AGS						
Cropping System (CS)	1	--	0.0001***	0.0013***	0.1552***	0.4797***
Fertilization (F)	1	--	0.0001***	0.0010***	0.0007***	0.0333***
CS x F	1	--	0.3163***	0.8054***	0.8140***	0.5490***
Year (Y)	1	--	0.0012***	0.0001***	0.0001***	0.0057***
CS x Y	1	--	0.8598***	0.0084***	0.0002***	0.1171***
F x Y	1	--	0.3686***	0.0831***	0.5945***	0.0009***
UNITUS						
Cropping System (CS)	1	0.0033***	0.0098***	0.9982***	0.0291***	0.1266***
Fertilization (F)	1	0.8243***	0.0457***	0.7513***	0.1144***	0.5813***
CS x F	1	0.8534***	0.2431***	0.7073***	0.0567***	0.4987***
Year (Y)	1	0.0002***	0.0001***	0.0008***	0.0494***	0.0001***
CS x Y	1	0.0080***	0.0028***	0.1579***	0.9922***	0.0100***
F x Y	1	0.8760***	0.0980***	0.8432***	0.7952***	0.2047***
INRA						
Cropping System (CS)	1	0.3834***	0.7685***	0.8806***	0.4328***	0.2576***
Fertilization (F)	1	0.2131***	0.0023***	0.0063***	0.0493***	0.9102***
CS x F	1	0.0829***	0.5019***	0.2616***	0.1770***	0.2345***
Year (Y)	1	0.0001***	0.0001***	0.0001***	0.0278***	0.0424***
CS x Y	1	0.6536***	0.5832***	0.2364***	0.1295***	0.2720***
F x Y	1	0.1529***	0.0315***	0.0465***	0.0307***	0.0192***
ICARDA						
Cropping System (CS)	1	0.3659***	0.9869***	0.8191***	0.4858***	0.3020***
Fertilization (F)	1	0.4859***	0.9443***	0.0154***	0.0452***	0.9061***
CS x F	1	0.0747***	0.0240***	0.0075***	0.3725***	0.6483***
Year (Y)	1	0.0001***	0.0170***	0.0843***	0.0467***	0.1206***
No significant interactions						

Figure 1. Weather conditions (monthly average of the daily temperatures and monthly total amount of rainfall) during the field experiments in 2012/2013 and 2013/2014 experimental years at the sites described in Table 1. Only one climate chart provided for the two sites at TUM as they were located near each other.

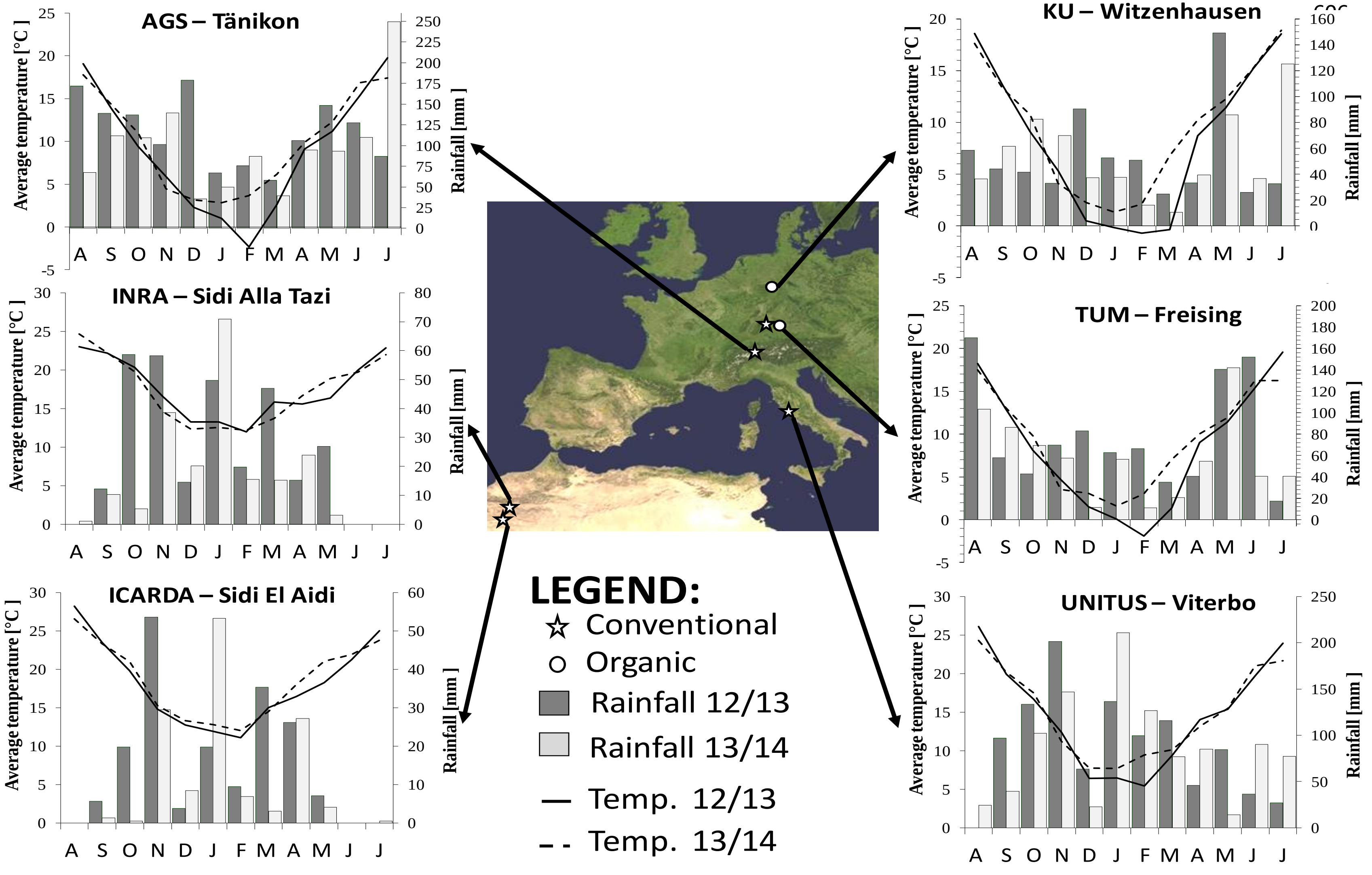

Figure 2. Main crop stages of wheat and subclover in the conventional managed field during the experimental periods represented on a calendar scale. Developmental stages: SW = Sowing of wheat; WE = wheat emergence [Zadoks (Z) scale 10]; WT = Wheat tillering (Z20); FL Flag leaf (Z45); WF = Wheat flowering (Z65); WR = Wheat seed ripening (Z75); WH = Wheat harvesting (Z100); SS = Subclover sowing; SE = Subclover emergence; SF = Subclover flowering; SSR = Subclover seed ripening. N on X axis indicate fertilizer-N application.

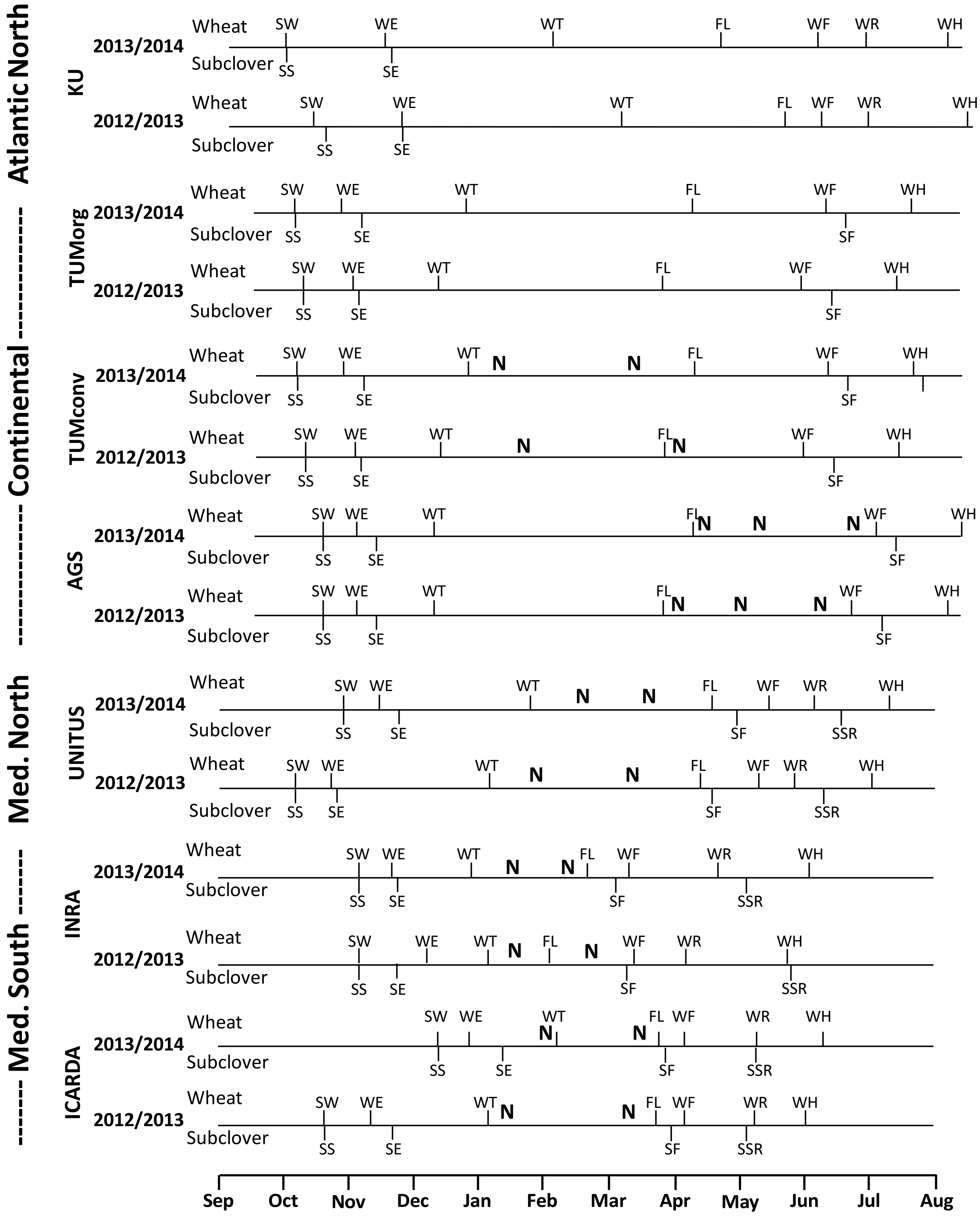
Atlantic North
KU
2013/2014
Wheat
SW WE WT FL WF WR WH
Subclover
SS SE
2012/2013
Wheat
SW WE WT FL WF WR WH
Subclover
SS SE
Continental
TUMorg
2013/2014
Wheat
SW WE WT FL WF WH
Subclover
SS SE SF
2012/2013
Wheat
SW WE WT FL WF WH
Subclover
SS SE SF
TUMconv
2013/2014
Wheat
SW WE WT N N FL WF WH
Subclover
SS SE SF
2012/2013
Wheat
SW WE WT N FL N WF WH
Subclover
SS SE SF
AGS
2013/2014
Wheat
SW WE WT FL N N N WF WH
Subclover
SS SE SF
2012/2013
Wheat
SW WE WT FL N N N WF WH
Subclover
SS SE SF
Med. North
UNITUS
2013/2014
Wheat
SW WE WT N N FL WF WR WH
Subclover
SS SE SF SSR
2012/2013
Wheat
SW WE WT N N FL WF WR WH
Subclover
SS SE SF SSR
Med. South
INRA
2013/2014
Wheat
SW WE WT N N FL WF WR WH
Subclover
SS SE SF SSR
2012/2013
Wheat
SW WE WT N FL N WF WR WH
Subclover
SS SE SF SSR
ICARDA
2013/2014
Wheat
SW WE WT N N FL WF WR WH
Subclover
SS SE SF SSR
2012/2013
Wheat
SW WE WT N N FL WF WR WH
Subclover
SS SE SF SSR
Sep
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug

Figure 3. Subclover aboveground biomass at subclover flowering in different nitrogen fertilization level. N+ = high N fertilization; N- = low N fertilization; N0 = no nitrogen fertilization; WC+ and WC- indicate treatments with and without application of compost at KU. Errors bars represent ± standard errors.

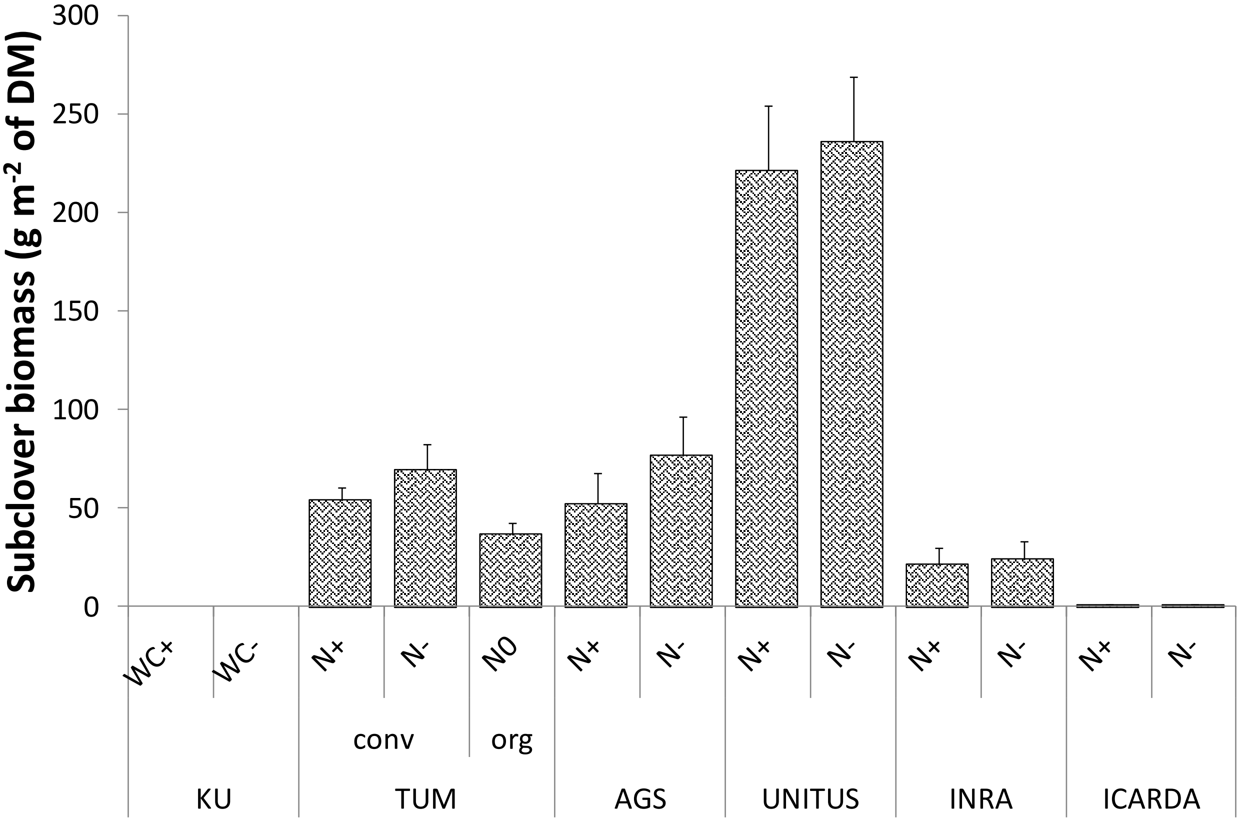

Figure 4. The main effects of cropping system on grain yield and yield component of wheat at different sites. Values belonging to the same variable followed by the same letter are not significantly different according to LSD (0.05). PW = Pure wheat; IW = Intercropped wheat; TGW = Thousand grain weight.

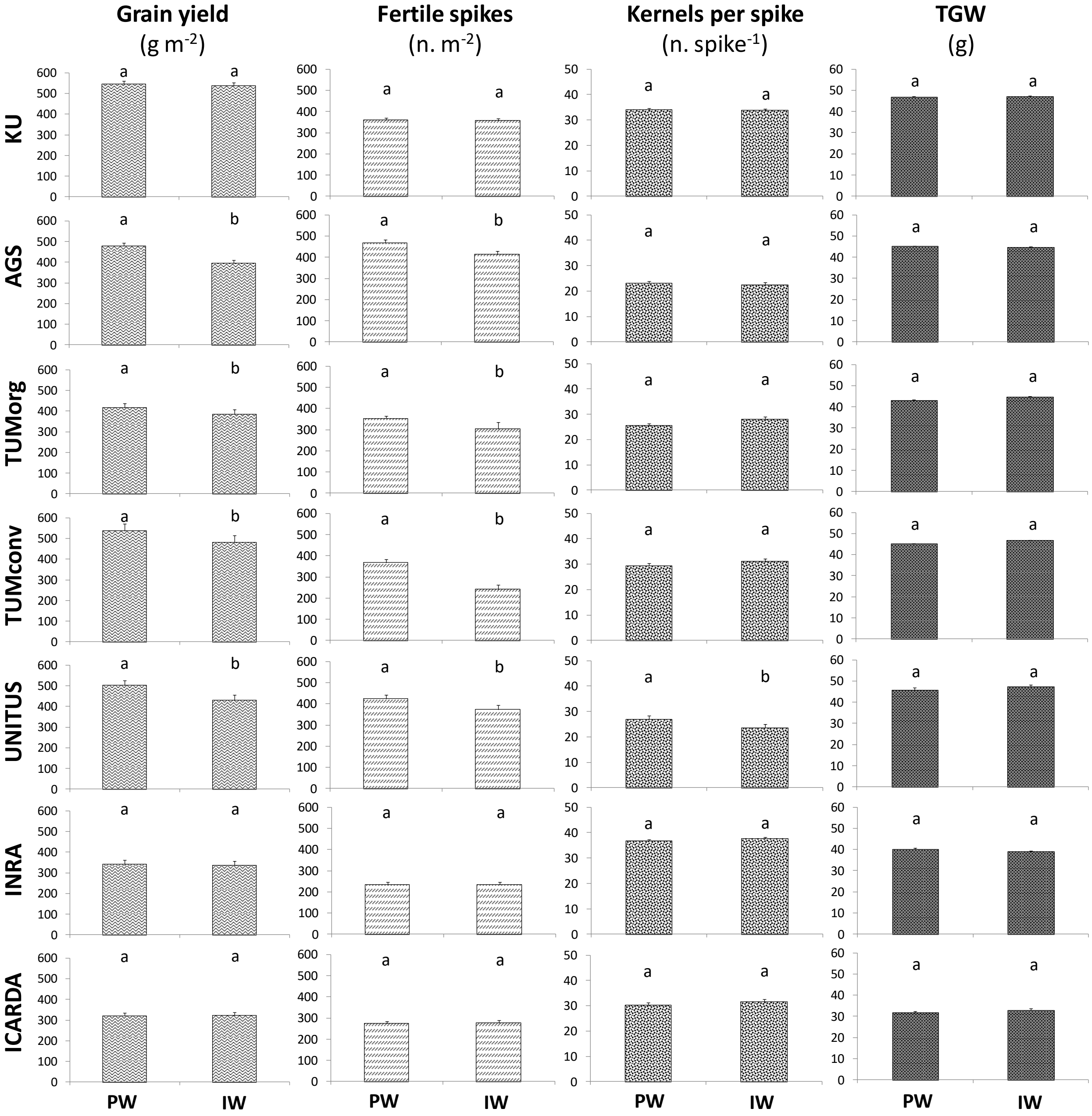
Grain yield
(g m^{-2})
Fertile spikes
(n. m^{-2})
Kernels per spike
(n. spike^{-1})
TGW
(g)
KU
a a a a a a a a
AGS
a b a b a a a a
TUMorg
a b a b a a a a
TUMconv
a b a b a a a a
UNITUS
a b a b a b a a
INRA
a a a a a a a a
ICARDA
a a a a a a a a
0 100 200 300 400 500 600
0 10 20 30 40 50
0 10 20 30 40 50 60
PW IW
PW IW
PW IW
PW IW

Figure 5. Percentage change in wheat grain yield comparing (a) intercropped to pure wheat in different agro-environmental zones and (b) fertilization management. Data are grouped by Continental (AGS, TUMconv and TUMorg), Mediterranean North (UNITUS), Mediterranean South (INRA and ICARDA), High N Input and Low N input (AGS, TUMconv, UNITUS, INRA and ICARDA). Error bars are 95% confidence intervals. The number of observations is indicated in parentheses. Significant change is denoted by * (where error bars do not overlap zero).

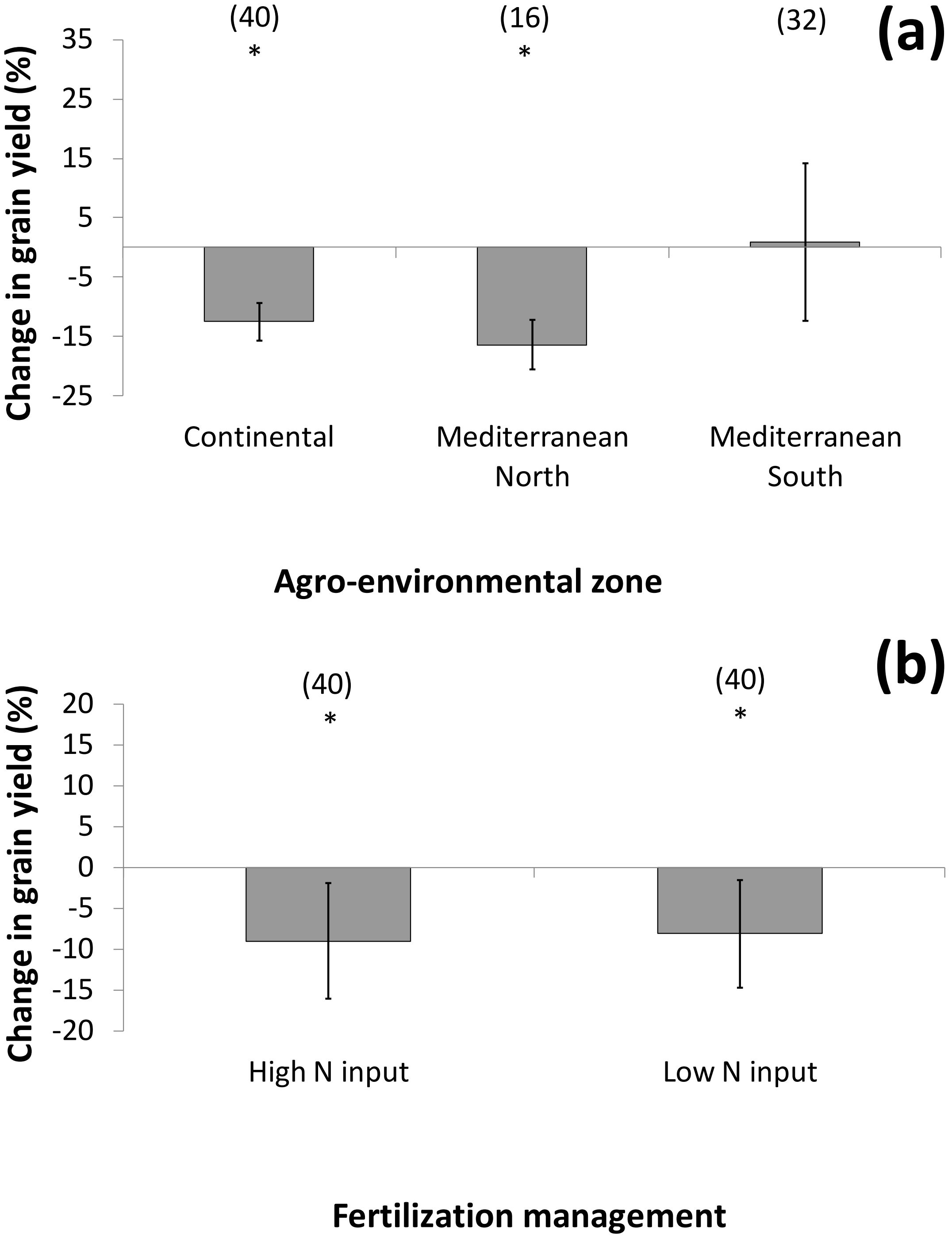

Figure 6. Percentage changes in wheat yield attributes comparing intercropped to pure wheat across different agro-environmental conditions and management agricultural practices. Error bars are 95% confidence intervals. The number of observations is indicated in parentheses. Significant changes are denoted by * (where error bars do not overlap zero).

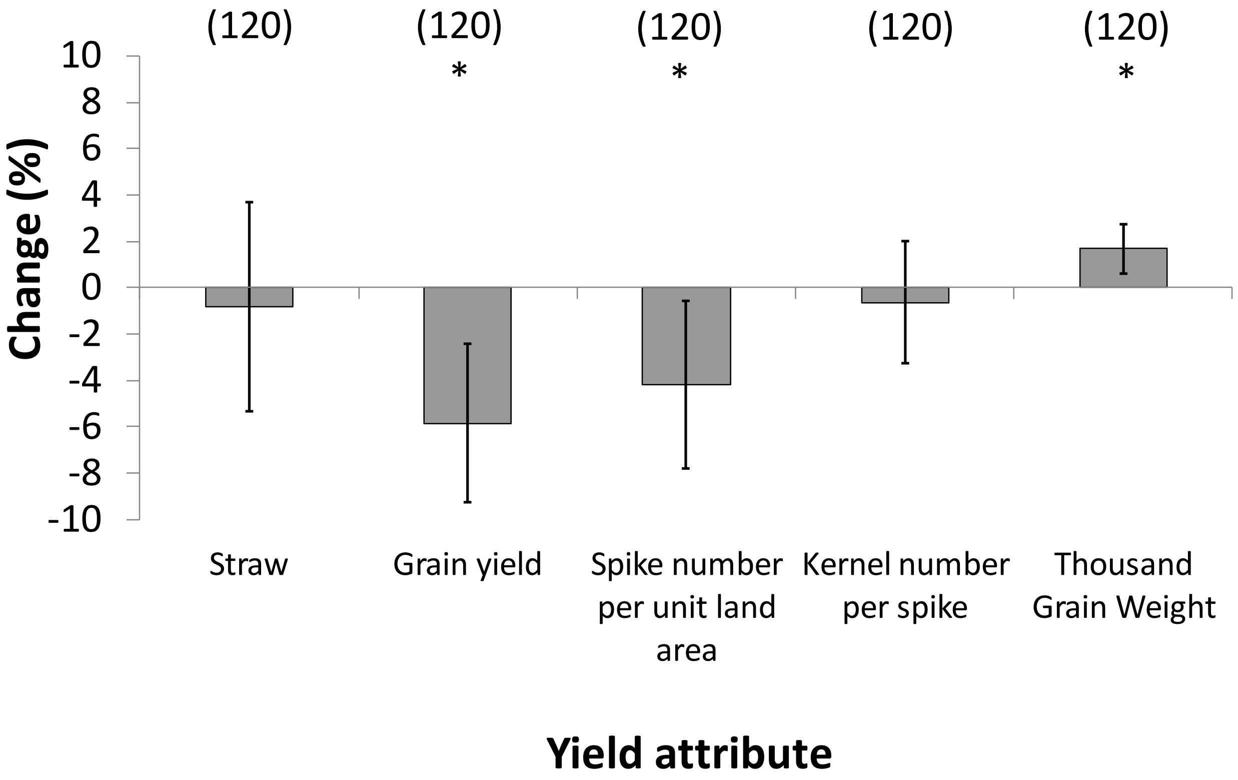

Figure 7. Relationship between the change in grain yield comparing intercropped to pure wheat and the subclover aboveground biomass (a) and the change in weed aboveground biomass comparing intercropped to pure wheat and the subclover aboveground biomass (b). The significance level is (*) significant at P ≤ 0.05.

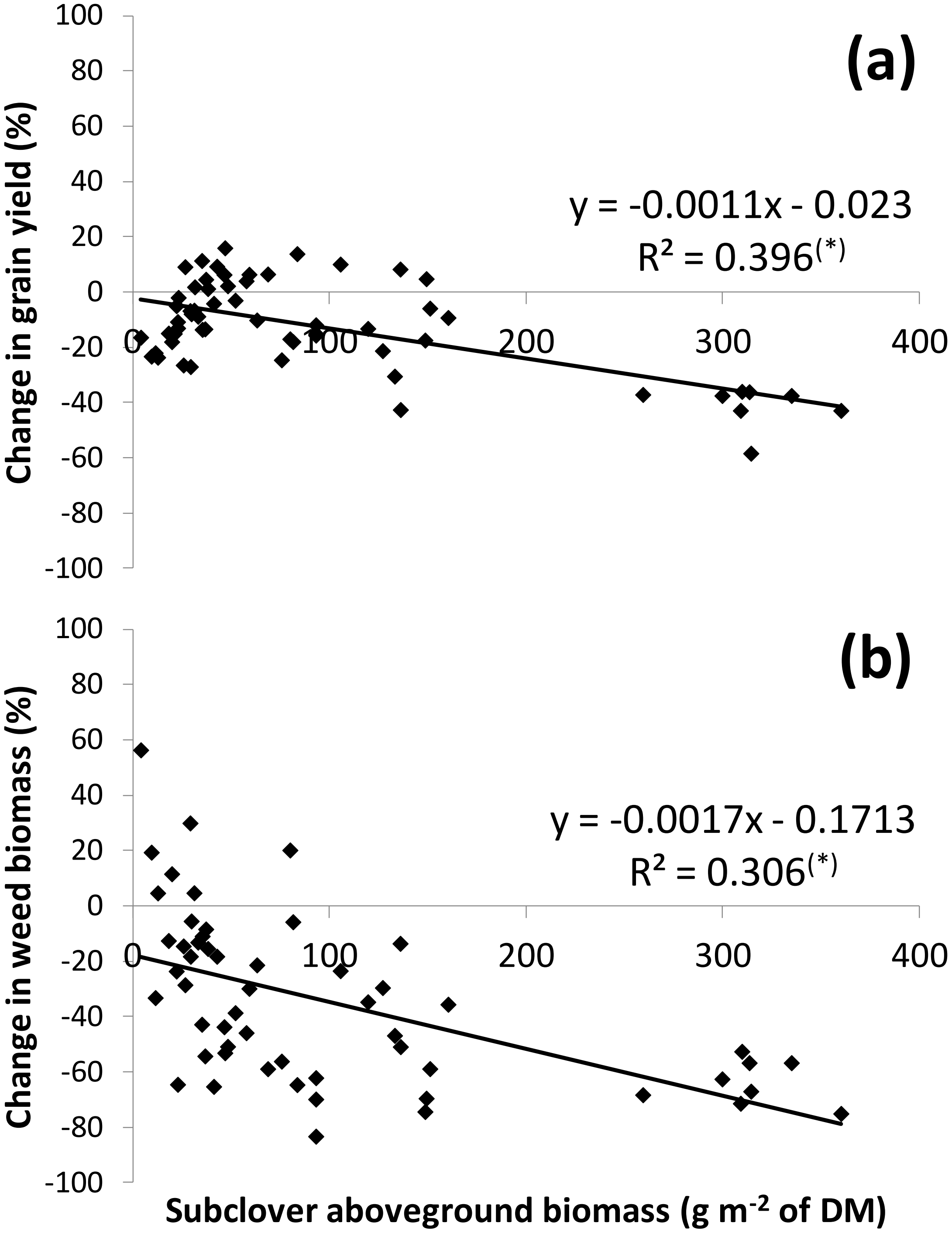

Figure 8. Percentage change in weed aboveground biomass comparing intercropped to pure wheat in different agro-environmental zones (a) and N fertilization level (b). Data used were those where the weeds were left to grow undisturbed. Data are grouped by Continental (TUMconv and TUMorg), Mediterranean North (UNITUS), Mediterranean South (INRA and ICARDA), High N Input and Low N input (TUMconv, UNITUS, INRA and ICARDA). Error bars are 95% confidence intervals. The number of observations is indicated in parentheses. Significant change is denoted by * (where error bars do not overlap zero).