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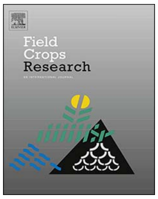

Single *vs* multiple agroecosystem services provided by common wheat cultivar mixtures: Weed suppression, grain yield and quality

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ABSTRACT

Cultivar mixtures are a well studied practice to improve common wheat performance by exploiting the potential of genetic diversity to buffer biotic and abiotic stresses. However, their ability to reduce weed interference is still unclear. In this work, crop-weed interactions were studied across two growing seasons under Mediterranean climatic conditions on nineteen common wheat stand types: twelve cultivars including modern and heritage varieties, four three-cultivar mixtures, two six-cultivar mixtures and one high diversity mixture with all twelve cultivars. Wheat morphological parameters, biomass accumulation of wheat and weeds, wheat yield, yield components and grain quality were assessed. Heritage cultivars showed the highest weed suppression (on average −67% weed biomass at harvest compared to modern cultivars) due probably to increased height, above ground biomass and leaf area index. No consistent mixture effects were detected for either weed suppression, grain yield or grain quality, when considered separately from one another. However, when considering the three agroecosystem services altogether based on a rank analysis, mixtures with higher number of components (six and twelve) tended to improve the overall crop performance compared to the average of less diverse wheat stand types. Although the observed benefits of mixtures *vs* component cultivars for individual agroecosystem services (i.e. weed suppression, yield and grain quality) were limited, cultivar mixtures appear as a potential tool to improve overall crop performance, especially with medium to high number of component cultivars. However, increased adoption of cultivar mixtures would require prior identification of key cultivar traits clearly associated with the provision of target agroecosystem services. Enhanced complementarity and synergy among these traits would maximize exploitation of the available genetic agrobiodiversity.

1. Introduction

Common wheat (*Triticum aestivum* L.) is the most widely grown cereal crop worldwide in terms of land extension and it is the staple food for more than one third of the human population (FAOSTAT, 2014). Being widespread in different geographical areas and farming systems, wheat growing ranges from small scale, labour intensive cultivation to large scale, extensive cultivation. Beyond this, wheat is a commodity whose price is determined on the international market, hence being characterized by increasing uncertainty and fluctuation (Haile et al., 2016). In this context, both conventional and organic farmers aim to decrease use of external inputs to keep wheat production costs low. Because of this, one major interest is to develop wheat management strategies able to cope with biotic and abiotic stresses.

Cultivar choice and hence breeding have often been proposed as major tools to improve crop performance under low-input and organic farming conditions (Lammerts van Bueren and Myers, 2012), especially concerning disease and weed reduction. This strategy has mainly been developed by targeted breeding programmes (Lammerts van Bueren et al., 2011), improved Value for Cultivation and Use (VCU) protocols (Löschenberger et al., 2008) and reintroduction of heritage cultivars, which are known to possess biotic and abiotic stress-tolerance traits that have been largely lost through modern breeding (Mason and Spaner, 2006).

Increasing attention is dedicated to the use of cultivar mixtures not only as a strategy to reduce biotic and abiotic stresses but also to stabilize and possibly increase yield (Kiær et al., 2009). The broader framework for cultivar mixture use is given by the widely recognised impact of diversity in ecosystem functioning. Although much of the results, as recently summarised by Barot et al. (2017), come from the ecological literature in which the effect of biodiversity on ecosystems has been investigated by focusing on species number, many biodiversity-related services can also be achieved by manipulating within-species diversity, i.e. utilising different cultivars of the same crop

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species. Services provided by agroecosystems are multiple, including among others food production, regulation of greenhouse gases, C storage, and soil health. Functional diversity exploitation at intra-specific, inter-specific or landscape level supports different services and/or can impact differently on the same services. In this study, we focused on a subset of agroecosystem services that can be provided by the use of intra-specific (i.e. genetic) functional diversity.

In cultivar mixtures, seeds from a certain number of cultivars are blended at sowing. The cultivars composing the mixture need to be similar for traits such as growing cycle length or end-use quality in order to be cultivated together (Wolfe, 1985). At the same time, they need to differ for traits related to the agroecosystem service expected to be improved, e.g. to carry different disease resistance traits (Garrett and Mundt, 1999).

Wheat cultivar mixtures have largely been studied for reducing the effect of airborne disease outbreaks (Cox et al., 2004; Finckh et al., 2000; Finckh and Mundt, 1992). Cultivar mixtures out-yielded single cultivar stands in different contexts and field experiments (Smithson and Lenné, 1996; Finckh et al., 2000; Gallandt et al., 2001; Cowger and Weisz, 2008; Kiær et al., 2009; Döring et al., 2015). Also, mixtures have been shown to stabilize yield over time (Smithson and Lenné, 1996; Finckh et al., 2000; Cowger and Weisz, 2008; Kaut et al., 2009; Mengistu et al., 2010; Döring et al., 2015). In some cases mixtures also improved grain protein content and bread-making quality (Finckh et al., 2000; Sarandon and Sarandon, 1995). Overall, use of cultivar mixtures appear as an insurance strategy for farmers, as they tend to buffer the impact of fluctuating environmental conditions on crop performance. Nevertheless, most of the experiments that studied the performance of wheat cultivar mixtures focused on a single agroecosystem service, e.g. yield, yield stability, quality or disease reduction. In these experiments, mixtures were assembled to ensure complementarity and synergy among component cultivars for just one target service. However, in real farming conditions wheat mixtures should be able to achieve results comparable to or better than those of the best available pure line varieties for a plurality of agroecosystem services. In fact, wheat cultivar mixture experiments did not always demonstrate a positive mixture effect. In some experiments, only few of the tested mixtures were successful (Finckh and Mundt, 1992; Kiær et al., 2012). In other experiments, mixtures did not outperform their individual components for yield (Finckh et al., 2000; Kaut et al., 2009), grain quality (Cowger and Weisz, 2008; Kaut et al., 2009) or disease reduction (Kaut et al., 2009). The wheat mixtures used by Dai et al. (2012) were unsuccessful for yield, grain quality and disease reduction at the same time. These outcomes make it difficult to promote use of cultivar mixtures by farmers until a clear approach on how to create successful mixtures in any growing conditions will be available (Kiær et al., 2012). In this work, several common wheat cultivar mixtures and single component cultivars were tested for their potential to provide target agroecosystem services, viz. weed suppression, grain yield and grain quality, under Mediterranean conditions. Weed suppression has rarely been investigated in wheat cultivar mixtures (Kaut et al., 2009) and never under Mediterranean conditions. Kaut et al. (2009) showed no evidence of weed suppression by any of the cultivar mixtures tested and the effects studied were more related to weed tolerance (reduced effect of weed competition on crop performance) than weed suppression (the ability of the crop to reduce weed abundance and/or biomass).

Variation in competitive ability against weeds has been observed in bread wheat germplasm (Coleman et al., 2001; Lemerle et al., 1996). The weed suppression ability of more competitive cultivars is usually not due to a single trait but rather to a series of interacting traits that need to coexist to determine suppression (Hoad et al., 2012). Andrew et al. (2015) described plant height, early vigour, tillering capacity and canopy architecture as the most important above ground traits that have been associated with wheat competitive ability against weeds. In Hoad et al. (2012), increased plant height, rapid growth rate, wide leaf laminae, high yield potential and allelopathy are listed among the desirable traits, whereas a planophile habit and high leaf area index are reported as highly desirable traits. Good plant establishment, high early season ground cover and high tillering capacity were mentioned as essential for good competition against weeds. Although developing a ranking system for competitiveness of wheat cultivars would be desirable (Andrew et al., 2015), studies that investigated the contribution of wheat traits to crop competitive ability against weeds are sporadic (Lemerle et al., 2006; Mason et al., 2008; Murphy et al., 2008).

In this work, the effect of wheat stand type on the interference with weeds was investigated by looking at the role of a series of competition-related traits in the provision of the weed suppression service.

We tested the following three hypotheses on selected common wheat cultivar mixtures and stands of single component cultivars:

(1) Weed suppression, grain yield or grain quality can be improved by introducing a given set of homogeneous traits into the wheat stand, according to the mass-ratio hypothesis (Grime, 1998). This hypothesis intended to test the role of functional identity (Costanzo and Bàrberi, 2014) in determining the weed suppression, grain yield or grain quality potential of the wheat stand types.

(2) Weed suppression, grain yield or grain quality can be improved by increasing the diversity of given traits within the crop stand through a niche differentiation effect, according to the diversity hypothesis (Fornara and Tilman, 2008). With this hypothesis we tested the effect of the functional composition of the mixtures on the weed suppression, grain yield, or grain quality potential (Costanzo and Bàrberi, 2014).

(3) Increasing the diversity of cultivars within the crop stand, and consequently their trait diversity, is expected to improve the overall crop performance, i.e. the provision of the three target agroecosystem services (weed suppression, grain yield and grain quality) altogether. With this hypothesis, we tested the effect of specific trait combinations on the provision of selected agroecosystem services (Barot et al., 2017).

2. Materials and methods

2.1. Study site and experimental design

The experiment consisted of a field trial replicated across two growing seasons (2013/14 and 2014/15) at the Interdepartmental Centre for Agri-environmental Research (CIRAA) of the University of

Table 1
Soil properties of the experimental fields used in 2013/14 and 2014/15.

	pH	conductivity microS	CSC meq 100 g^{-1}	total N[a] mg kg^{-1}	organic matter[b] %	P[c] ppm	clay %	silt %	sand %
2013/14	8.03	101.17	2.97	1.43	2.03	6.32	17.51	47.54	34.95
2014/15	8.15	85.20	2.22	1.77	2.64	7.39	27.40	38.14	34.46

[a] Kjeldahl method.

[b] Walkley-Black method.

[c] Olsen method. Samples collected on 06/11/2013 on both fields.

Pisa, (43°41′02.8″N, 10°20′35.0″E) on an alkaline loamy soil (Table 1). It was arranged in a randomised complete block design with three replicates. Crop management simulated an organic cropping system, with no application of herbicides, fungicides and mineral fertilizers. The only fertilization applied was 1 t ha^{-1} of organic fertilizer (NUTEX, i.e. commercial pelleted mixture of manure from different sources with 32–34% organic C, 3% N and 3% P_2O_5) incorporated into the soil before sowing.

In the first year, the experiment was sown as a wheat crop following wheat and a previous 5-year lucerne (*Medicago sativa* L.) ley. Wheat in the season preceding our experiment was managed conventionally with one post emergence application of herbicide and fungicide and application of 200 kg ha^{-1} of ammonium nitrate, 50% at tillering and 50% at stem elongation. In the second year, wheat followed a broad bean (*Vicia faba* L. var. *minor*) crop. No herbicides, fungicides and mineral fertilizers were applied to the broad bean crop. The lucerne ley growing in both field from 2 to 7 years before the onset our experiment was managed without any herbicide, fungicide and fertilizer.

The seedbed was prepared by ploughing at 25 cm depth and subsequent disc harrowing at 7–10 cm depth. Wheat was mechanically sown in 1.5 × 7 m plots at a density adjusted to 400 viable seeds m^{-2} in 15 cm spaced rows.

The experiment was sown on 14 November 2013 and 30 October 2014 and combine harvested on 2 July 2014 and 30 June 2015. Total rainfall from sowing to harvest was 986 mm in the first year and 891 mm in the second (Fig. 1). The highest rainfall was measured in January 2014 (355 mm) during the first year and in November 2014 (290 mm) during the second year. From the sowing date to the beginning of April, maximum temperature was < 20 °C in both years (Fig. 1). Minimum temperature was < 5 °C from November to the end of March in the first year and from December to the end of March in the second. Temperature in April was higher in the second than in the first year (Fig. 1).

2.2. Selection of cultivars and constitution of mixtures

Twelve single cultivars, four three-cultivar mixtures, two six-cultivar mixtures and one twelve-cultivar mixture were compared, constituting the levels of the wheat stand type factor. All mixtures were prepared by mixing component cultivars with equivalent seed rates (the ratio between total seed number m^{-2} and the number of component cultivars) in both years. Cultivars included nine modern and three heritage cultivars. According to the official end-use classification in Italy (Foca et al., 2007), modern cultivars included three superior bread-making (cvs Albachiara, Blasco and Bolero), three ordinary bread-making (cvs A416, Isengrain and Katou) and three biscuit-making cultivars (cvs Altezza, Artico and Bramante). Heritage cultivars included cvs Autonomia A, Gentil Rosso and Verna, selected to represent the group of traditionally cultivated cultivars in Tuscany.

Mixtures were constituted as follows:

- Four mixtures of three cultivars each belonging to the same end-use category.
- Two mixtures of six cultivars each: one from the heritage and biscuit-making groups (3 + 3 cultivars) and the other from bread-making cultivars.
- One mixture including all twelve cultivars.

The three-component mixtures were kept uniform in terms of end-use quality. For the six components mixtures, we decided to mix the heritage cultivars with the biscuit-making cultivars in order to balance the predicted low productivity and high grain protein content of the first group with the predicted high productivity and low grain protein content of the second. Overall, 19 wheat stand types were tested (Table 2).

2.3. Data collection

Wheat phenology was monitored throughout the crop cycle according to the BBCH growth scale (GS) (Meier et al., 2009). Growing Degree Days (GDD) to heading were calculated assuming 0 °C as base temperature and heading date was registered and adapted to a 1–5 scale (very early, early, intermediate, late, very late). Winter growth habit was determined in a 1–5 scale from erectophile narrow to planophile spread as in Hoad et al. (2012). Crop establishment was measured ca. one month after sowing in three 25 × 30 cm quadrates per plot. Weed density and number of wheat tillers were assessed at end of the tillering stage in one quadrate per plot of 25 × 30 cm in 2014 and of 45 × 50 cm in 2015. The ratio between the number of tillers at end of the tillering phase and the number of emerged seedlings was used as tillering index. The ratio between number of spikes and emerged seedlings was used as fertile tiller index.

Crop height was measured in February, April and at crop harvest in both years on 10 random plants per plot. Leaf area index (LAI) was indirectly measured twice per year (in April and May) with a SunScan Canopy analyser (Delta-T Devices Ltd, UK). Above ground weed and crop biomass was collected three times per year: at the end of winter (BBCH GS 30), in spring (BBCH GS 60/69) and at crop physiological

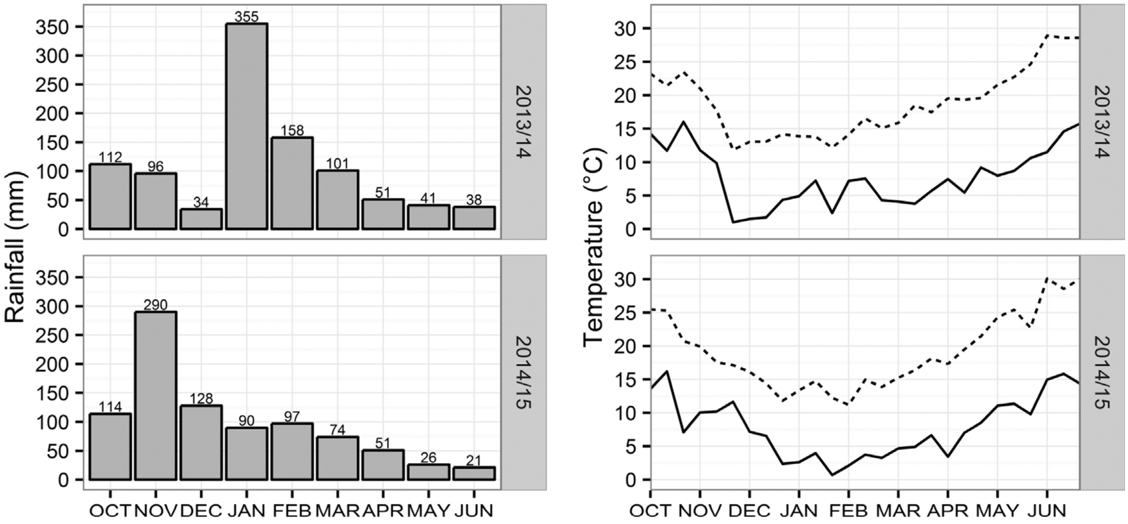

Fig. 1. Monthly rainfall (left) and maximum (dashed line) and minimum (solid line) daily temperatures (right) during the two growing seasons. Numbers on top of bars are the total rainfall amounts per month.

Table 2
Wheat stand types tested and corresponding codes.

	Code[a]	End-use class[b]	Component cultivars
1	ALB	Superior bread-making	Albachiara
2	BLA	Superior bread-making	Blasco
3	BOL	Superior bread-making	Bolero
4	M3_Br1	Superior bread-making	Albachiara + Blasco + Bolero
5	A41	Ordinary bread-making	A416
6	ISE	Ordinary bread-making	Isengrain
7	KAT	Ordinary bread-making	Katou
8	M3_Br2	Ordinary bread-making	A416 + Isengrain + Katou
9	M6_Br	Superior + ordinary bread-making	Albachiara + Blasco + Bolero + A416 + Isengrain + Katou
10	ALT	Biscuit-making	Altezza
11	ART	Biscuit-making	Artico
12	BRA	Biscuit-making	Bramante
13	M3_Bi	Biscuit-making	Altezza + Artico + Bramante
14	AUT	Heritage cultivar	Autonomia A
15	GRO	Heritage cultivar	Gentil Rosso
16	VER	Heritage cultivar	Verna
17	M3_He	Heritage cultivars	Autonomia A + Gentil Rosso + Verna
18	M6_HeBi	Biscuit-making + heritage cultivars	Altezza + Artico + Bramante + Autonomia A + Gentil Rosso + Verna
19	M12	All types of cultivars	All 12 cultivars

[a] In the mixture codes, the first part refer to the number of component cultivars (M3, M6 and M12 for three-, six-, and twelve-cultivar mixtures respectively) and the second to the end-use class of component cultivars (Br, Bi and He for bread-making, biscuit-making and heritage cultivars respectively; different numbers indicate different mixtures).

[b] The end-use class has been assigned based on Foca et al. (2007), except for the heritage cultivars that, having peculiar flour characteristics, could not be included in any of the given end-use classes.

maturity. Biomass sampling was performed in one quadrat per plot of 25 × 30 cm for the first sampling, 45 × 50 cm for the second, and 1 × 1 m for the third sampling. Dry biomass weights were obtained by oven-drying samples at 60 °C for the first sampling and 100 °C for the other samplings until constant weight. N percentage (Kjeldahl method) in the biomass harvested at the first sampling date was determined in both years. Sampling date, BBCH growth stage range and GDD from sowing for each variable listed above are described in Table 3. The presence of brown rust (*Puccinia recondita* L. f. sp. *tritici*), leaf spot complex (*Mycosphaerella graminicola* and *Helminthosporium* leaf blight) and *Fusarium* head blight was monitored in the field in both seasons as indicator of possible problems on grain yield quality. No statistical analysis is presented on these data because the field layout was not appropriately planned for a disease study.

At crop physiological maturity (BBCH GS 89), the number of spikes m^{-2} and straw and grain biomass (g) were determined in one 1 × 1 m quadrat per plot, and samples were oven-dried at 100 °C. Wheat test weight (TW, kg hl^{-1}) and thousand kernel weight (TKW, g) were measured for each plot on grain samples taken from the combine harvester. An Infratec 1241 Grain analyser (Foss, DK) was used to analyse whole grain protein and starch content on these latter samples.

2.4. Data analysis

All statistical analyses were conducted using R environment for statistical computing, version 3.3.1 (R Core Team, 2016). R/vegan (Oksanen et al., 2015) was used for principal components analysis (PCA). PCA biplots were created using variable-focused scaling; angle size between variable vectors is negatively correlated with the strength of their association. The PCA was done using the measurements of ten morphological traits on the twelve single-cultivar wheat stand types to summarise differences among cultivars. Pearson correlation values

Table 3
Growth stage (BBCH GS), date and Growing Degree Days (GDD) for the variables sampled in the two seasons.

Variable	Description	2013/2014			2014/2015		
		BBCH GS range	Date 2014	GDD from sowing	BBCH GS range	Date 2015	GDD from sowing
H_Feb	plant height in February (cm)	2. –	14/02	820	2. –	26/02	1200
H_Apr	plant height in April (cm)	3. – 45	02/04	1338	3. – 45	14/04	1712
H_fin	final plant height excluding awns (cm)	89	02/07	2897	89	30/06	3153
B_Mar	wheat above ground biomass in March (g m^{-2})	2. – 32	12/03	1095	2. – 32	10/03	1316
B_May	wheat above ground biomass in May (g m^{-2})	7. – 8.	21/05	2040	3. – 4. – 5.	29/04	1930
S_harv	wheat straw biomass at harvest (g m^{-2})	89	02/07	2897	89	30/06	3153
LAI_Apr	leaf area index in April	2. – 3.	18/03	1163	3. – 4.-5.	15/04	1727
LAI_May	leaf area index in May	47–75	05/05	1787	71–75	13/05	2176
WB_Mar	weed biomass in March (g m^{-2})	2. – 32	12/03	1095	2. – 32	10/03	1316
WB_May	weed biomass in May (g m^{-2})	7. – 8.	21/05	2040	3. – 4. – 5.	29/04	1930
WB_harv	weed biomass at harvest (g m^{-2})	89	02/07	2897	89	30/06	3153
Tillers	No. tillers plant^{-1}	2. – 30	11/03	1085	2. – 30	05/03	1270
G_hab	canopy growth habit in winter	2. –	14/02	820	2. –	26/02	1200
W_den	weeds density (No. weeds m^{-2})	2. – 32	11/03	1085	2. – 30	05/03	1270

Table 4
Set of orthogonal linear contrasts, type of comparison and corresponding research hypothesis addressed. For wheat stand type codes see Table 2.

	Comparison	Type of comparison	Research hypothesis
1	M12 *vs* all other treatments	Higher diversity mixture vs all other treatments	(2)
2	M6_Br *vs* components	M6_Br *vs* ALB + BLA + BOL+ KAT + A41 + ISE + M3_Br1 + M3_Br2	(2)
3	M3_Br1 *vs* components	Mixture effect	(2)
4	ALB *vs* BLA + BOL	Cultivar identity	(1)
5	BLA *vs* BOL	Cultivar identity	(1)
6	M3_Br2 *vs* components	Mixture effect	(2)
7	KAT *vs* A41 + ISE	Cultivar identity	(1)
8	ISE *vs* A41	Cultivar identity	(1)
9	M6_HeBi *vs* components	M6_HeBi vs ART + ALT + BRA + AUT + GRO + VER + M3_Bi + M3_He	(2)
10	M3_Bi *vs* components	Mixture effect	(2)
11	ART *vs* ALT + BRA	Cultivar identity	(1)
12	ALT *vs* BRA	Cultivar identity	(1)
13	M3_He *vs* components	Mixture effect	(2)
14	AUT *vs* GRO + VER	Cultivar identity	(1)
15	GRO *vs* VER	Cultivar identity	(1)
16	Br1 + Br2 *vs* Bi + He	Bread-making cultivars and relative mixtures *vs* biscuit-making and heritage cultivars and relative mixtures	(1)
17	M3_Br1 + components *vs* M3_Br2 + components	Differences between the two groups of bread-making cultivars	(1)
18	M3_Bi + components *vs* M3_He + components	Differences between the biscuit-making and the heritage cultivar groups	(1)

Research hypotheses: (1) test the role of functional identity; (2) test the role of functional composition.

among all traits in the PCA were calculated on the full two-year dataset to study the relationship among groups of highly correlated variables and the growing phase in which they were measured. The mean of the correlation values (using absolute values) was calculated by transforming correlations into Fisher-Z-values weighted by the number of cases before averaging and retransforming with an inverse Fisher-Z. Variables assessed through measures on individual random plants in each plot (e.g. height) were analysed only for single cultivar stand types, since component cultivars in mixtures were not distinguished from one another in the samplings.

A cumulative analysis of variance (ANOVA) for each explanatory variable was performed using a mixed effect model. The model was formulated as:

$$Y_{ijk} = \mu + WST_i + GS_j + (WST{:}GS)_{ij} + BLK_k + \varepsilon_{ijk} \quad (1)$$

where Y_{ijk} is the variable value for the wheat stand type i (WST_i) in the growing season j (GS_j) and in the block k (BLK_k), WST:GS represents the interaction of the i-th WST in the j-th GS; μ represents the grand mean and ε_{ijk} is the residual error. The model was run with WST, GS, and WST:GS as fixed effects and BLK as random effect. In the case of significant WST:GS interaction, differences among treatments were investigated separately in each growing season. In that case, the model formulation was the same except for the absence of the GS and WST:GS terms.

Count data were analysed with a generalized linear mixed model R/lme4 using the Poisson distribution (Bates et al., 2015). For the case of weed density, which is highly affected by patchiness in the field, spatial autocorrelation was tested with the Mantel test (as implemented in R/ade4). In the case of significant spatial autocorrelation, in order to cope with spatial variability the row and column position of each plot in the grid of the experimental plan was used as random effect.

Continuous variables were analyzed with linear mixed model in R/nlme (Pinheiro et al., 2013). In the linear mixed models fitted in R/nlme, an adjustment for different variances per stratum was used to take the heterogeneity of variance among the different levels of the wheat stand type factor into account (Zuur et al., 2009). The dependent variable was appropriately transformed when the requirements for the linear model were not met, upon graphical check.

For each response variable, the wheat stand type factor was split into a set of 18 orthogonal linear contrasts, in order to test:

- The effect of the highest diversity mixture (twelve cultivars);
- The effect of each six-cultivar mixture;
- The effect of each three-cultivar mixture;
- The single-cultivar identity effects.

The complete list of orthogonal linear contrasts and their relationship with the research hypotheses is shown in Table 4. To highlight the mixture effect, the value of each parameter as measured in the mixture was compared with the average value of the same parameter for all the related wheat stand types (hereafter defined as 'mid-value'). Each comparison considered the error structure within the mixed model by taking into account the blocking factor. Specifically, the value of a three-cultivar mixture was compared with the average value of the three component cultivars when cultivated as single stand. The value of a six-cultivar mixture was compared with the average value of all mono-cultivar stands constituting the mixture and the two respective three-cultivar mixtures. Finally, the value of the twelve-cultivar mixture was compared with the average value of the twelve component cultivars when cultivated as single stand and that of all other mixtures. With the comparison of the three-cultivar mixtures to the average of their components, we tested the simple mixture effect. Testing the six and twelve-cultivar mixtures *vs* their components cultivars plus the lowest diversity mixtures, we aimed to study the effect of increasing diversity within the crop stand.

For each end-use group of three cultivars, the most different cultivar in terms of earliness/height was compared to the other two and then the two cultivars with more similar behaviour were compared with each other. These comparisons were set in order to help discern the role of the identity of the single cultivar in delivering a specific agroecosystem service. The last three comparisons aim to investigate the effect

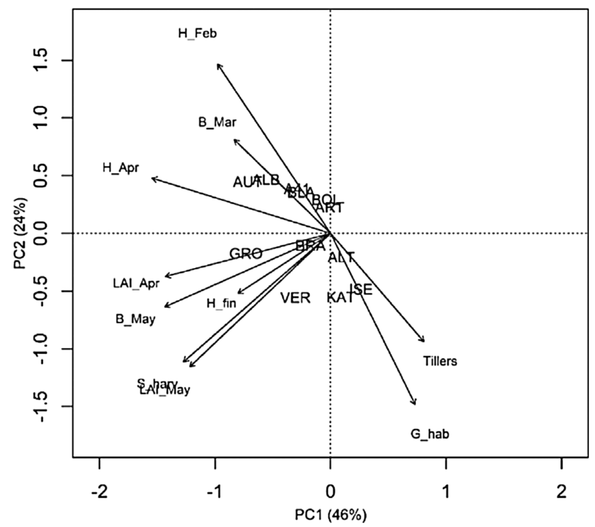

Fig. 2. PCA based on 10 morphological traits (two-year pooled data). Arrows represent traits and three-letter codes represent cultivars (A41 = A416, ALB = Albachiara, ALT = Altezza, ART = Artico, AUT = Autonomia A, BLA = Blasco, BOL = Bolero, BRA = Bramante, GRO = Gentil Rosso, ISE = Isengrain, KAT = Katou, VER = Verna). The traits used in the analysis are: B_Mar: wheat above ground biomass in March; H_Feb: plant height in February; Tillers: No. tillers plant^{-1} measured before the onset of the stem elongation phase; G_hab: canopy growth habit in winter; H_Apr: plant height in April; B_May: wheat above ground biomass in May; S_harv: wheat straw biomass at harvest; H_fin: final plant height excluding awns; LAI_Apr, LAI_May: Leaf area indices in April and May respectively.

of the end-use categories. The 18 comparisons selected do not comprise all possible data queries, (e.g., we do not have a specific comparison of the twelve-cultivar mixture against the average of the single components). This choice was taken in order to keep the comparisons independent (for having orthogonal contrasts) and for getting a set for queries that, although not exhaustive, can be used for a clear and synthetic presentation of results.

Pearson correlation between weed density and weed biomass for all competition-related traits were calculated on the original data to study the impact of specific traits on weed suppression.

The overall performance of wheat stand types (research hypothesis 3) was tested using the Friedman test, equivalent to a non-parametric ANOVA for an RCB design, as implemented in R/agricolae (Mendiburu, 2014). Within each year, all plots were ranked according to a series of indicators for each target agroecosystem service, namely (i) weed biomass at the three sampling dates for weed suppression; (ii) yield (t ha^{-1}), number of spikes m^{-2}, number of seeds spike^{-1} and thousand kernel weight for production; (iii) test weight, percent whole grain protein and starch content for grain quality. These indicators were ranked such that a higher value indicates higher provision of the corresponding service. To avoid different minimum and/or maximum rank values among the indicators due to the presence of ties, the rank was standardized upon the maximum value of each indicator to have all ranks ranging between 0 and 1. The overall weighted mean for each plot in each year was then calculated. The three services (weed suppression, yield production and yield quality) were given the same weight in the analysis as well as each indicator within service. A Friedman test of these ranks was performed on the two-year pooled data. A Wilcoxon rank-sum test, the non-parametric equivalent of a *t*-test (as implemented in R/stats), was used to compare the ranking of (i) the three-cultivar mixtures against all the single-cultivar stands, and of (ii) the six- or twelve-cultivar mixture components against all single cultivars and the three-cultivar mixtures.

3. Results

The crop showed pronounced differences between the first and second year of trial, with, overall, a better performance in 2013/14 than in 2014/15. Mean grain yield was 77% higher in 2014 (3.80 ± 0.08 t ha^{-1}) than in 2015 (2.15 ± 0.07 t ha^{-1}) ($P < 0.05$). Similarly, fertile tillers, i.e. number of spikes plant^{-1} (1.11 ± 0.03 in 2013/14 vs 0.83 ± 0.02 in 2014/15) and number of seeds spike^{-1} (41.42 ± 0.82 in 2013/14 vs 33.23 ± 0.86 in 2014/15) were higher in the first than in the second year. Total weed density at the end of winter was lower in 2013/14 than in 2014/15 (116 ± 7.97 vs 208 ± 8.26 plants m^{-2}). Regarding crop nutritional status, the percentage of N in the above ground wheat biomass (at end of tillering phase) ranged between 1.07–1.44% in 2013/14 and 1.20–1.64% in 2014/15, without any consistent mixture effect. Brown rust, Leaf spot complex and *Fusarium* head blight symptoms were detected in both years. The three diseases occurred in the field in both seasons with differentiated level of infection among cultivar stand types (e.g. cv. A416 suffered of high rust infection, cv. Blasco had a prominent leaf spot infection and cvs. A416, Artico and Bolero were visibly affected by *Fusarium* head blight).

3.1. Wheat morphological traits

3.1.1. Differences among cultivars

The PCA plot in Fig. 2 summarises the differences among single cultivar wheat stand types for 10 morphological traits over the two years. The first two principal components explained 70% of the total variability for the studied traits among the single cultivars in our dataset. Table 5 shows the correlation values between traits included in the PCA.

Table 5
Pairwise Pearson correlation coefficients between the morphological traits used to differentiate cultivars (two-year pooled data). B_Mar: wheat above ground biomass in March; H_Feb: plant height in February; Tillers: No. tillers plant^{-1} measured before the onset of the stem elongation phase; G_hab: canopy growth habit in winter; H_Apr: plant height in April; B_May: wheat above ground biomass in May; S_harv: wheat straw biomass at harvest; H_fin: final plant height excluding awns; LAI_Apr, LAI_May: Leaf Area Indices in April and May respectively.

	Group1					Group2				
	B_Mar	H_Feb	Tillers	G_hab	H_Apr	B_May	S_harv	H_fin	LAI_Apr	LAI_May
B_Mar	–	0.60	ns	−0.45	0.50	0.35	0.24	ns	0.31	ns
H_Feb		–	−0.59	−0.73	0.71	0.23	ns	ns	0.35	ns
Tillers			–	0.49	−0.56	−0.23	ns	ns	−0.33	ns
G_hab				–	−0.54	ns	ns	ns	ns	ns
H_Apr					–	0.70	0.53	0.36	0.73	0.51
B_May						–	0.82	0.32	0.77	0.77
S harv							–	0.54	0.67	0.84
H_fin								–	0.26	0.42
LAI_Apr									–	0.74
LAI_May										–

Table 6
Heading date and winter growth habit for the twelve single cultivar crop stands.

Cultivar	GDD to heading 2013/14	GDD to heading 2014/15	Earliness class	Growth habit
A416	1602	1853	3	2
Albachiara	1504	1740	1	2
Altezza	1602	1853	3	3
Artico	1602	1853	3	2
Autonomia A	1504	1740	1	1
Blasco	1647	1898	3	2
Bolero	1647	1898	3	2
Bramante	1602	1853	3	2
Gentil Rosso	1848	2140	5	2
Isengrain	1703	1944	4	4
Katou	1816	2085	4	4
Verna	1881	2177	5	3

GDD = Growing Degree Days assuming 0 °C as base temperature. Earliness classes: 1 = very early, 2 = early, 3 = intermediate, 4 = late, 5 = very late. Growth habit in scale 1–5, from erectophile narrow (1) to planophile spread (5) as in Hoad et al. (2012).

According to the relationship among all traits as shown in the PCA and the correlations (Fig. 2 and Table 5), wheat morphological traits can be grouped in two sets:

- a first group of traits representative of the winter growth phase, including above-ground biomass at end of winter, plant height in February, tillering index and growth habit;
- a second group of traits representative of the spring growth phase, including above-ground biomass at spring and harvest time, plant height at physiological maturity and LAI in April and May.

Traits belonging to one group had stronger correlation with traits within the same group than with those of the other group (Table 5). The mean correlation coefficient between pair of traits within the first or second group was 0.60 and 0.65 respectively. Instead, the mean correlation between the traits of the first and the second group was 0.29. Plant height in April was highly correlated with both trait groups (average r = 0.58).

The twelve cultivars largely differed for heading date and growth habit in winter. Heading date ranged from very early to very late, and the growth habit in winter varied from erectophile narrow to planophile (Table 6).

Overall, the tillering index was higher in 2014/15 (on average 2.55 ± 0.14 tillers plant^{-1}) than in 2013/14 (2.04 ± 0.05 tillers plant^{-1}) ($P < 0.05$). Tiller number was positively associated with planophile habit (shown by cvs Isengrain and Katou), and negatively associated with winter canopy height and winter above ground biomass (Fig. 2 and Table 5).

Crop establishment was on average higher in 2014/15 (240 ± 8.19 seedlings m^{-2}) than in 2013/14 (218 ± 5.57 seedlings m^{-2}) ($P < 0.05$). At both sampling dates, LAI was on average higher in 2014 (2.30 ± 0.08 and 3.12 ± 0.11 for the first and second date respectively) than in 2015 (1.12 ± 0.09 and 1.22 ± 0.08) ($P < 0.05$). LAI was positively correlated with final plant height and crop above ground biomass in spring and at harvest (Fig. 2 and Table 5).

By combining results of the PCA and correlation analysis with cultivar identity, four groups of cultivars emerged:

(i) The two heritage cultivars Gentil Rosso and Verna were associated with high biomass accumulation at flowering and physiological maturity, high LAI and high final plant height.
(ii) A group of modern cultivars (Albachiara, Blasco, Bolero, Artico, A146) and the heritage cv. Autonomia A, were associated with high plant height and high biomass accumulation earlier in the season, negatively associated with the tillering index and unrelated to final plant height and biomass accumulation at physiological maturity.
(iii) Another group of modern cultivars (Isengrain, Katou and Altezza) was positively associated with tillering index, had prostrate growth habit, low biomass accumulation and low canopy height early in the season and were unrelated to final plant height and biomass accumulation at physiological maturity.
(iv) Cv. Bramante did not appear to cluster with the others, showing intermediate values for all traits included in the PCA.

3.1.2. Differences between mixtures and their component cultivars

The same traits used to differentiate the cultivars were used to study the effect of diversity in the wheat mixtures. Although mixtures were planned by taking into account end-use quality classes, none of them was completely homogeneous for heading date, as clearly shown by the earliness index (Table 6). Among the three-cultivar mixtures, the one composed of heritage cultivars was the most heterogeneous in terms of heading date, with cv. Autonomia A showing a very early heading date and cvs Gentil Rosso and Verna a very late one. The six- and twelve-cultivar mixtures were also highly heterogeneous in terms of heading date.

In both years, winter growth habit (erectophile to prostrate) of the mixtures corresponded to the most frequent growth habit among the component cultivars. Indeed, all the mixtures had an erectophile growth habit (value = 2) except the three-cultivar mixture composed of bread-making cultivars which had an intermediate growth habit (value = 3), because two of the component cultivars were planophile.

Above-ground wheat biomass in the mixtures did not differ from the mid-value in March, May and at harvest in both years (Fig. 3), except for a few inconsistent mixture effects occasionally detected (Appendix A, Table A1). In the first year, the twelve-cultivar mixture had on average a 12.6% higher biomass than all other treatments at the three sampling dates, but this effect was not confirmed in the second year. A significant mixture effect was detected in seven more cases, of which four indicated an increase of wheat biomass in the mixture compared to the mid-value and three a decrease. Single stands of heritage cultivars and their mixture had an average 28% higher biomass than single stands of biscuit-making cultivars and their mixture ($P < 0.05$ for all three samplings in year 1 and for the third in year 2). In both years, at the third sampling date, biscuit-making and heritage cultivars taken together had an average 18% higher biomass than wheat stand types composed of bread-making cultivars ($P < 0.05$).

Wheat stands showed limited differences in LAI in April and May (Appendix A, Table A2). The only mixture that showed significantly higher LAI than its mid-value was the three-cultivar mixture of superior bread-making cultivars (+65.8%) but only in year 2. Two additional significant mixture effects were detected, both positive (Appendix A, Table A2).

Clearer differences in LAI were related to a cultivar identity effect. Within heritage cultivars, cvs Gentil Rosso and Autonomia A had an average 60% higher LAI in April than cv. Verna ($P < 0.05$ in year 1 and $P = 0.08$ in year 2). However, in May cvs Verna and Gentil Rosso had an average 59% higher LAI than cv. Autonomia A ($P < 0.05$). On average, heritage cultivars had a significantly higher ($P < 0.05$) LAI than biscuit-making cultivars in April and than cultivars belonging to all three end-use groups in May.

3.2. Weed suppression

As far as weed suppression is concerned, in terms of crop's ability to reduce both weed density in winter and weed biomass throughout the

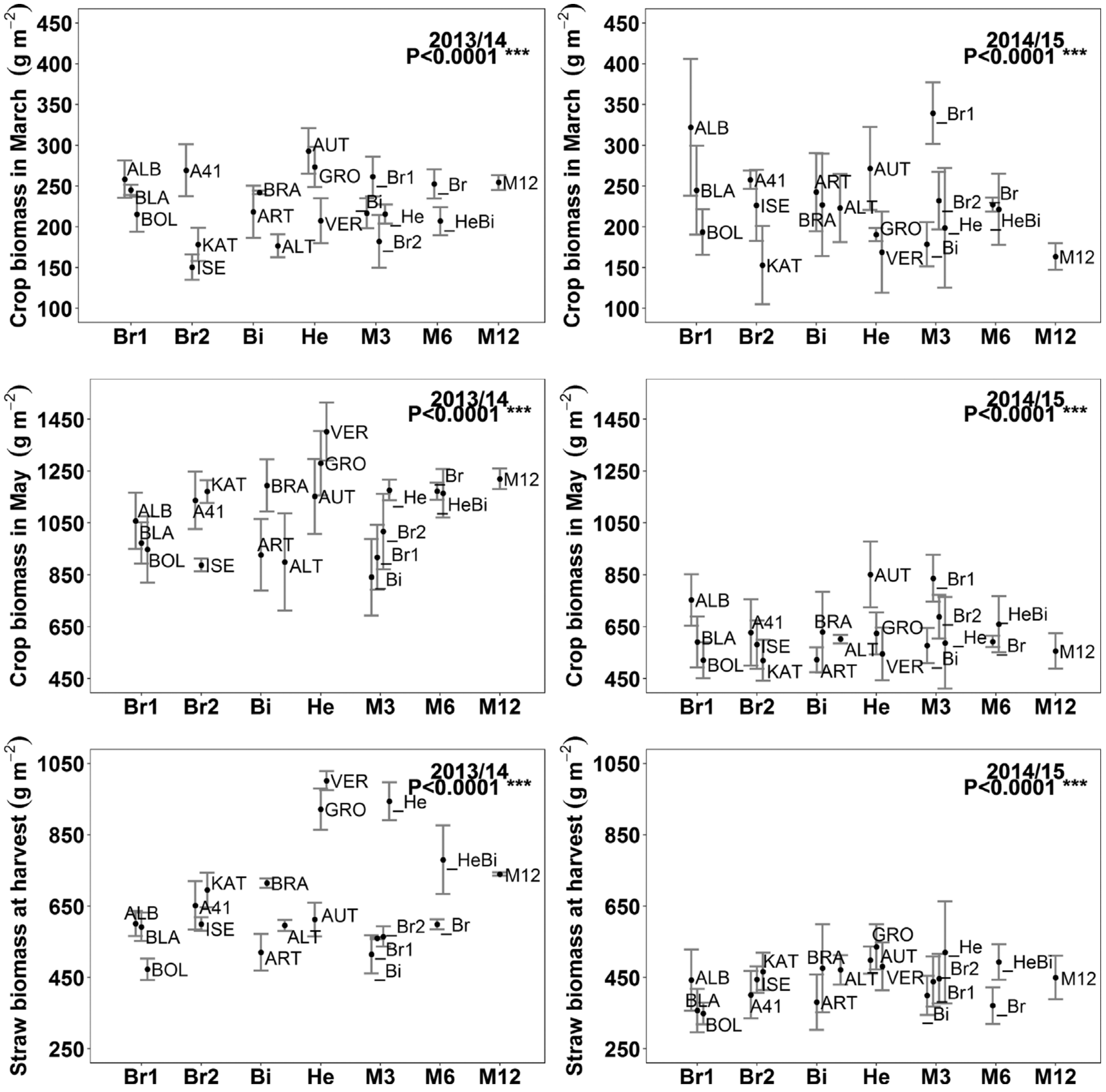

Fig. 3. Above ground wheat biomass (g m^{-2}) in March (top graphs) and May (middle graphs), and straw biomass at harvest (bottom graphs) in the 2013/14 and 2014/15 growing seasons. Codes on the y axis represent different cultivar end use classes (Br1 = superior bread-making cultivars, Br2 = ordinary bread-making cultivars, Bi = biscuit-making cultivars, He = heritage cultivars, M3 = three-cultivar mixtures, M6 = six-cultivar mixtures, M12 = twelve-cultivar mixture). Codes in the graphs represent cultivars and mixtures (ALB = Albachiara, BLA = Blasco, BOL = Bolero, M3_Br1 = Albachiara + Blasco + Bolero, A41 = A416, ISE = Isengrain, KAT = Katou, M3_Br2 = A416 + Isengrain + Katou, M6_Br = Albachiara + Blasco + Bolero + A416 + Isengrain + Katou, ALT = Altezza, ART = Artico, BRA = Bramante, M3_Bi = Altezza + Artico + Bramante, AUT = Autonomia A, GRO = Gentil Rosso, VER = Verna, M3_He = Autonomia A + Gentil Rosso + Verna, M6_HeBi = Altezza + Artico + Bramante + Autonomia A + Gentil Rosso + Verna, M12 = all 12 cultivars). Within mixture, symbols and error bars representing individual cultivars are not aligned vertically to improve figure readability. Bars are standard errors of the means. ***$P < 0.001$.

growing season, only a few significant differences that could be ascribed to a mixture effect emerged.

Weed density was influenced by wheat stand type in both years ($P < 0.05$). In particular, a clear effect emerged within heritage cultivars. As a two-year average, weed density was 24% and 44% lower in cv. Gentil Rosso than in cv. Verna and cv. Autonomia A, respectively ($P = 0.07$). Moreover, in year 2 heritage cultivars had on average 11% lower weed density than the other three end-use classes ($P < 0.05$). The other significant effects detected on weed density were inconsistent between years (Appendix B, Table B1).

On average, total weed biomass was 18% lower in year 2 compared to year 1 at BBCH 30 (4.47 ± 0.40 g m^{-2} *vs* 5.49 ± 0.85 g m^{-2}) whilst an opposite pattern was observed at later stages. In year 1 weed biomass was 15% lower at BBCH 60/69 (8.27 ± 1.57 g m^{-2} *vs* 9.69 ± 0.94 g m^{-2} in year 2) and 56% lower at wheat physiological maturity (10.63 ± 1.27 g m^{-2} vs 24.01 ± 4.30 g m^{-2} in year 2). Wheat stand type affected total weed biomass ($P < 0.05$) at all sampling times except in spring of the first year (Fig. 4 and Appendix B, Table B2).

In year 2, the twelve-cultivar mixture had on average 65% lower weed biomass at physiological maturity than the other wheat stand types ($P < 0.05$). None of the six- and three-cultivar mixtures suppressed weed biomass better than their mid-value in either years except the six-cultivar mixture between heritage and biscuit-making cultivars at the first sampling, but with inconsistent effects between years (Appendix B, Table B2). In contrast, in both years the suppressive effect of cv. Gentil Rosso and cv. Verna was confirmed also in terms of weed biomass. On average, these two cultivars reduced weed biomass by 85 and 72% compared to cv. Autonomia A in year 1 and 2 respectively ($P < 0.05$ in 2013/14 and $P = 0.10$ in 2014/15). In year 2 weed

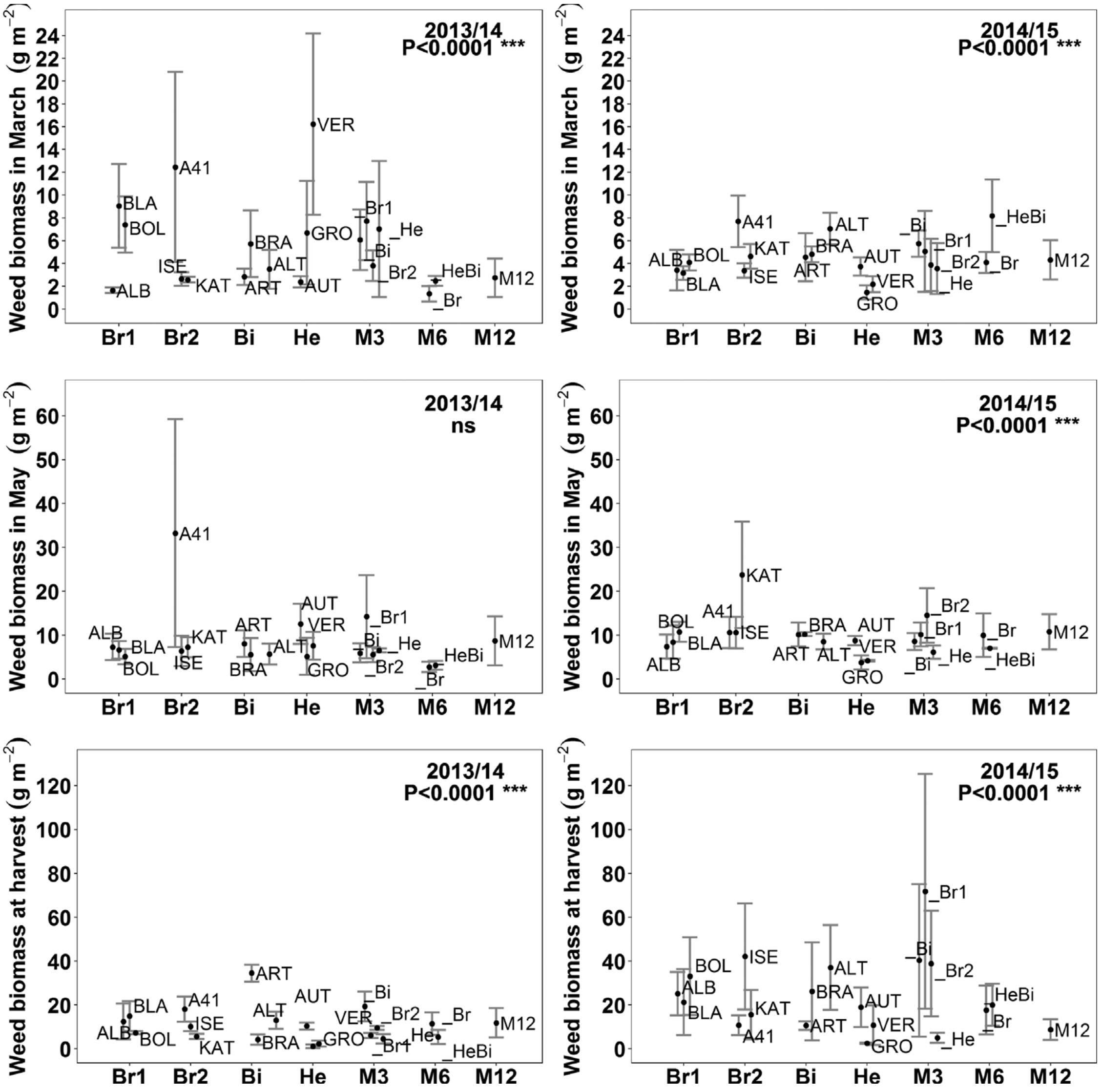

Fig. 4. Weed biomass (g m^{-2}) in March (top graphs), May (middle graphs), and at harvest (bottom graphs) in the 2013/14 and 2014/15 growing seasons. Br1 = superior bread-making cultivars, Br2 = ordinary bread-making cultivars, Bi = biscuit-making cultivars, He = heritage cultivars, M3 = three-cultivar mixtures, M6 = six-cultivar mixtures, M12 = twelve-cultivar mixture. The codes on the y axis represent different cultivar end use classes (Br1 = superior bread-making cultivars, Br2 = ordinary bread-making cultivars, Bi = biscuit-making cultivars, He = heritage cultivars, M3 = three-cultivar mixtures, M6 = six-cultivar mixtures, M12 = twelve-cultivar mixture). The codes on the graphs represent cultivars and mixtures (ALB = Albachiara, BLA = Blasco, BOL = Bolero, M3_Br1 = Albachiara + Blasco + Bolero, A41 = A416, ISE = Isengrain, KAT = Katou, M3_Br2 = A416 + Isengrain + Katou, M6_Br = Albachiara + Blasco + Bolero + A416 + Isengrain + Katou, ALT = Altezza, ART = Artico, BRA = Bramante, M3_Bi = Altezza + Artico + Bramante, AUT = Autonomia A, GRO = Gentil Rosso, VER = Verna, M3_He = Autonomia A + Gentil Rosso + Verna, M6_HeBi = Altezza + Artico + Bramante + Autonomia A + Gentil Rosso + Verna, M12 = all 12 cultivars). Within mixture, symbols and error bars representing individual cultivars are not aligned vertically to improve figure readability. Bars are standard errors of the means. ***$P < 0.001$; ns = non significant.

biomass at harvest was 38% lower in the biscuit-making or heritage cultivars and their mixtures than in the bread-making cultivars and their mixtures ($P = 0.05$). Among the first, the three-component mixture composed of heritage and the single stand heritage cultivars had an average 75% lower weed biomass than single stand biscuit-making cultivars and their mixture ($P < 0.05$ in both years). This effect was shown throughout the growing season in year 2 and only at harvest in year 1 (Appendix B, Table B2).

Weed density and weed biomass were negatively correlated with LAI, plant height at different growth stages and crop biomass (Table 7). Unexpectedly, weed density and weed biomass at the first sampling date were positively correlated with the tillering index, and weed biomass was positively correlated with straw biomass at harvest.

3.3. Yield and yield components

Grain yield of the twelve-cultivar mixture was 13.5% higher than the average of all other wheat stand types in year 1 ($P < 0.05$, Fig. 5). There was no other significant mixture effect except for the six-cultivar mixture with superior and ordinary bread-making cultivars, which had a 9.4% higher yield than its mid-value in year 1 (Appendix C, Table C1). Differences among cultivar types were less evident in year 2, when grain yield was uniformly low. On average, biscuit-making cultivars and its three-component mixture yielded 40% more than heritage cultivars and its three-component mixture ($P < 0.05$). Within each end-use class, there was further variability in grain yield. In the biscuit-making class cvs Altezza and Bramante showed a significantly higher

Table 7
Pearson correlation coefficients (r) between crop traits and weed density or biomass (only r values significant at $P < 0.05$ are shown, ns = non significant).

	Weed density GS30	Weed biomass GS30	Weed biomass GS60/69	Weed biomass GS92
B_Mar	ns	ns	ns	ns
H_Feb	ns	ns	ns	ns
Tillers	0.20	ns	ns	0.26
G_hab	ns	ns	ns	ns
H_Apr	−0.43	ns	ns	−0.24
B_May	−0.47	ns	ns	−0.28
S_harv	−0.44	0.20	ns	−0.40
H_fin	ns	ns	ns	−0.28
LAI_Apr	−0.54	ns	ns	−0.36
LAI_May	−0.63	ns	ns	−0.34
W_se	0.17	ns	ns	ns

B_Mar: wheat above ground biomass in March; H_Feb: plant height in February; Tillers: No. tillers plant^{-1} measured before the onset of the stem elongation phase; G_hab: canopy growth habit in winter; H_Apr: plant height in April; B_May: wheat above ground biomass in May; S_harv: wheat straw biomass at harvest; H_fin: final plant height excluding awns; LAI_Apr, LAI_May: Leaf area indices in April and May respectively; W_se: emerged seedlings m^{-2}.

yield than cv. Artico (on average +28%, $P < 0.05$). In the heritage class cv. Autonomia A yielded more than cvs Verna and Gentil Rosso in both years (on average +29%, $P < 0.05$). These latter two were consistently ranked among the less-yielding cultivars (Fig. 5).

When pooling data of the two years, weed density and weed biomass at harvest were negatively correlated with wheat grain yield (−0.44 and −0.19 respectively), with values in the correspondent linear regression of $P < 0.05$, slope = −0.006, $R^2 = 0.18$, and $P = 0.05$, slope = −0.008, $R^2 = 0.03$. Weed density and weed biomass were also negatively correlated to number of spikes m^{-2} (−0.26 and −0.22 respectively), with values in the correspondent linear regression of $P < 0.05$, slope = −0.23, $R^2 = 0.06$, and $P < 0.05$, slope = −0.59, $R^2 = 0.04$. In both years, mixtures did not perform differently from their mid-values for TKW (Appendix C, Table C2) except the twelve-cultivar mixture (higher TKW than all other wheat stand types), the six-cultivar mixture with heritage and biscuit-making cultivars (higher TKW than its mid-value), and the mixture of three heritage cultivars (lower TKW than its mid-value in year 2). The other yield differences observed between cultivars likely depended on the genetic basis of the trait and on their different susceptibility to fusarium head blight, which was present in both years (data not shown).

3.4. Grain quality

In both years, wheat mixtures did not perform differently from their mid-value for test weight, except the mixture of three biscuit-making cultivars, which showed higher values in 2014 (+4.9%, $P < 0.05$) and lower values in 2015 (−1.2%, $P < 0.05$), and the twelve-cultivar mixture, which outperformed all other wheat stand types in 2015 (+1.2%, $P < 0.05$) (Appendix D, Table D1). Test weight of each wheat stand type is shown in Fig. 6. Unlike 2014, in 2015 values were always above the commercial threshold of 75 kg 100 L^{-1}.

Regarding whole grain protein content (Fig. 6 and Appendix D, Table D2), there was no significant mixture effect in 2014. Instead, in 2015 the twelve-cultivar mixture and the mixture of three ordinary bread-making cultivars had 6.5 and 6.3% higher protein content than their mid-value respectively ($P < 0.05$). Wheat stand types containing only heritage cultivars had the highest whole grain protein content in both years (Fig. 6 and Appendix D, Table D2).

Regarding grain starch content (Fig. 6, Appendix D, Table D3) the only significant effect ascribed to mixture was found in year 1, when the mixture of three ordinary bread-making cultivars had a 1.3% higher starch content than its mid-value. Averaged over years, wheat stand types containing bread-making or biscuit-making cultivars had a 3.7% higher whole grain starch content ($P < 0.05$) than those containing only heritage cultivars.

3.5. Overall performance

The Friedman test on the pooled data of the two years was significant ($P = 0.02$), meaning that there were differences in the overall performance of wheat stand types (Fig. 7). Wheat stand ranking based on the traits taken into account for the target agroecosystem services showed that both extremes were represented by monovarietal stands. The best performing genotype was cv. Albachiara while cv. Artico was the worst. There was no evidence of a better overall performance of the three-cultivar mixtures compared to their components (M3 *vs* CV in Fig. 7, Wilcoxon test, $P > 0.05$), and their position in the ranking was intermediate. In contrast, the mixtures of higher diversity (six- and twelve-cultivar) had usually a higher ranking compared to the average rank of all monovarietal stands and of the three-cultivar mixtures (M6 + M12 *vs* M3 + CV in Fig. 7, Wilcoxon test, $P = 0.06$).

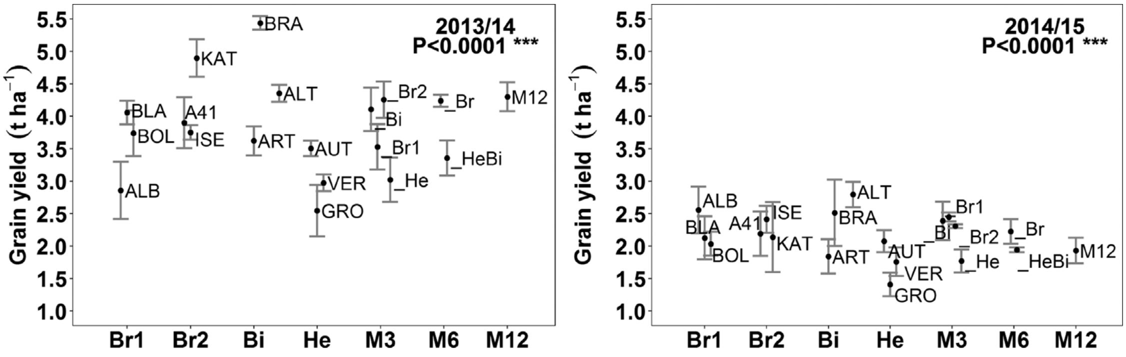

Fig. 5. Wheat grain yield in the 2013/14 and 2014/15 growing seasons. Br1 = superior bread-making cultivars, Br2 = ordinary bread-making cultivars, Bi = biscuit-making cultivars, He = heritage cultivars, M3 = three-cultivar mixtures, M6 = six-cultivar mixtures, M12 = twelve-cultivar mixture. The codes on the y axis represent different cultivar end use classes (Br1 = superior bread-making cultivars, Br2 = ordinary bread-making cultivars, Bi = biscuit-making cultivars, He = heritage cultivars, M3 = three-cultivar mixtures, M6 = six-cultivar mixtures, M12 = twelve-cultivar mixture). The codes on the graphs represent cultivars and mixtures (ALB = Albachiara, BLA = Blasco, BOL = Bolero, M3_Br1 = Albachiara + Blasco + Bolero, A41 = A416, ISE = Isengrain, KAT = Katou, M3_Br2 = A416 + Isengrain + Katou, M6_Br = Albachiara + Blasco + Bolero + A416 + Isengrain + Katou, ALT = Altezza, ART = Artico, BRA = Bramante, M3_Bi = Altezza + Artico + Bramante, AUT = Autonomia A, GRO = Gentil Rosso, VER = Verna, M3_He = Autonomia A + Gentil Rosso + Verna, M6_HeBi = Altezza + Artico + Bramante + Autonomia A + Gentil Rosso + Verna, M12 = all 12 cultivars). Within mixture, symbols and error bars representing individual cultivars are not aligned vertically to improve figure readability. Bars are standard errors of the means. ***$P < 0.001$.

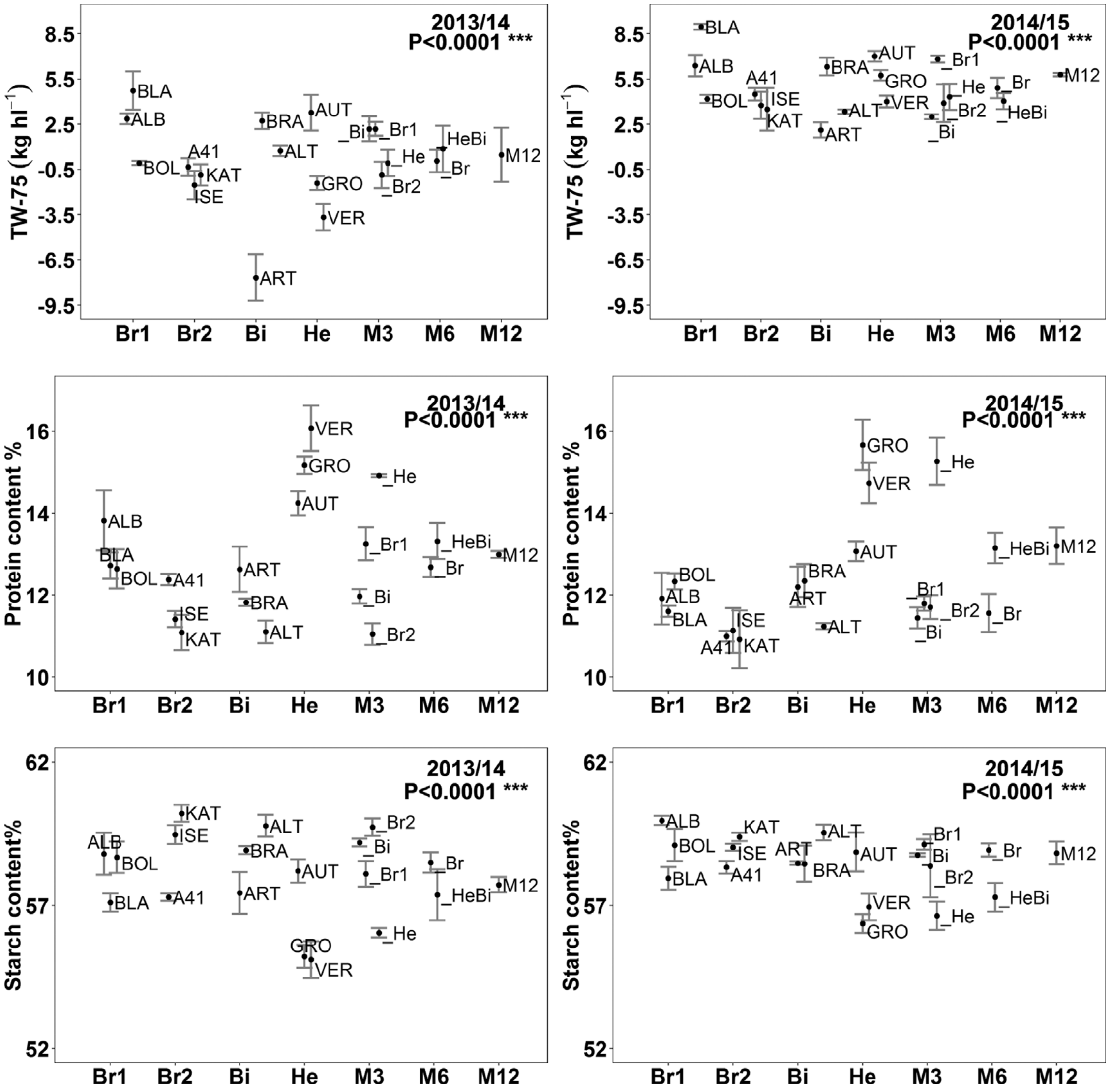

Fig. 6. Test weight (top graphs), whole grain protein content (middle graphs) and whole grain starch content (bottom graphs) in the 2013/14 and 2014/15 growing seasons. Br1 = superior bread-making cultivars, Br2 = ordinary bread-making cultivars, Bi = biscuit-making cultivars, He = heritage cultivars, M3 = three-cultivar mixtures, M6 = six-cultivar mixtures, M12 = twelve-cultivar mixtures. The codes on the y axis represent different cultivar end use classes (Br1 = superior bread-making cultivars, Br2 = ordinary bread-making cultivars, Bi = biscuit-making cultivars, He = heritage cultivars, M3 = three-cultivar mixtures, M6 = six-cultivar mixtures, M12 = twelve-cultivar mixture). The codes on the graphs represent cultivars and mixtures (ALB = Albachiara, BLA = Blasco, BOL = Bolero, M3_Br1 = Albachiara + Blasco + Bolero, A41 = A416, ISE = Isengrain, KAT = Katou, M3_Br2 = A416 + Isengrain + Katou, M6_Br = Albachiara + Blasco + Bolero + A416 + Isengrain + Katou, ALT = Altezza, ART = Artico, BRA = Bramante, M3_Bi = Altezza + Artico + Bramante, AUT = Autonomia A, GRO = Gentil Rosso, VER = Verna, M3_He = Autonomia A + Gentil Rosso + Verna, M6_HeBi = Altezza + Artico + Bramante + Autonomia A + Gentil Rosso + Verna, M12 = All 12 cultivars). Within mixture, symbols and error bars representing individual cultivars are not aligned vertically to improve figure readability. Bars are standard errors of the means. ***P < 0.001.

4. Discussion and conclusion

Overall, our results show that, in the context studied and with the component compositions tested, cultivar mixtures are unlikely to improve provision of single agro-ecosystem services when compared to their individual component cultivars. Significant differences that could be ascribed to the mixture effect in terms of weed suppression, grain yield and grain quality were limited and not always consistent between years. These results do not allow a clear-cut conclusion on the diversity hypothesis, according to which increased diversity for a given trait in the crop stand should improve the provision of the target service through a niche differentiation effect.

However, when the overall crop performance (i.e. weed suppression, yield and grain quality taken altogether) was analysed, a clearer relationship between diversity and provision of the target agroecosystem services was enlightened. In particular, cultivar mixtures appeared a good strategy to improve overall crop performance but only when high diversity mixtures (of six or twelve cultivars) were used. These results answer positively to the hypothesis that increasing trait diversity in the crop stand can support the improvement of overall crop performance.

For weed suppression, instead, the identity of individual cultivars had a stronger effect than diversity. In particular, two heritage cultivars showed a steadily high above-ground biomass and a taller canopy from the stem extension phase onwards. These were the most suppressive crop stand types in our experiment. These results support the role of functional identity and mass-ratio hypothesis in the case of weed suppression.

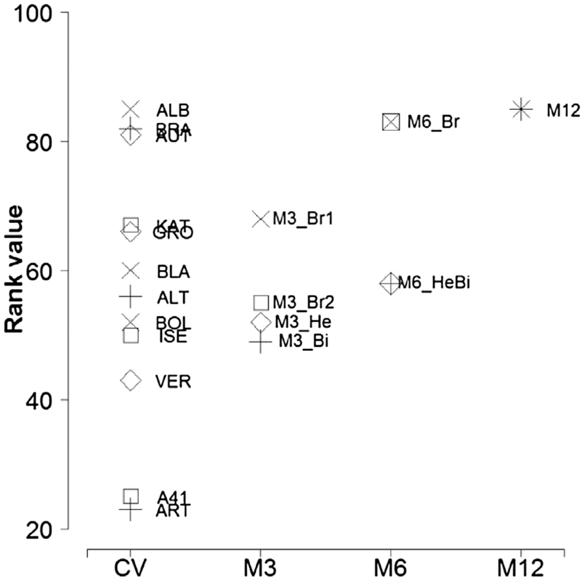

Fig. 7. Average rank value based on overall wheat performance (i.e. by taking the three target agroecosystem services altogether) for single cultivars (CV), three-cultivar mixtures (M3), six-cultivar mixtures (M6) and the twelve-cultivar mixture (M12). The codes on the graph represent cultivars and mixtures (ALB = Albachiara, BLA = Blasco, BOL = Bolero, M3_Br1 = Albachiara + Blasco + Bolero, A41 = A416, ISE = Isengrain, KAT = Katou, M3_Br2 = A416 + Isengrain + Katou, M6_Br = Albachiara + Blasco + Bolero + A416 + Isengrain + Katou, ALT = Altezza, ART = Artico, BRA = Bramante, M3_Bi = Altezza + Artico + Bramante, AUT = Autonomia A, GRO = Gentil Rosso, VER = Verna, M3_He = Autonomia A + Gentil Rosso + Verna, M6_HeBi = Altezza + Artico + Bramante + Autonomia A + Gentil Rosso + Verna, M12 = All 12 cultivars). The analysed dataset contained 114 entries, so the maximum rank value for a single plot is 114. For detailed explanation on the calculation, refer to materials and methods.

Negative correlations between weed growth (both density and biomass) on one side and wheat final height, above ground biomass accumulation and LAI on the other side confirm that these traits are good predictors of weed suppressive ability (Asif et al., 2014; Hoad et al., 2012). Unexpectedly, the tillering index was (although slightly) positively correlated with both weed density and biomass, unlike what has been reported in previous works (Challaiah et al., 1986; Wicks et al., 2004). This might be explained by two findings highlighted in our work:

(i) The association of high tillering index with low early growth rate can be more detrimental to weed suppressive ability in Mediterranean than in cooler climates, where most previous studies were carried out;
(ii) The negative correlation between wheat establishment and tillering index suggest that both higher tillering and higher weed abundance may have been a consequence of suboptimal crop establishment.

Overall, our results suggest that the most important phase for crop-weed competition, especially in terms of weed suppression ability, in autumn-sown common wheat under Mediterranean conditions is around the stem elongation phase. The ability of wheat to occupy space in this phase determines the main effects on weed suppression ability. In particular, a high soil cover, linked to higher crop biomass and height, is necessary for successfully competing with weeds for light. This is in line with the current agronomic practice in the study area where, both in conventional and in organic farming, weed control (either chemical or mechanical) is applied − if weather conditions allow − just before the onset of the stem elongation phase (Geminiani and Campagna, 2015). In our experiment, successful weed suppression was only achieved by cv. Gentil Rosso − whose superiority over the other cultivars was already evident before the stem elongation phase − and by cv. Verna. However, these two cultivars represent an ideotype in which high competitive ability is associated with very tall canopy at maturity and low harvest index, two traits which either increase the risk of lodging or do not match with current expectations by most European farmers that, for example, have little or no interest in straw as a by-product.

To our knowledge, the only other available study on weed suppression in wheat mixtures is the one of Kaut et al. (2009) in western Canada. In that experiment total weed biomass did not differ between entries and the mixtures did not suppress weeds better than their individual components. However, this work reported weed tolerance effects associated with higher early vigour and tillering. Instead, in our work morphological traits associated with the stem extension phase were likely more important.

Sage (1971) reported that a wheat mixture with components highly differing in height and earliness would better compete with weeds, at least at a low sowing rate. Our experiment, although limited to a standard sowing rate, did not confirm this trend. The three-cultivar mixture composed of heritage cultivars was highly diversified for earliness, and the six-cultivar mixture composed of heritage and biscuit-making cultivars was highly diversified for both height and earliness, but neither of them suppressed weeds any better than their respective components.

In our study, there was a marked difference in grain yield between the two years, with higher values in the first than in the second. Lower yield in year 2 depended upon a combination of lower number of fertile tillers, lower number of spikes at harvest and lower number of seeds spike^{-1} although, at end of the tillering phase, the N status of the crop was better in 2014/15 than in the previous season. Furthermore, wheat stands in year 2 were characterised by less uniform crop establishment and higher weed infestation. Suboptimal soil and weather conditions may have contributed to this yield decrease. In fact, in the 2014/15 field the soil had ca. 10% more clay that caused evident soil cracking. Moreover, average temperatures in April were higher in year 2, possibly contributing to reduced spikelet fertility due to heat stress (Dolferus et al., 2011).

For grain yield and grain quality the dominant effects were in line with the expectations based on end use cultivar groups. Bread-making cultivars yielded less than biscuit-making ones (Foca et al., 2007). Similarly, heritage cultivars showed lower grain yield than modern cultivars (Ormoli et al., 2015). In contrast, grain protein content was, as expected, higher in heritage cultivars (Ormoli et al., 2015), and lower in bread-making and biscuit-making cultivars. However, modern cultivars had higher whole grain starch content than heritage cultivars, likely resulting in higher flour production during milling. No consistent mixture effects were detected as far as grain quality was concerned.

Although wheat mixtures did not show an evident effect in terms of either weed suppression, grain yield or grain quality taken alone, their positive effect was evident when looking at the overall crop performance, i.e. considering the three target agroecosystem services altogether. Also all mixtures performed better than the worst performing cultivars for each variable considered. These results support the existence of a diversity-driven ‘buffering effect’, which is important for farmers wishing to minimise the unpredictable effect of season on crop performance. This buffering effect may suggest the existence of an ‘insurance effect’, that can stabilise wheat performance across environments and climatic conditions (Yachi and Loreau, 1999). This could not be tested here due to lack of a time series. Cv Albachiara had a rank value for overall performance similar to that of the twelve-cultivar mixture; however, if we consider the overall performance and buffering effect together, the use of the mixture should be preferred to that of the single cultivar to exploit the buffering effect. As reported in Mundt et al. (1995) and in Newton et al. (1997) for disease control and yield, wheat mixtures with a higher number of components show a tendency to perform better. In our case, only the mixtures of six and twelve cultivars outperformed the average of individual components. As to this, we have to consider the constraint of our study due to the use of cultivars of the same end-use group in the three-cultivar mixtures and the minimization of end-use diversity in the six-cultivar mixtures. This constraint reduced the available diversity for the target traits, especially in the three-component mixtures arrangement. This decision was driven by the aim to study mixtures whose grain yield would be acceptable for sale on the general market. If wheat production would be directed to alternative food networks, short food supply chains or local food systems, that are increasingly attractive for organic agricultural products (Favilli et al., 2015), it could be possible to overcome the constraints posed by current end-use categories for marketing wheat grain and/or flour. Alternative food systems are not oriented to industrial transformation and do not need highly standardized ingredients for transformation (Migliorini et al., 2016). They are rather oriented to local organic markets where most consumers are sensitive to buying artisanal products with clear links with a territory and with sustainable production methods (Seyfang, 2006). In these types of food systems, consumers are keener to accept products that change according to the specific biotic and abiotic conditions encountered in each growing season. In this context, a higher wheat stand diversity may be achieved by mixing cultivars belonging to different end-use categories and, as such, by better exploiting diversity in the available bread wheat germplasm without necessarily increasing the number of components in the mixture

Moreover, in our experiment the crop was grown in rows (the common practice), while weed suppression is maximised when the crop plants are placed at equal distance from each other (Olsen et al., 2012). In our case, the seeds of each cultivar are not randomly present on the soil surface and there might be clusters where one cultivar is over- or under-abundant. This implies that the effects reported on weed suppression from our case might not be the highest obtainable with the mixtures studied. Nevertheless, it is worth mentioning that patchy mixtures (with seeds of component varieties deliberately not mixed up into the driller hopper) proved to reduce diseases better than randomly sown mixtures (Newton and Guy, 2009). Overall, sowing patterns and (un)evenness of component varieties distribution are open doors to further investigate how cultivar mixtures can improve agro-ecosystem services including weed reduction.

Our work also highlights how the choice of the component cultivars in common wheat mixtures should be targeted to the environment and the priority agroecosystem services (Costanzo and Bàrberi, 2014). In literature, cultivar mixtures have proven useful in improving specific outputs (e.g. yield, yield stability, grain quality or disease reduction), although this was not always the case and not with any component combination, and in stabilizing yield. Given the high variability encountered in agriculture and the unpredictable conditions of a growing season, it would always be advisable to use a more diverse crop stand for relying on the insurance effect. However, this is not a sufficient reason to advocate blanket adoption of this strategy. For example, mixing a high number of cultivars (≥6), as suggested by our results, may be practically difficult. In this case, farmers would be supposed to buy seeds of different cultivars or save seeds from individual cultivars to re-constitute their mixture. Re-sowing seeds harvested from a highly diverse mixture could be an option, as far as evolutionary processes do not drive mixture composition towards an undesirable pattern (Knapp et al., 2013). As far as mixtures with a smaller number of cultivars are concerned, our study suggests that one would better not just rely on redundancy effects. Therefore, a clear methodology to predict mixtures performance starting from the selection of key traits of component cultivars to improve provision of target agroecosystem services through enhanced complementarity and synergy is required.

Acknowledgements

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Appendix A. Supplemental material to: 3.1.2 Differences between mixtures and components cultivars

Supplemental material to: 3.1.2 Differences between mixtures and components cultivars

Table A1

Analysis of variance of wheat above ground biomass in March and May, straw biomass at harvest (g m^{-2}). Separate models for 2013/14 and 2014/15 growing seasons were run. The wheat stand type factor was split into the set of eighteen orthogonal linear contrasts presented in Table 4. In bold the significant values ($P < 0.05$) for the contrasts testing the mixture effect. Only models in which the wheat stand type factor was significant ($P < 0.001$) are presented.

	2013/14 Wheat above ground biomass in March					2014/15 Wheat above ground biomass in March				
	Value	Std.Error	DF	t-value	p-value	Value	Std.Error	DF	t-value	p-value
(Intercept)	5.4065	0.0590	36	91.6321	0.0000	5.3600	0.1479	36	36.2346	0.0000
M12 *vs* all other treatments	0.0075	0.0015	36	4.8482	**0.0000**	−0.0148	0.0115	36	−1.2891	0.2056
M6_Br *vs* components plus M3_Br1 and M3_Br2	0.0182	0.0045	36	4.0808	**0.0002**	−0.0016	0.0138	36	−0.1173	0.9072
M3_Br1 *vs* components	0.0220	0.0186	36	1.1786	0.2463	0.0860	0.0420	36	2.0455	**0.0482**
ALB *vs* BLA + BOL	0.0384	0.0213	36	1.8038	0.0796	0.1206	0.0513	36	2.3496	0.0244
BLA *vs* BOL	0.0692	0.0311	36	2.2263	0.0323	0.1048	0.0817	36	1.2824	0.2079
M3_Br2 *vs* components	−0.0203	0.0357	36	−0.5695	0.5725	0.0326	0.0572	36	0.5701	0.5722
KAT *vs* A41 + ISE	−0.0409	0.0315	36	−1.2990	0.2022	−0.1747	0.1278	36	−1.3667	0.1802
ISE *vs* A41	−0.2881	0.0687	36	−4.1959	0.0002	−0.0833	0.0827	36	−1.0083	0.3200
M6_HeBi *vs* components plus M3_Bi and M3_He	−0.0103	0.0088	36	−1.1704	0.2495	0.0066	0.0235	36	0.2788	0.7820
M3_Bi *vs* components	0.0077	0.0188	36	0.4096	0.6846	−0.0563	0.0184	36	−3.0618	**0.0041**
ART *vs* ALT + BRA	0.0109	0.0418	36	0.2614	0.7953	0.0302	0.0525	36	0.5757	0.5684
ALT *vs* BRA	−0.1599	0.0697	36	−2.2947	0.0277	0.0098	0.0892	36	0.1102	0.9128
M3_He *vs* components	−0.0399	0.0159	36	−2.5168	**0.0164**	−0.0302	0.0533	36	−0.5666	0.5745
AUT *vs* GRO + VER	0.0701	0.0377	36	1.8611	0.0709	0.1395	0.0479	36	2.9107	0.0062
GRO *vs* VER	0.1423	0.0884	36	1.6101	0.1161	0.0981	0.1063	36	0.9229	0.3622
Br1 + Br2 *vs* Bi + He	−0.0142	0.0198	36	−0.7150	0.4792	0.0674	0.0379	36	1.7786	0.0838
M3_Br1 + components *vs* M3_Br2 + components	0.1296	0.0287	36	4.5207	0.0001	0.1151	0.0648	36	1.7771	0.0840
M3_Bi + components *vs* M3_He + components	−0.0712	0.0328	36	−2.1728	0.0365	0.0373	0.0472	36	0.7900	0.4347

	2013/14 Wheat above ground biomass in May					2014/15 Wheat above ground biomass in May				
	Value	Std.Error	DF	t-value	p-value	Value	Std.Error	DF	t-value	p-value
(Intercept)	6.9645	0.0367	36	189.7071	0.0000	6.4037	0.1104	36	57.9892	0.0000
M12 *vs* all other treatments	0.0079	0.0026	36	3.0783	**0.0040**	−0.0053	0.0071	36	−0.7434	0.4621
M6_Br *vs* components plus M3_Br1 and M3_Br2	0.0178	0.0038	36	4.6512	**0.0000**	−0.0047	0.0130	36	−0.3589	0.7218
M3_Br1 *vs* components	−0.0208	0.0335	36	−0.6213	0.5383	0.0796	0.0153	36	5.2035	**0.0000**
ALB *vs* BLA + BOL	0.0329	0.0380	36	0.8657	0.3924	0.1032	0.0342	36	3.0197	0.0046
BLA *vs* BOL	0.0193	0.0764	36	0.2526	0.8020	0.0576	0.0880	36	0.6541	0.5172
M3_Br2 *vs* components	−0.0143	0.0380	36	−0.3767	0.7086	0.0491	0.0571	36	0.8610	0.3950
KAT *vs* A41 + ISE	0.0522	0.0170	36	3.0768	0.0040	−0.0473	0.0620	36	−0.7628	0.4506
ISE *vs* A41	−0.1194	0.0440	36	−2.7170	0.0101	−0.0330	0.1416	36	−0.2328	0.8172
M6_HeBi *vs* components plus M3_Bi and M3_He	0.0082	0.0075	36	1.0967	0.2800	0.0089	0.0118	36	0.7510	0.4575
M3_Bi *vs* components	−0.0442	0.0443	36	−0.9978	0.3251	−0.0004	0.0281	36	−0.0148	0.9883
ART *vs* ALT + BRA	−0.0371	0.0540	36	−0.6864	0.4969	−0.0482	0.0384	36	−1.2546	0.2177
ALT *vs* BRA	−0.1585	0.1011	36	−1.5686	0.1255	0.0051	0.0845	36	0.0600	0.9525
M3_He *vs* components	−0.0173	0.0166	36	−1.0468	0.3022	−0.0451	0.0487	36	−0.9260	0.3606
AUT *vs* GRO + VER	−0.0532	0.0405	36	−1.3134	0.1974	0.1273	0.0376	36	3.3848	0.0017
GRO *vs* VER	−0.0472	0.0631	36	−0.7476	0.4596	0.0770	0.1023	36	0.7530	0.4564
Br1 + Br2 *vs* Bi + He	−0.0322	0.0241	36	−1.3354	0.1901	0.0131	0.0310	36	0.4213	0.6761
M3_Br1 + components *vs* M3_Br2 + components	−0.0396	0.0344	36	−1.1515	0.2571	0.0530	0.0516	36	1.0282	0.3107
M3_Bi + components *vs* M3_He + components	−0.1415	0.0415	36	−3.4112	0.0016	−0.0401	0.0434	36	−0.9226	0.3624

	2013/14 Wheat straw biomass at harvest					2014/15 Wheat straw biomass at harvest				
	Value	Std.Error	DF	t-value	p-value	Value	Std.Error	DF	t-value	p-value
(Intercept)	6.4786	0.0162	36	400.1940	0.0000	6.0649	0.0906	36	66.9284	0.0000
M12 *vs* all other treatments	0.0072	0.0008	36	8.7855	**0.0000**	0.0016	0.0083	36	0.1912	0.8495
M6_Br *vs* components plus M3_Br1 and M3_Br2	0.0022	0.0031	36	0.7116	0.4813	−0.0127	0.0137	36	−0.9282	0.3595
M3_Br1 *vs* components	0.0046	0.0095	36	0.4825	0.6324	0.0354	0.0246	36	1.4367	0.1595
ALB *vs* BLA + BOL	0.0427	0.0256	36	1.6691	0.1038	0.0673	0.0506	36	1.3291	0.1922
BLA *vs* BOL	0.1116	0.0447	36	2.4979	0.0172	0.0009	0.0724	36	0.0124	0.9902
M3_Br2 *vs* components	−0.0335	0.0159	36	−2.1055	**0.0423**	0.0034	0.0617	36	0.0555	0.9560
KAT *vs* A41 + ISE	0.0357	0.0273	36	1.3102	0.1984	0.0347	0.0521	36	0.6651	0.5102
ISE *vs* A41	−0.0365	0.0527	36	−0.6922	0.4932	0.0598	0.0899	36	0.6653	0.5101

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Table A1 (*continued*)

	2013/14 Wheat straw biomass at harvest					2014/15 Wheat straw biomass at harvest				
	Value	Std.Error	DF	t-value	p-value	Value	Std.Error	DF	t-value	p-value
M6_HeBi *vs* components plus M3_Bi and M3_He	0.0101	0.0132	36	0.7671	0.4480	0.0082	0.0203	36	0.4047	0.6881
M3_Bi *vs* components	−0.0426	0.0272	36	−1.5629	0.1268	−0.0195	0.0237	36	−0.8223	0.4163
ART *vs* ALT + BRA	−0.0785	0.0318	36	−2.4665	0.0185	−0.0741	0.0460	36	−1.6093	0.1163
ALT *vs* BRA	−0.0910	0.0174	36	−5.2418	0.0000	0.0217	0.0823	36	0.2638	0.7934
M3_He *vs* components	0.0331	0.0156	36	2.1206	**0.0409**	−0.0062	0.0481	36	−0.1280	0.8988
AUT *vs* GRO + VER	−0.1511	0.0274	36	−5.5197	0.0000	−0.0019	0.0187	36	−0.1017	0.9196
GRO *vs* VER	−0.0433	0.0377	36	−1.1493	0.2580	0.0581	0.0547	36	1.0632	0.2948
Br1 + Br2 *vs* Bi + He	−0.0934	0.0155	36	−6.0283	0.0000	−0.0646	0.0297	36	−2.1771	0.0361
M3_Br1 + components *vs* M3_Br2 + components	−0.0601	0.0214	36	−2.8027	0.0081	−0.0582	0.0482	36	−1.2090	0.2346
M3_Bi + components *vs* M3_He + components	−0.1939	0.0232	36	−8.3592	0.0000	−0.0861	0.0379	36	−2.2730	0.0291

Model fitted: lme() with fixed = log(dw_cropX + 1) ~ trt, random = ~1|blk, weights = varIdent(form = ~1|trt), in which trt = wheat stand type level, blk = block, dw_crop = crop biomass.

Table A2

Analysis of variance of Leaf Area Index in April and May. Separate models for 2013/14 and 2014/15 growing seasons were run. The wheat stand type factor was split into the set of eighteen orthogonal linear contrasts presented in Table 4. In bold the significant values ($P < 0.05$) for the contrasts testing the mixture effect. Only models in which the wheat stand type factor was significant ($P < 0.001$) are presented.

	2013/14 Leaf area index in April					2014/15 Leaf area index in April				
	Value	Std.Error	DF	t-value	p-value	Value	Std.Error	DF	t-value	p-value
(Intercept)	1.1742	0.0962	36	12.2056	0.0000	0.7106	0.0976	36	7.2784	0.0000
M12 *vs* all other treatments	0.0088	0.0023	36	3.7365	**0.0006**	−0.0008	0.0051	36	−0.1613	0.8728
M6_Br *vs* components plus M3_Br1 and M3_Br2	0.0092	0.0080	36	1.1442	0.2601	−0.0131	0.0067	36	−1.9645	0.0572
M3_Br1 *vs* components	−0.0061	0.0154	36	−0.3961	0.6944	0.0871	0.0372	36	2.3430	**0.0248**
ALB *vs* BLA + BOL	0.0417	0.0383	36	1.0887	0.2835	0.0883	0.0638	36	1.3833	0.1751
BLA *vs* BOL	0.0808	0.0647	36	1.2484	0.2200	0.0901	0.0967	36	0.9321	0.3575
M3_Br2 *vs* components	−0.0207	0.0136	36	−1.5183	0.1377	−0.0228	0.0678	36	−0.3368	0.7382
KAT *vs* A41 + ISE	0.0214	0.0196	36	1.0965	0.2801	0.0040	0.0605	36	0.0663	0.9475
ISE *vs* A41	−0.0099	0.0572	36	−0.1738	0.8630	0.0917	0.1330	36	0.6895	0.4949
M6_HeBi *vs* components plus M3_Bi and M3_He	−0.0006	0.0088	36	−0.0699	0.9447	0.0154	0.0221	36	0.6966	0.4905
M3_Bi *vs* components	−0.0249	0.0278	36	−0.8945	0.3770	0.0058	0.0304	36	0.1919	0.8489
ART *vs* ALT + BRA	−0.0463	0.0439	36	−1.0534	0.2992	−0.0004	0.0347	36	−0.0118	0.9906
ALT *vs* BRA	−0.1126	0.0293	36	−3.8495	0.0005	−0.0800	0.0964	36	−0.8301	0.4119
M3_He *vs* components	−0.0047	0.0214	36	−0.2204	0.8268	−0.0464	0.0441	36	−1.0506	0.3004
AUT *vs* GRO + VER	−0.0224	0.0245	36	−0.9143	0.3666	0.0777	0.0355	36	2.1855	0.0354
GRO *vs* VER	0.1425	0.0490	36	2.9084	0.0062	0.1655	0.0919	36	1.8017	0.0800
Br1 + Br2 *vs* Bi + He	−0.0014	0.0176	36	−0.0807	0.9361	0.0266	0.0343	36	0.7753	0.4432
M3_Br1 + components *vs* M3_Br2 + components	0.0874	0.0255	36	3.4331	0.0015	0.1161	0.0599	36	1.9384	0.0604
M3_Bi + components *vs* M3_He + components	−0.0976	0.0276	36	−3.5423	0.0011	−0.0895	0.0420	36	−2.1292	0.0402

	2013/14 Leaf area index in May					2014/15 Leaf area index in May				
	Value	Std.Error	DF	t-value	p-value	Value	Std.Error	DF	t-value	p-value
(Intercept)	1.3999	0.0226	36	61.8345	0.0000	0.7650	0.0515	36	14.8562	0.0000
M12 *vs* all other treatments	0.0063	0.0037	36	1.6870	0.1003	0.0024	0.0036	36	0.6630	0.5115
M6_Br *vs* components plus M3_Br1 and M3_Br2	0.0099	0.0027	36	3.6323	**0.0009**	−0.0088	0.0083	36	−1.0585	0.2969
M3_Br1 *vs* components	−0.0078	0.0098	36	−0.7924	0.4333	0.0621	0.0221	36	2.8097	**0.0080**
ALB *vs* BLA + BOL	0.0322	0.0224	36	1.4355	0.1598	0.0484	0.0217	36	2.2299	0.0321
BLA *vs* BOL	−0.0190	0.0324	36	−0.5878	0.5604	0.0123	0.0306	36	0.4019	0.6901
M3_Br2 *vs* components	−0.0054	0.0153	36	−0.3511	0.7276	−0.0207	0.0575	36	−0.3600	0.7210
KAT *vs* A41 + ISE	0.0475	0.0347	36	1.3693	0.1794	0.1212	0.0271	36	4.4689	0.0001
ISE *vs* A41	0.0466	0.0265	36	1.7557	0.0876	0.1247	0.0813	36	1.5334	0.1339
M6_HeBi *vs* components plus M3_Bi and M3_He	0.0009	0.0059	36	0.1443	0.8861	0.0174	0.0120	36	1.4454	0.1570
M3_Bi *vs* components	−0.0173	0.0124	36	−1.3901	0.1730	0.0004	0.0254	36	0.0145	0.9885

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Table A2 (*continued*)

	2013/14 Leaf area index in May					2014/15 Leaf area index in May				
	Value	Std.Error	DF	t-value	p-value	Value	Std.Error	DF	t-value	p-value
ART *vs* ALT + BRA	−0.0636	0.0357	36	−1.7830	0.0830	−0.0865	0.0414	36	−2.0891	0.0438
ALT *vs* BRA	−0.0871	0.0339	36	−2.5690	0.0145	−0.2097	0.1140	36	−1.8403	0.0740
M3_He *vs* components	−0.0059	0.0174	36	−0.3409	0.7351	0.0160	0.0549	36	0.2913	0.7725
AUT *vs* GRO + VER	−0.1356	0.0366	36	−3.6991	0.0007	−0.0740	0.0273	36	−2.7097	0.0102
GRO *vs* VER	0.0578	0.0666	36	0.8671	0.3917	0.0626	0.0672	36	0.9324	0.3574
Br1 + Br2 *vs* Bi + He	−0.0630	0.0146	36	−4.3154	0.0001	−0.0587	0.0266	36	−2.2056	0.0339
M3_Br1 + components *vs* M3_Br2 + components	−0.0183	0.0192	36	−0.9538	0.3466	−0.0278	0.0376	36	−0.7393	0.4645
M3_Bi + components *vs* M3_He + components	−0.1435	0.0260	36	−5.5241	0.0000	−0.1478	0.0442	36	−3.3471	0.0019

Model fitted: lme() with fixed = fixed = log(LAIX + 1) ~ trt, random = ~1|blk, weights = varIdent(form = ~1|trt), in which trt = wheat stand type level, blk = block, LAI = Leaf area index.

Appendix B. Supplemental material to: 3.2 Weed suppression

Supplemental material to: 3.2 Weed suppression

Table B1

Analysis of variance of total weed density at end of winter (plants m^{-2}). Separate models for 2013/14 and 2014/15 growing seasons were run. The wheat stand type factor was split into the set of eighteen orthogonal linear contrasts presented in Table 4. In bold the significant values ($P < 0.05$) for the contrasts testing the mixture effect. Only models in which the wheat stand type factor was significant ($P < 0.001$) are presented.

	2013/14 Weed density at GS30				2014/15 Weed density at GS30			
	Estimate	Std. Error	value	Pr(> |z|)	Estimate	Std. Error	value	Pr(> |z|)
(Intercept)	4.6445	0.1429	32.4900	< 2e-16	5.2996	0.0739	71.7000	< 2e-16
M12 *vs* all other treatments	−0.0122	0.0042	−2.8900	**0.0039**	0.0064	0.0027	2.3700	**0.0180**
M6_Br *vs* components plus M3_Br1 and M3_Br2	0.0110	0.0087	1.2600	0.2091	0.0026	0.0060	0.4400	0.6615
M3_Br1 *vs* components	0.0128	0.0191	0.6700	0.5013	−0.0677	0.0159	−4.2600	**0.0000**
ALB *vs* BLA + BOL	−0.0329	0.0288	−1.1400	0.2532	−0.0597	0.0206	−2.9000	0.0037
BLA *vs* BOL	0.0892	0.0458	1.9500	0.0512	−0.3103	0.0374	−8.3100	< 2e-16
M3_Br2 *vs* components	−0.0868	0.0194	−4.4900	**0.0000**	0.0096	0.0155	0.6200	0.5353
KAT *vs* A41 + ISE	0.0207	0.0272	0.7600	0.4469	0.0338	0.0209	1.6200	0.1061
ISE *vs* A41	−0.0138	0.0469	−0.2900	0.7685	−0.1711	0.0465	−3.6800	0.0002
M6_HeBi *vs* components plus M3_Bi and M3_He	−0.0033	0.0082	−0.4000	0.6861	0.0149	0.0060	2.4900	**0.0128**
M3_Bi *vs* components	0.0267	0.0169	1.5700	0.1153	0.0452	0.0121	3.7200	**0.0002**
ART *vs* ALT + BRA	−0.0025	0.0254	−0.1000	0.9220	0.1399	0.0184	7.5900	0.0000
ALT *vs* BRA	0.0473	0.0469	1.0100	0.3124	0.1077	0.0376	2.8600	0.0042
M3_He *vs* components	0.0267	0.0191	1.4000	0.1617	−0.0607	0.0163	−3.7100	**0.0002**
AUT *vs* GRO + VER	0.1948	0.0249	7.8300	0.0000	0.0597	0.0226	2.6400	0.0083
GRO *vs* VER	−0.0928	0.0496	−1.8700	0.0613	−0.0747	0.0416	−1.7900	0.0728
Br1 + Br2 *vs* Bi + He	−0.0095	0.0146	−0.6500	0.5131	−0.0297	0.0121	−2.4500	0.0142
M3_Br1 + components *vs* M3_Br2 + components	0.0482	0.0262	1.8400	0.0660	−0.0045	0.0165	−0.2800	0.7826
M3_Bi + components *vs* M3_He + components	0.0040	0.0303	0.1300	0.8962	0.0770	0.0174	4.4300	0.0000

Model fitted: glmer() with fixed = weed.density ~trt, random = ~(1|col) + (1|row), family = poisson in which trt = wheat stand type level; col = column in the field; row = row in the field weed.density = weed density m^{-2} at the end of winter.

Table B2

Analysis of variance of total weed biomass at end of winter (BBCH GS 30), in spring (BBCH GS 60/69) and at harvest (BBCH GS 92) in g m^{-2}. Separate models for 2013/14 and 2014/15 growing seasons were run. The wheat stand type factor was split into the set of eighteen orthogonal linear contrasts presented in Table 4. In bold the significant values ($P < 0.05$) for the contrasts testing the mixture effect. Only models in which the wheat stand type factor is significant ($P < 0.001$) are presented.

	2013/14 Weed biomass at GS30					2014/15 Weed biomass at GS30				
	Value	Std.Error	DF	t-value	p-value	Value	Std.Error	DF	t-value	p-value
(Intercept)	1.5328	0.1042	36	14.7154	0.0000	1.5475	0.1907	36	8.1171	0.0000
M12 *vs* all other treatments	−0.0234	0.0248	36	−0.9453	0.3508	0.0013	0.0088	36	0.1440	0.8863
M6_Br *vs* components plus M3_Br1 and M3_Br2	−0.0956	0.0370	36	−2.5866	**0.0139**	0.0057	0.0153	36	0.3712	0.7127
M3_Br1 *vs* components	0.0448	0.1612	36	0.2781	0.7825	0.0089	0.1134	36	0.0784	0.9379
ALB *vs* BLA + BOL	−0.3657	0.1043	36	−3.5072	0.0012	−0.0834	0.1749	36	−0.4767	0.6364
BLA *vs* BOL	0.0166	0.2991	36	0.0553	0.9562	−0.1038	0.1449	36	−0.7167	0.4782
M3_Br2 *vs* components	−0.0171	0.1133	36	−0.1510	0.8808	−0.0930	0.1592	36	−0.5842	0.5628
KAT *vs* A41 + ISE	−0.1292	0.1407	36	−0.9180	0.3647	−0.0248	0.0734	36	−0.3382	0.7372
ISE *vs* A41	−0.3929	0.4168	36	−0.9427	0.3521	−0.3134	0.2187	36	−1.4332	0.1604
M6_HeBi *vs* components plus M3_Bi and M3_He	−0.0437	0.0240	36	−1.8181	0.0774	0.0702	0.0262	36	2.6836	**0.0109**
M3_Bi *vs* components	0.0917	0.1023	36	0.8961	0.3761	0.0303	0.0593	36	0.5103	0.6130
ART *vs* ALT + BRA	−0.0765	0.1176	36	−0.6505	0.5195	−0.1425	0.2087	36	−0.6829	0.4990
ALT *vs* BRA	−0.1620	0.2930	36	−0.5529	0.5837	0.1559	0.1951	36	0.7991	0.4295
M3_He *vs* components	−0.0841	0.2144	36	−0.3923	0.6972	0.0138	0.1810	36	0.0761	0.9398
AUT *vs* GRO + VER	−0.2919	0.1637	36	−1.7833	0.0830	0.1859	0.0622	36	2.9913	0.0050
GRO *vs* VER	−0.5380	0.4662	36	−1.1539	0.2562	−0.1409	0.1864	36	−0.7559	0.4546
Br1 + Br2 *vs* Bi + He	−0.0233	0.1070	36	−0.2175	0.8290	−0.0007	0.0838	36	−0.0082	0.9935
M3_Br1 + components *vs* M3_Br2 + components	0.1174	0.1566	36	0.7502	0.4580	−0.1088	0.1300	36	−0.8364	0.4084
M3_Bi + components *vs* M3_He + components	−0.0735	0.1784	36	−0.4118	0.6829	0.3101	0.1343	36	2.3095	0.0268

	2013/14 Weed biomass at GS60/69		2014/15 Weed biomass at GS60/69			
		Value	Std.Error	DF	t-value	p-value
(Intercept)	**Model**	2.1666	0.0852	36	25.4393	0.0000
M12 *vs* all other treatments	**P > 0.05**	0.0082	0.0148	36	0.5568	0.5879
M6_Br *vs* components plus M3_Br1 and M3_Br2		−0.0049	0.0524	36	−0.0935	0.9270
M3_Br1 *vs* components		0.0144	0.0724	36	0.1996	0.8452
ALB *vs* BLA + BOL		−0.0919	0.1254	36	−0.7333	0.4775
BLA *vs* BOL		−0.1614	0.2737	36	−0.5896	0.5664
M3_Br2 *vs* components		0.0001	0.1187	36	0.0008	0.9994
KAT *vs* A41 + ISE		0.1003	0.2260	36	0.4440	0.6649
ISE *vs* A41		−0.0072	0.1943	36	−0.0372	0.9709
M6_HeBi *vs* components plus M3_Bi and M3_He		0.0032	0.0099	36	0.3256	0.7503
M3_Bi *vs* components		−0.0242	0.0291	36	−0.8299	0.4228
ART *vs* ALT + BRA		0.0457	0.0595	36	0.7679	0.4574
ALT *vs* BRA		−0.0948	0.0630	36	−1.5057	0.1580
M3_He *vs* components		0.0199	0.0629	36	0.3157	0.7577
AUT *vs* GRO + VER		0.1770	0.0478	36	3.7001	0.0030
GRO *vs* VER		−0.1168	0.1433	36	−0.8148	0.4311
Br1 + Br2 *vs* Bi + He		0.1380	0.0687	36	2.0095	0.0675
M3_Br1 + components *vs* M3_Br2 + components		−0.1808	0.1338	36	−1.3510	0.2016
M3_Bi + components *vs* M3_He + components		0.2568	0.0596	36	4.3048	0.0010

	2013/14 Weed biomass a GS92 (harvest)					2014/15 Weed biomass a GS92 (harvest)				
	Value	Std.Error	DF	t-value	p-value	Value	Std.Error	DF	t-value	p-value
(Intercept)	2.1105	0.0922	36	22.8882	0.0000	2.5324	0.4032	36	6.2810	0.0000
M12 *vs* all other treatments	0.0066	0.0318	36	0.2065	0.8375	−0.0269	0.0097	36	−2.7848	**0.0085**
M6_Br *vs* components plus M3_Br1 and M3_Br2	0.0087	0.0519	36	0.1684	0.8672	−0.0400	0.0525	36	−0.7627	0.4506
M3_Br1 *vs* components	−0.0752	0.0925	36	−0.8124	0.4219	0.1078	0.2216	36	0.4864	0.6296
ALB *vs* BLA + BOL	−0.0953	0.2951	36	−0.3228	0.7487	0.0278	0.3617	36	0.0768	0.9392
BLA *vs* BOL	0.2103	0.2633	36	0.7987	0.4297	−0.3434	0.5490	36	−0.6255	0.5356
M3_Br2 *vs* components	−0.0022	0.0428	36	−0.0514	0.9593	0.1657	0.2331	36	0.7107	0.4819
KAT *vs* A41 + ISE	−0.2474	0.0892	36	−2.7717	0.0088	−0.1287	0.1662	36	−0.7742	0.4439
ISE *vs* A41	−0.2248	0.2094	36	−1.0735	0.2902	0.4171	0.3619	36	1.1524	0.2567
M6_HeBi *vs* components plus M3_Bi and M3_He	−0.0454	0.0587	36	−0.7726	0.4448	0.0392	0.1347	36	0.2907	0.7729
M3_Bi *vs* components	0.1035	0.1011	36	1.0239	0.3127	−0.0168	0.2219	36	−0.0755	0.9402
ART *vs* ALT + BRA	0.5368	0.1151	36	4.6655	0.0000	−0.1072	0.1682	36	−0.6374	0.5279

(*continued on next page*)

Table B2 (*continued*)

	2013/14 Weed biomass a GS92 (harvest)					2014/15 Weed biomass a GS92 (harvest)				
	Value	Std.Error	DF	t-value	p-value	Value	Std.Error	DF	t-value	p-value
ALT *vs* BRA	0.5796	0.3286	36	1.7637	0.0863	0.2719	0.4442	36	0.6121	0.5443
M3_He *vs* components	0.0558	0.1070	36	0.5219	0.6049	−0.0527	0.0667	36	−0.7902	0.4346
AUT *vs* GRO + VER	0.5318	0.1015	36	5.2373	0.0000	0.3442	0.2053	36	1.6767	0.1023
GRO *vs* VER	−0.2204	0.2727	36	−0.8080	0.4244	−0.2657	0.2951	36	−0.9001	0.3740
Br1 + Br2 *vs* Bi + He	0.1554	0.0915	36	1.6984	0.0981	0.3094	0.1557	36	1.9873	0.0545
M3_Br1 + components *vs* M3_Br2 + components	−0.1015	0.1385	36	−0.7326	0.4686	0.1559	0.2523	36	0.6177	0.5407
M3_Bi + components *vs* M3_He + components	0.5945	0.1263	36	4.7066	0.0000	0.4076	0.1846	36	2.2082	0.0337

Model fitted: lme() with fixed = log(dw_weedsX + 1) ~ trt, random = ~1|blk, weights = varIdent(form = ~1|trt), in which trt: wheat stand type level; blk = block dw_weeds1: weeds dry weight g m^{-2} at end of winter (crop BBCH GS 30); dw_weeds2: weeds dry weight g m^{-2} in spring (crop BBCH GS 60/69); dw_weeds3: weeds dry weight g m^{-2} and at crop maturity (crop BBCH GS 92).

Appendix C. Supplemental material to: 3.3 Yield and yield components

Supplemental material to: 3.3 Yield and yield components

Table C1
Analysis of variance of grain yield (t ha^{-1}). Separate models for 2013/14 and 2014/15 growing seasons were run. The wheat stand type factor was split into the set of eighteen orthogonal linear contrasts presented in Table 4. In bold the significant values ($P < 0.05$) for the contrasts testing the mixture effect. Only models in which the wheat stand type factor is significant ($P < 0.001$) are presented.

	2013/14 yield (t ha^{-1})					2014/15 yield (t ha^{-1})				
	Value	Std.Error	df	t-value	p-value	Value	Std.Error	df	t-value	p-value
(Intercept)	381.3668	6.2875	36	60.6546	0.0000	215.1123	18.5596	36	11.5903	0.0000
M12 *vs* all other treatments	2.7005	1.2238	36	2.2067	0.0338	−1.2114	1.5637	36	−0.7747	0.4436
M6_Br *vs* components plus M3_Br1 and M3_Br2	4.0502	1.6171	36	2.5047	0.0169	−0.5773	2.3575	36	−0.2449	0.8080
M3_Br1 *vs* components	−0.4786	10.0502	36	−0.0476	0.9623	5.1983	5.4354	36	0.9564	0.3453
ALB *vs* BLA + BOL	−34.6322	16.0648	36	−2.1558	0.0379	15.8100	9.6590	36	1.6368	0.1104
BLA *vs* BOL	15.9267	19.8288	36	0.8032	0.4271	4.5400	9.3768	36	0.4842	0.6312
M3_Br2 *vs* components	1.8100	8.1300	36	0.2226	0.8251	1.4781	7.3049	36	0.2023	0.8408
KAT *vs* A41 + ISE	35.6983	11.7882	36	3.0283	0.0045	−5.4322	17.0599	36	−0.3184	0.7520
ISE *vs* A41	−7.3917	20.3513	36	−0.3632	0.7186	11.0300	22.2488	36	0.4958	0.6231
M6_HeBi *vs* components plus M3_Bi and M3_He	−3.7801	3.1648	36	−1.1944	0.2401	−1.3703	2.3351	36	−0.5868	0.5610
M3_Bi *vs* components	−9.1347	8.6643	36	−1.0543	0.2988	0.1589	4.8515	36	0.0328	0.9741
ART *vs* ALT + BRA	−42.3961	7.9796	36	−5.3131	0.0000	−27.1006	8.5094	36	−3.1848	0.0030
ALT *vs* BRA	−54.2917	8.4203	36	−6.4477	0.0000	14.2650	17.7343	36	0.8044	0.4265
M3_He *vs* components	0.4006	9.2702	36	0.0432	0.9658	0.5936	2.1443	36	0.2768	0.7835
AUT *vs* GRO + VER	24.8678	8.0239	36	3.0992	0.0038	16.4072	4.4256	36	3.7073	0.0007
GRO *vs* VER	−21.5967	20.8511	36	−1.0358	0.3072	−17.5550	12.7257	36	−1.3795	0.1763
Br1 + Br2 *vs* Bi + He	12.8052	6.5198	36	1.9640	0.0573	10.8244	5.3208	36	2.0344	0.0493
M3_Br1 + components *vs* M3_Br2 + components	−32.7554	11.1824	36	−2.9292	0.0059	1.4729	9.6466	36	0.1527	0.8795
M3_Bi + components *vs* M3_He + components	68.3696	8.7937	36	7.7749	0.0000	31.5504	6.1956	36	5.0924	0.0000

Model fitted: lme() with fixed = yield ~ trt, random = ~1|blk, weights = varIdent(form = ~1|trt), in which trt: wheat stand type level; blk = block yield: dry grain production t ha^{-1}.

Table C2

Analysis of variance of thousand kernel weight (g). Separate models for 2013/14 and 2014/15 growing seasons were run. The wheat stand type factor was split into the set of eighteen orthogonal linear contrasts presented in Table 4. In bold the significant values ($P < 0.05$) for the contrasts testing the mixture effect. Only models in which the wheat stand type factor is significant ($P < 0.001$) are presented.

	2013/14 thousand kernel weight (g)					2014/15 thousand kernel weight (g)				
	Value	Std.Error	DF	t-value	p-value	Value	Std.Error	DF	t-value	p-value
(Intercept)	34.3658	0.4987	36	68.9132	0.0000	38.7354	0.1833	36	211.3602	0.0000
M12 *vs* all other treatments	0.1239	0.0753	36	1.6460	0.1085	0.0666	0.0255	36	2.6084	**0.0132**
M6_Br *vs* components plus M3_Br1 and M3_Br2	−0.1221	0.1712	36	−0.7130	0.4805	0.0914	0.0889	36	1.0279	0.3108
M3_Br1 *vs* components	−0.3869	0.2926	36	−1.3224	0.1944	0.1528	0.2186	36	0.6990	0.4890
ALB *vs* BLA + BOL	3.0711	0.1821	36	16.8606	0.0000	4.7556	0.5366	36	8.8624	0.0000
BLA *vs* BOL	−0.9667	0.5245	36	−1.8431	0.0736	−0.7600	0.5201	36	−1.4612	0.1526
M3_Br2 *vs* components	0.6244	0.7250	36	0.8613	0.3948	−0.0800	0.2394	36	−0.3342	0.7401
KAT *vs* A41 + ISE	−0.3061	0.6675	36	−0.4586	0.6493	−1.9167	0.4152	36	−4.6160	0.0000
ISE *vs* A41	−4.7517	1.1947	36	−3.9772	0.0003	−2.0633	0.3984	36	−5.1790	0.0000
M6_HeBi *vs* components plus M3_Bi and M3_He	0.0729	0.4602	36	0.1584	0.8751	0.1714	0.0558	36	3.0728	**0.0040**
M3_Bi *vs* components	−0.1869	0.1987	36	−0.9409	0.3530	0.3628	0.2105	36	1.7234	0.0934
ART *vs* ALT + BRA	−3.3739	0.5820	36	−5.7967	0.0000	−2.3944	0.2759	36	−8.6775	0.0000
ALT *vs* BRA	1.0717	0.9151	36	1.1711	0.2492	2.5767	0.8141	36	3.1652	0.0031
M3_He *vs* components	−0.3136	0.6701	36	−0.4680	0.6426	−0.3811	0.1735	36	−2.1962	**0.0346**
AUT *vs* GRO + VER	3.2561	0.5076	36	6.4151	0.0000	−0.7022	0.2415	36	−2.9074	0.0062
GRO *vs* VER	−0.1450	1.0901	36	−0.1330	0.8949	2.9333	0.5003	36	5.8629	0.0000
Br1 + Br2 *vs* Bi + He	−0.0419	0.4124	36	−0.1015	0.9197	−1.7200	0.1919	36	−8.9648	0.0000
M3_Br1 + components *vs* M3_Br2 + components	1.5188	0.5325	36	2.8519	0.0072	1.0442	0.3189	36	3.2743	0.0023
M3_Bi + components *vs* M3_He + components	0.2133	0.5302	36	0.4024	0.6898	−2.5592	0.2710	36	−9.4424	0.0000

Model fitted: lme() with fixed = TKW ~ trt, random = ~1|blk, weights = varIdent(form = ~1|trt), in which trt: wheat stand type level; blk = block. TKW: thousand kernel weight in g.

Appendix D. Supplemental material to: 3.4 Grain quality

Supplemental material to: 3.4 Grain quality

Table D1

Analysis of variance of grain test weight (kg 100 L^{-1}). Separate models for 2013/14 and 2014/15 growing seasons were run. The wheat stand type factor was split into the set of eighteen orthogonal linear contrasts presented in Table 4. In bold the significant values ($P < 0.05$) for the contrasts testing the mixture effect. Only models in which the wheat stand type factor is significant ($P < 0.001$) are presented.

	2013/14 test weight (kg 100 L^{-1})					2014/15 test weight (kg 100 L^{-1})				
	Value	Std.Error	DF	t-value	p-value	Value	Std.Error	DF	t-value	p-value
(Intercept)	75.1930	0.2182	36	344.6212	0.0000	79.8703	0.2179	36	366.5673	0.0000
M12 *vs* all other treatments	0.0152	0.0949	36	0.1601	0.8737	0.0510	0.0138	36	3.7028	**0.0007**
M6_Br *vs* components plus M3_Br1 and M3_Br2	−0.0778	0.0874	36	−0.8897	0.3795	−0.0378	0.0921	36	−0.4110	0.6835
M3_Br1 *vs* components	−0.0778	0.1599	36	−0.4864	0.6296	0.0794	0.1150	36	0.6910	0.4940
ALB *vs* BLA + BOL	0.1778	0.2436	36	0.7297	0.4703	−0.0624	0.2751	36	−0.2270	0.8217
BLA *vs* BOL	2.4000	0.6399	36	3.7504	0.0006	2.3900	0.1049	36	22.7737	0.0000
M3_Br2 *vs* components	0.0111	0.2441	36	0.0455	0.9639	−0.0038	0.3561	36	−0.0106	0.9916
KAT *vs* A41 + ISE	0.0222	0.2987	36	0.0744	0.9411	−0.2089	0.5355	36	−0.3901	0.6988
ISE *vs* A41	−0.6000	0.5500	36	−1.0908	0.2826	−0.3720	0.4502	36	−0.8262	0.4141
M6_HeBi *vs* components plus M3_Bi and M3_He	0.1500	0.1747	36	0.8588	0.3962	−0.0483	0.0518	36	−0.9329	0.3571
M3_Bi *vs* components	0.9000	0.2502	36	3.5968	**0.0010**	−0.2339	0.0534	36	−4.3828	**0.0001**
ART *vs* ALT + BRA	−3.1333	0.5231	36	−5.9898	0.0000	−0.9018	0.1694	36	−5.3246	0.0000
ALT *vs* BRA	−1.0000	0.3197	36	−3.1277	0.0035	−1.4920	0.2175	36	−6.8593	0.0000
M3_He *vs* components	0.1333	0.2535	36	0.5260	0.6021	−0.3173	0.2566	36	−1.2365	0.2243
AUT *vs* GRO + VER	1.9333	0.4279	36	4.5185	0.0001	0.7113	0.1760	36	4.0415	0.0003
GRO *vs* VER	1.1333	0.4942	36	2.2934	0.0278	0.8673	0.2762	36	3.1408	0.0034
Br1 + Br2 *vs* Bi + He	0.5111	0.2077	36	2.4610	0.0188	0.3914	0.1610	36	2.4308	0.0202
M3_Br1 + components *vs* M3_Br2 + components	1.6667	0.2637	36	6.3206	0.0000	1.3385	0.2987	36	4.4814	0.0001
M3_Bi + components *vs* M3_He + components	−0.0167	0.3213	36	−0.0519	0.9589	−0.7878	0.1726	36	−4.5649	0.0001

Model fitted: lme() with fixed = TW ~ trt, random = ~1|blk, weights = varIdent(form = ~1|trt), in which trt: wheat stand type level; blk = block. TW: test weight in kg 100l^{-1}.

Table D2

Analysis of whole grain protein content (%). Separate models for 2013/14 and 2014/15 growing seasons were run. The wheat stand type factor was split into the set of eighteen orthogonal linear contrasts presented in Table 4. In bold the significant values ($P < 0.05$) for the contrasts testing the mixture effect. Only models in which the wheat stand type factor is significant ($P < 0.001$) are presented.

	2013/14 whole grain protein content (%)					2014/15 whole grain protein content (%)				
	Value	Std.Error	DF	t-value	p-value	Value	Std.Error	DF	t-value	p-value
(Intercept)	12.9075	0.1150	36	112.1965	0.0000	12.4352	0.1970	36	63.1312	0.0000
M12 *vs* all other treatments	0.0044	0.0043	36	1.0045	0.3218	0.0426	0.0185	36	2.3006	**0.0273**
M6_Br *vs* components plus M3_Br1 and M3_Br2	0.0427	0.0237	36	1.8011	0.0801	0.0011	0.0470	36	0.0234	0.9815
M3_Br1 *vs* components	0.0481	0.1102	36	0.4370	0.6647	−0.0395	0.0425	36	−0.9295	0.3588
ALB *vs* BLA + BOL	0.3795	0.2341	36	1.6211	0.1137	−0.0176	0.1563	36	−0.1125	0.9110
BLA *vs* BOL	0.0396	0.2362	36	0.1677	0.8678	−0.3664	0.1166	36	−3.1428	0.0033
M3_Br2 *vs* components	−0.1450	0.0935	36	−1.5511	0.1296	0.1722	0.0754	36	2.2849	**0.0283**
KAT *vs* A41 + ISE	−0.2702	0.1243	36	−2.1749	0.0363	−0.0492	0.2393	36	−0.2056	0.8382
ISE *vs* A41	−0.4861	0.1048	36	−4.6366	0.0000	0.0696	0.2101	36	0.3312	0.7424
M6_HeBi *vs* components plus M3_Bi and M3_He	−0.0194	0.0576	36	−0.3357	0.7390	−0.0106	0.0277	36	−0.3817	0.7049
M3_Bi *vs* components	0.0296	0.0706	36	0.4196	0.6773	−0.1216	0.0810	36	−1.5019	0.1418
ART *vs* ALT + BRA	0.3890	0.2114	36	1.8400	0.0740	0.1352	0.2389	36	0.5660	0.5749
ALT *vs* BRA	−0.3595	0.0970	36	−3.7061	0.0007	−0.5556	0.3147	36	−1.7653	0.0860
M3_He *vs* components	−0.0619	0.0584	36	−1.0616	0.2955	0.1939	0.1248	36	1.5545	0.1288
AUT *vs* GRO + VER	−0.4598	0.1297	36	−3.5446	0.0011	−0.7107	0.1068	36	−6.6527	0.0000
GRO *vs* VER	−0.4526	0.2636	36	−1.7168	0.0946	0.4654	0.2843	36	1.6372	0.1103
Br1 + Br2 *vs* Bi + He	−0.5673	0.0823	36	−6.8915	0.0000	−0.8414	0.0881	36	−9.5530	0.0000
M3_Br1 + components *vs* M3_Br2 + components	0.8127	0.1295	36	6.2738	0.0000	0.3616	0.1204	36	3.0040	0.0048
M3_Bi + components *vs* M3_He + components	−1.6099	0.1144	36	−14.0779	0.0000	−1.4382	0.1469	36	−9.7893	0.0000

Model fitted: lme() with fixed = Protein ~ trt, random = ~1|blk, weights = varIdent(form = ~1|trt), in which trt: wheat stand type level; blk = block. Protein: whole grain protein content%.

Table D3

Analysis of whole grain starch content (%). Separate models for 2013/14 and 2014/15 growing seasons were run. The wheat stand type factor was split into the set of eighteen orthogonal linear contrasts presented in Table 4. In bold the significant values ($P < 0.05$) for the contrasts testing the mixture effect. Only models in which the wheat stand type factor is significant ($P < 0.001$) are presented.

	2013/14 whole grain starch content (%)					2014/15 whole grain starch content (%)				
	Value	Std.Error	DF	t-value	p-value	Value	Std.Error	DF	t-value	p-value
(Intercept)	58.0424	0.1042	36	556.9664	0.0000	58.4365	0.1024	36	570.7433	0.0000
M12 *vs* all other treatments	−0.0180	0.0153	36	−1.1745	0.2479	0.0217	0.0217	36	0.9979	0.3250
M6_Br *vs* components plus M3_Br1 and M3_Br2	−0.0198	0.0431	36	−0.4585	0.6494	0.0032	0.0317	36	0.1022	0.9192
M3_Br1 *vs* components	−0.0235	0.1378	36	−0.1706	0.8655	0.0316	0.0743	36	0.4254	0.6731
ALB *vs* BLA + BOL	0.3029	0.2668	36	1.1354	0.2637	0.4798	0.1265	36	3.7932	0.0005
BLA *vs* BOL	−0.7894	0.3169	36	−2.4912	0.0175	−0.5788	0.3437	36	−1.6841	0.1008
M3_Br2 *vs* components	0.1863	0.0849	36	2.1931	**0.0348**	−0.1344	0.2758	36	−0.4872	0.6290
KAT *vs* A41 + ISE	0.6118	0.1164	36	5.2579	0.0000	0.2384	0.0625	36	3.8129	0.0005
ISE *vs* A41	1.0905	0.1781	36	6.1235	0.0000	0.3496	0.1216	36	2.8740	0.0068
M6_HeBi *vs* components plus M3_Bi and M3_He	−0.0129	0.1003	36	−0.1283	0.8987	−0.0801	0.0583	36	−1.3752	0.1776
M3_Bi *vs* components	0.1194	0.0775	36	1.5413	0.1320	−0.0142	0.0590	36	−0.2401	0.8116
ART *vs* ALT + BRA	−0.6401	0.2524	36	−2.5356	0.0157	−0.1757	0.1159	36	−1.5156	0.1384
ALT *vs* BRA	0.4261	0.2036	36	2.0932	0.0434	0.5440	0.3424	36	1.5885	0.1209
M3_He *vs* components	−0.0327	0.0823	36	−0.3977	0.6932	−0.1878	0.1440	36	−1.3042	0.2005
AUT *vs* GRO + VER	1.0127	0.1851	36	5.4707	0.0000	0.7374	0.2449	36	3.0103	0.0047
GRO *vs* VER	0.0575	0.3777	36	0.1523	0.8798	−0.2929	0.2835	36	−1.0331	0.3084
Br1 + Br2 *vs* Bi + He	0.5891	0.1090	36	5.4057	0.0000	0.4930	0.1058	36	4.6604	0.0000
M3_Br1 + components *vs* M3_Br2 + components	−0.5022	0.1505	36	−3.3368	0.0020	0.1270	0.1686	36	0.7532	0.4562
M3_Bi + components *vs* M3_He + components	1.3454	0.1520	36	8.8503	0.0000	0.8033	0.1532	36	5.2435	0.0000

Model fitted: lme() with fixed = Starch ~ trt, random = ~1|blk, weights = varIdent(form = ~1|trt), in which trt: wheat stand type level; blk = block. Starch: whole grain starch content in%.

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