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Multifaceted functional diversity for multifaceted crop yield: towards ecological assembly rules for varietal mixtures

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Keywords

assembly rules, community-weighted mean, durum wheat, functional diversity, functional trait, grain protein content, grain yield, varietal mixtures

Abstract

1. Ecological theories suggest that higher plant genetic diversity can increase productivity in natural ecosystems. So far, varietal mixtures, *i.e.* the cultivation of different genotypes within a field, have shown contrasting results, notably for grain yield where both positive and negative mixing effects have been reported. Such discrepancy between ecological theories and agronomical applications calls for a better understanding of plant-plant interactions in crops.
2. Using durum wheat (*Triticum turgidum* ssp. *durum*) as a model species, we investigated the effect of functional trait composition on productivity and grain quality of varietal mixtures by growing 179 highly diverse genotypes in pure stands and 197 two-way mixtures in field conditions. We quantified the agronomic performance of the mixtures relative to their components grown in pure stands on two variables related to productivity, vegetative biomass yield and grain yield, and one variable related to grain quality, grain protein content. We then analysed the relationship between the relative performance of the mixtures and their functional composition that we characterized with trait means and trait differences on 19 above- and belowground traits.
3. We found that biomass and grain yield increased by 4% overall in mixtures relative to single varieties, but that mixing effects were non-significant for grain protein content. The combined effects of trait means and trait differences explained 12%, 17%, and 22% of the variability of relative grain yield, biomass yield, and grain protein content, respectively, with different traits affecting productivity and grain quality. Clustering varieties into functional groups allowed us to identify the most beneficial associations for multifaceted agronomic performance.
4. *Synthesis and applications.* Functional traits explained a significant part of the relative agronomic performance of mixtures compared to monocultures (12 to 22%, depending on the yield component). They can thus serve as a basis to identify groups of varieties whose combinations are expected to generate positive mixing effects, especially for productivity, and without compromising grain quality. Selection could then target convergence between groups for some traits and divergence between groups for other traits using empirically derived relationships between functional traits and agronomic performance as a guideline.

28 Introduction

29 Numerous studies have shown a positive relationship between biodiversity and productivity in natural plant
30 communities (Hooper et al., 2005; Loreau et al., 2001; Tilman et al., 2001). Although most of these effects were first
31 attributed to species richness, intraspecific diversity is now being recognized as an important driver of ecological
32 processes, including productivity (Bolnick et al., 2011; Hughes, Inouye, Johnson, Underwood, & Vellend, 2008). In
33 crops, the effect of genetic diversity on agronomic performance has been studied for decades in the context of varietal
34 mixtures (Gustafsson, 1953). Considering grain yield, the most widely studied variable, mixing effects are slightly
35 positive on average, but are also highly variable and can be negative (Kiær, Skovgaard, & Østergård, 2009; Reiss &
36 Drinkwater, 2018; Smithson & Lenné, 1996). Such results contrast with expectations based on ecological theories and
37 call for a better understanding of the mechanisms that drive the biodiversity-productivity relationship in crops (Barot
38 et al., 2017).

39 The expected benefits of mixtures lie on a central and seminal theory of ecology: niche complementarity.
40 This theory assumes that organisms that differ in their ecological niche experience reduced competition because they
41 use resources differently, which leads to enhanced productivity (MacArthur & Levins, 1967). In crops, niche
42 complementarity has long been proposed as the basis for productivity gains in intercropping, *i.e.* the practice of
43 growing two or more species in the same field (Vandermeer, 1992). For example, diversity in root foraging strategies
44 between maize, bean, and squash has been shown to promote complementarity effects in this ancient intercrop known
45 as “the three sisters” (Zhang, Postma, York, & Lynch, 2014). Similarly, complementary nitrogen acquisition strategies
46 between cereals and legumes, *i.e.* soil nitrogen acquisition for cereals *vs* atmospheric nitrogen fixation for legumes,
47 contribute to yield gains in cereal-legume intercrops (Bedoussac et al., 2015). While the theoretical and applied
48 knowledge on the potential benefits of multi-species intercropping are well developed, our understanding of how
49 niche complementarity owing to within-species or within-genotype variability remains less well explored (but see
50 Montazeaud et al., 2018).

51 In contrast with the predictions from the niche complementarity theory, several experiments in ecology have
52 shown that average community characteristics may be more important than differences between individuals in
53 explaining processes such as productivity (Garnier, Navas, & Grigulis, 2015). Such results are in line with the “mass
54 ratio hypothesis” (MRH, Grime, 1998), extended in the Trait Driver Theory (TDT, Enquist et al., 2015), which
55 predicts that ecosystem processes are mainly determined by the dominant species in the community. In addition, such
56 average characteristics are even more informative when they are weighted according to the relative biomass of the
57 species or genotypes in the mixture (Garnier et al., 2004). Yet, the MRH remains unexplored in the context of varietal
58 mixtures, where the potential effects of individual trait values on growth and yield are often considered negligible in
59 comparison to varietal and/or phenotypic trait variation (Barot et al., 2017; Prieto et al., 2015).

60 In this context, which traits are the most important drivers of mixture productivity and which combination of
61 phenotypes optimize crop performance remain open questions (Barot et al., 2017; Borg et al., 2018). In natural
62 communities, recent results suggest that processes such as productivity could be explained by a multivariate
63 combination of trait means and trait differences for traits ranging from organ-level to whole plant-level (Cadotte,
64 2017; Kraft, Godoy, & Levine, 2015). Such traits relate to resource-use strategies and size, the two main dimensions

65 of phenotypic variation among vascular plants (Díaz et al., 2016; Westoby, Falster, Moles, Vesk, & Wright, 2002).
66 Importantly, some of these studies and others (Bardgett, Mommer, & De Vries, 2014) have highlighted the major
67 contribution of root traits in driving such processes. In crops, root traits are rarely considered when studying varietal
68 mixtures (Kiær et al., 2009), whereas belowground interactions such as resource-use complementarity are often
69 advanced as potential mechanisms for productivity gains in mixtures (Barot et al., 2017; Litrico & Violle, 2015).
70 Improving belowground trait characterization might then help better understand the ecological mechanisms driving
71 plant-plant interactions and agronomic performance in varietal mixtures.

72 In this study, we used a trait-based approach to determine which facets of intra-specific diversity drive
73 agronomic performance in varietal mixtures of durum wheat, *Triticum turgidum* ssp. *durum*. Using a set of 179 highly
74 genetically diverse inbred lines, we grew 179 single varieties and 197 two-way mixtures under field conditions. We
75 assessed mixture performance relative to single varieties for two productivity variables, biomass yield and grain yield,
76 and for one quality variable, grain protein content. We assessed the diversity-performance relationship by quantifying
77 trait means and trait differences on 19 functional traits including 11 root traits. Then, we used a multivariate approach
78 to understand the relationship between the different components of agronomic performance and mixture trait
79 composition. Finally, we clustered genotypes into functional groups to identify assembly rules that allow maximizing
80 mixing effects on several dimensions of agronomic performance.

81 **Material and Methods**

82 **Experimental design**

83 Our objective was to assess the quantitative effect of trait combinations on productivity and quality rather than
84 qualitative effects such as the number of genotypes in the mixture or the genotypes' identities. Therefore, we used
85 two-component mixtures and we did not replicate single-variety and mixture plots to maximize the number of
86 observed trait combinations. We set up a 400-plots experiment at Mauguio, France (INRAE – UE DIASCOPE -
87 43°37'02''N, 3°51'18''E, 12 m above the sea level). We worked with 180 durum wheat inbred lines from an highly
88 diversified evolutionary pre-breeding population developed at INRAE Montpellier, France (David et al., 2014,
89 Appendix S1 in Supporting Information). We grew the 180 lines in single-variety plots and we randomly selected 220
90 pairwise combinations for mixture plots. We excluded pairs having more than three weeks' difference in heading date,
91 assuming that larger time lag would not be acceptable in real cultivation conditions. Single-variety and mixture plots
92 were randomly arranged in a grid of 11 x 41 plots (Figure 1). Each plot consisted of six 1.5 m long rows with 20 cm
93 between rows and 2-3 cm between plants of the same row. 60 seeds were sown on each row, resulting in a planting
94 density of 240 plants.m². The inter-plot distance was 30 cm in the horizontal direction and 2 m in the vertical
95 direction. In mixtures, one genotype was grown on rows 1, 3 and 5, and the other on rows 2, 4, and 6 (Figure 1). Such
96 spatial arrangement allowed us to individualize measurements for each mixture component. Biotic damages and
97 resource limitations were prevented by applying pesticides and fertilizers. Detailed information on plant growth
98 conditions can be found in Appendix S1. We discarded 24 plots with incomplete data due to sowing or sampling
99 problems. We thus analysed data from 179 single-variety plots and 197 mixtures.

100 **Plant trait and performance measurements**

101 Detailed information on plant trait and performance measurements is provided in Appendix S1. We characterized the
102 179 genotypes in single-variety plots. Except for phenological traits that we assessed at the plot level, all trait
103 measurements described below were replicated within each plot and then averaged to obtain a single value per
104 genotype and plot. At the end of the tillering stage, we collected two soil cores (10 cm diameter and 15 cm depth) per
105 plot from which we separated above and belowground biomass. We then counted the number of plants in each sample
106 and computed early biomass per capita (Ear. bio.) and tiller number per capita (Till. nb.). In addition, leaf nitrogen
107 content (LNC) and specific leaf area (SLA) were measured by sampling one foliar disc of 6 mm diameter on four
108 randomly selected leaves per sample. For each root sample, we separated the seminal and the adventitious root
109 systems and we quantified morphological traits through image analysis (WinRHIZO pro Version 2009; Regent
110 Instrument, Quebec, Canada). We computed mean root diameters ($Diam_{sem}$ and $Diam_{adv}$), specific root length (SRL_{sem}
111 and SRL_{adv} , root length / root dry mass), root tissue density (RTD_{sem} and RTD_{adv} , root dry mass / root volume), root
112 length density (RLD_{sem} and RLD_{adv} , root length / soil volume), and root branching intensity (RBI_{sem} and RBI_{adv} ,
113 number of root tips / total root length). At heading, we measured the angle between the two most distant tillers
114 ($Angle_{aer}$) on two plants per plot. At maturity, we collected three plants per plot to measure the angle between their
115 two most distant roots ($Angle_{root}$), and we measured plant height (Height) on three plants per plot.

116 We characterized phenology by recording heading date (Heading) and maturity date (Maturity) at the plot
117 level. For each genotype, heading date and maturity date were defined as the date at which spikes were visible for
118 50% of the plants and the date at which 50% of the peduncles were ripe, respectively.

119 We measured agronomic performance in single-variety plots and in mixture plots. At maturity, we collected
120 aboveground biomass on the four central rows of each plot on 70 cm length, leaving 40 cm on each side to avoid edge
121 effects. For each single-variety plot, we collected two samples by pooling rows 2 and 3, and rows 4 and 5 (Figure 1).
122 In mixtures, we collected two samples per genotype by separating rows 2 and 4 for the first component, and rows 3
123 and 5 for the second component. We then quantified agronomic performance for each sample with three variables:
124 vegetative biomass yield (BY, $g \cdot m^{-2}$), *i.e.* leaf and stem dry weight per unit area; grain yield (GY, $g \cdot m^{-2}$), *i.e.* grain dry
125 weight per unit area; and grain protein content, *i.e.* mass fraction of protein in the grain (GPC, %). GPC was predicted
126 using a spectroscopic method (see Appendix S1). GPC has an important contribution to the end-use quality of durum
127 wheat. Indeed, most of the durum wheat production is used to make semolina and pasta, and GPC is one of the main
128 variables determining the cooking quality of these food products (Troccoli, Borrelli, De Vita, Fares, & Di Fonzo,
129 2000).

130 **Computation of trait indices**

131 We characterized the functional composition of the mixtures with both trait means and trait differences between
132 components. We quantified these two facets of functional diversity using the community-weighted mean (CWM) and
133 the Rao quadratic diversity (D), respectively, two indices that are commonly used in functional ecology (Ricotta &
134 Moretti, 2011).

135 Following Garnier et al. (2004), we computed CWMs by weighing the trait values of each component with their
 136 respective total biomass, including vegetative biomass (stems and leaves) and reproductive biomass (spikes):

$$137 \quad [1] \quad CWM_{ij} = \frac{B_i x_i + B_j x_j}{B_i + B_j}$$

138 Where CWM_{ij} is the community-weighted mean of the mixture containing genotypes i and j , B_i and B_j are the total
 139 biomass of genotypes i and j measured in mixture, and x_i and x_j are the trait values of genotypes i and j measured in
 140 single-variety plots, respectively.

141 For each trait, we also computed the Rao quadratic diversity index, which quantifies the expected difference between
 142 two genotypes sampled at random within the mixture (Rao, 1982):

$$143 \quad [2] \quad D_{ij} = d_{ij} \times \frac{B_i}{B_i + B_j} \times \frac{B_j}{B_i + B_j}$$

144 Where D_{ij} is the functional difference between genotypes i and j , and d_{ij} is a measure of phenotypic dissimilarity
 145 between genotypes i and j that we quantified with the Euclidian distance using trait values measured in single-variety
 146 plots..

147 **Relative agronomic performance**

148 We computed mixture relative performance using Relative Yield Total (RYT) indices (de Wit & van den Bergh,
 149 1965). RYT_{BY} for biomass yield and RYT_{GY} for grain yield were calculated as follows:

$$150 \quad [3] \quad RYT_{ij} = \frac{\frac{Y_{imixt}}{Y_{imonoc}} + \frac{Y_{jmixt}}{Y_{jmonoc}}}{2}$$

151 Where RYT_{ij} is the Relative Yield Total of the mixture containing genotypes i and j , Y_{imixt} and Y_{jmixt} are the grain or
 152 biomass yield of genotypes i and j in mixture, and Y_{imonoc} and Y_{jmonoc} are the grain or biomass yield of genotypes i and j
 153 in pure stands. For GPC, we adapted this formula to account for the fact that mixture components do not produce the
 154 same quantity of grains, even when sown in equal proportions. We thus weighted the contribution of one component
 155 to the grain protein content of the mixture by its grain yield relative to the total grain yield of the mixture. RYT_{GPC}
 156 was calculated as follows:

$$157 \quad [4] \quad RYT_{ij} = \frac{\frac{GY_{imixt} \times GPC_{imixt} + GY_{jmixt} \times GPC_{jmixt}}{GY_{imixt} + GY_{jmixt}}}{\frac{GY_{imonoc} \times GPC_{imonoc} + GY_{jmonoc} \times GPC_{jmonoc}}{GY_{imonoc} + GY_{jmonoc}}}$$

158 Where GY_{imixt} and GY_{jmixt} are the grain yield of genotypes i and j in mixture, GY_{imonoc} and GY_{jmonoc} are the grain yield of
 159 genotypes i and j in pure stands, GPC_{imixt} and GPC_{jmixt} are the grain protein content of genotypes i and j in mixture, and
 160 GPC_{imonoc} and GPC_{jmonoc} are the grain protein content of genotypes i and j in pure stands.

161 Under no mixing effects, RYT equals 1. $RYT > 1$ means that the mixture performed better than the average of the two
 162 monocultures (positive mixing effect), whereas $RYT < 1$ means that the mixture performed worse (negative mixing
 163 effect).

164 **Statistical analysis**

165 All statistical analysis were performed with R v. 3.5.3 (R Core Team, 2019).

166 We first processed the data to obtain a single value of agronomic performance for each genotype in each plot. To
167 do so, we fitted a linear mixed model to account for both spatial autocorrelation and identity of genotype combination,
168 with agronomic performance (BY, GY, and GPC) set as the response variable. To account for spatial autocorrelation,
169 we used the P-splines method implemented in the *SpATS* package (Rodríguez-Álvarez et al., 2018). We included row
170 identity and column identity as random effects, as well as a smooth bivariate surface function to model the deviation
171 to the linear trends along rows and columns (Rodríguez-Álvarez et al., 2018, Figure S2). Row identity was defined as
172 the coordinate of the plot along the smallest dimension of the grid and thus ranged from 1 to 11 (Figure 1 & Figure
173 S2). As agronomic performance measurements were individualized for the four central rows of each plot, each of the
174 41 columns of the grid was divided into four sub-columns. Column identity was defined as the sub-column where
175 agronomic performance was measured, and ranged from 1 to 164 (4*41) (Figure 1 & Figure S2). In order to estimate
176 the effect of each genotype on agronomic performance after accounting for spatial effects, we also included a dummy
177 variable corresponding to the combination of the identity of the focal genotype, *i.e.* the one for which agronomic
178 performance is measured, and the identity of the neighbour genotype, as a random effect. For each performance
179 variable, we extracted the BLUPs (Best Linear Unbiased Predictions) corresponding to the genotypic effect and thus
180 ended up with one value per genotype per plot that we used to compute RYT.

181 To assess how functional traits affect mixing effects, we fitted a full linear model with all trait CWMs and all trait
182 Ds as independent variables and RYT as the dependent variable (*lm()* function from the *stats* package). We
183 standardized all dependent and independent variables ($\mu = 0$, $\sigma = 1$), and we then ran a backward model selection
184 (*glmulti()* function from the *glmulti* package). We used the second-order Akaike Information Criterion (AIC_C,
185 Sugiura, 1978) to rank the models and performed model-averaging inference based on the top-ten models using the
186 *coef()* function from the *glmulti* package. We report parameter estimates and their 95% unconditional confidence
187 interval computed as ± 1.96 unconditional sampling standard deviation (Burnham & Anderson, 2002). We also report
188 variable importance and adjusted R-squared averaged over the top-ten models (Tables S2, S3 and S4).

189 Finally, we clustered the 179 genotypes into functional groups to test whether the structure of the phenotypic
190 variability allowed creating beneficial trait associations for multiple components of agronomic performance. We used
191 a standardized ($\mu = 0$, $\sigma = 1$) genotype-by-trait matrix to compute a pairwise Euclidian distance matrix. We then
192 performed Ascending Hierarchical Clustering (ACH) with the *agnes()* function from the *cluster* package using the
193 pairwise Euclidian distance matrix and the Ward aggregation criterion (Ward, 1963). We chose the number of groups
194 based on the height of the tree branches (Figure S3).

195 **Results**

196 **Functional trait variability**

217 The 19 functional traits showed contrasted patterns of variability among genotypes (Table 1). Ear. bio. and Till. nb.
218 were the most variable traits aboveground with respective CVs of 0.42 and 0.26, and mean values of 0.88 g.ind-1 and
219 3.49 tillers.ind-1. Heading and Maturity were the least variable traits, the difference between the earliest and the latest
220 genotype being 250 GDD for both traits, which showed CVs of 0.03 and 0.02, respectively. Angle_{aer}, SLA, LNC, and
221 Height displayed intermediate levels of variability. Of belowground traits, RLD was the most variable trait, with a CV
222 of 0.48 for seminal roots and 0.46 for adventitious roots, and about a ten-fold difference between minimum and
223 maximum values: 0.07 to 0.65 cm.cm-3 and 0.04 to 0.47 cm.cm-3 for seminal and adventitious roots, respectively.
224 Diam, RTD, and RBI were the least variable traits with CVs around 0.10 for both root types. Angle_{root} and SRL had
225 intermediate levels of variability (Table 1).

226 **Relative agronomic performance of mixtures**

227 The mean RYTs for biomass yield and grain yield both equaled 1.04 and were significantly larger than 1 (Fig. 2 a and
228 b), meaning that mixtures produced on average 4% more biomass and grains than expected from single varieties. RYT
229 variation was high for both variables ($sd \geq 0.14$), with values ranging from 0.61 to 1.53 for RYT_{BY} and from 0.56 to
230 1.56 for RYT_{GY}. Mixing effects were non-significant for grain protein content (mean RYT_{GPC} = 1.00) (Fig. 2 c). RYT
231 variation was lower for grain protein content ($sd = 0.04$), RYT_{GPC} values ranging from 0.90 to 1.13.

232 **Relationship between functional trait composition and relative agronomic performance of** 233 **mixtures**

234 Functional trait composition explained 17%, 12%, and 22% of the variability observed on RYT_{BY}, RYT_{GY}, and
235 RYT_{GPC}, respectively (Fig. 3). Depending on the performance component, four (RYT_{BY}, RYT_{GY}) to five (RYT_{GPC})
236 variables were important to explain mixture performance. A mix of aboveground, belowground, and phenological
237 traits significantly affected RYT_{BY} and RYT_{GPC} (Fig. 3 a and c), whereas phenological traits had a limited effect on
238 RYT_{GY} (Fig. 3 b). Both CWMs and Ds contributed to explain the observed variability in relative mixture performance
239 for all three components. Yet, CWMs were overrepresented in the set of important variables for RYT_{GPC} (Fig. 3 c).
240 Two variables involving root traits had a large positive effect on RYT_{BY} and RYT_{GY}: RBI_{sem} D and RTD_{adv} CWM
241 (Fig. 3 a and b). Till. nb. D had a negative effect on both variables. RYT_{BY} increased with increasing Heading CWM,
242 whereas it decreased with increasing LNC CWM (Fig. 3 a). RYT_{GY} decreased with increasing Ear. bio. CWM, and, to
243 a lesser extent, increased with increasing Till. nb. CWM (Fig. 3 b). Till. nb. CWM also had a positive effect on
244 RYT_{GPC} (Fig. 3 c). Maturity CWM, Angle_{aer} CWM, and Angle_{root} CWM affected positively RYT_{GPC}, but had no effect
245 on RYT_{BY} and RYT_{GY}. Diam_{sem} D had a negative effect on RYT_{GPC}.

246 **Functional clustering and identification of the most favourable functional associations**

247 We identified three functional groups that differed on several functional traits (Figure 4 & Table S5). Aboveground,
248 genotypes from group 1 had few tillers, high SLA and LNC, and produced low biomass compared to groups 2 and 3.
249 They were early for heading and maturity. Belowground, they had an intermediate root angle, produced low root
250 length per unit soil volume, and their roots had low-density tissues. Genotypes from group 2 were tall, produced
251 numerous tillers and high biomass. They had low SLA and low leaf nitrogen content, and were early at heading but

232 late at maturity. They had a narrow root angle, but produced more root length per unit soil volume. Their roots were
233 thin and had a low branching intensity. Finally, genotypes from group 3 were short, had a wide aerial angle, and
234 produced intermediate amounts of tillers and biomass. They had low SLA and low LNC. They were late for heading
235 and maturity. Unlike group 2, they had a wide root angle, their roots were thick and dense, and they had high
236 branching intensity.

237 Mixtures associating genotypes from groups 2 and 3 had the highest RYT_{BY} and RYT_{GY} , and showed a
238 significant positive mixing effect on both components (Fig. 5 a and b). Mixtures associating genotypes from group 2
239 had a significant positive mixing effect on biomass yield. Mixtures associating genotypes from group 1 had a
240 significant positive mixing effect on grain yield but a significant negative mixing effect on grain protein content (Fig.
241 5 b and c). Other functional associations did not show significant mixing effects for any of the three components of
242 agronomic performance

243 Discussion

244 Mixing effects detected on grain yield and biomass yield are consistent with values previously reported in the
245 literature (Kjær et al., 2009; Reiss & Drinkwater, 2018; Smithson & Lenné, 1996). The average and the variability of
246 the mixing effects detected on grain protein content were weak, strengthening sparse data observed on such
247 components (Gallandt, Dofing, Reisenauer, & Donaldson, 2001; Sammons & Baenziger, 1985). However, most of
248 these experiments did not explain mixing effect variability. Here, multivariate combinations of trait means and trait
249 differences explain from 12% to 22% of the variation in mixing effects, this proportion being larger for quality-related
250 mixing effects than for quantity-related mixing effects. However, in this study, the distance between plants was much
251 smaller on the row than between rows, leading to stronger intra-genotypic interactions than inter-genotypic
252 interactions in mixture plots. Diversity effects might thus have been stronger with reduced spacing between rows.

253 Root traits had a strong effect on relative agronomic performance measured on productivity variables:
254 relative biomass and grain yields were both affected by a joint positive effect of trait difference on seminal root
255 branching intensity and community-weighted mean on adventitious root tissue density. High root branching intensity
256 is expected to provide intense soil exploration around the principal roots, while low branching intensity is expected to
257 lead to more extensive soil foraging (Richardson et al., 2011). Mixing both foraging strategies could result in
258 belowground spatial complementarity, as observed in the historic “three sisters” (maize/bean/squash) polyculture
259 (Zhang et al., 2014). The positive effect of community-weighted mean of adventitious root tissue density on biomass
260 and grain yield RYTs might result from improved soil anchoring at a stage where plants are sensitive to lodging. Root
261 morphological traits, including root tissue density, determine the biomechanical properties of the roots, which are
262 known to be involved in sensitivity to lodging (Ennos & Fitter, 1992; Liu, Li, Zhu, & Song, 2018). This interpretation
263 was partly supported by single-variety data, where we detected a marginal negative correlation between adventitious
264 root tissue density and lodging score ($P = 0.06$, Figure S5). The joint effect of these two variables on both biomass
265 and grain yield RYT suggests that the effect on grain yield arose primarily from an increase in spike density. This was
266 supported by further analyses showing that these two variables had a positive effect on RYT measured on the number
267 of spikes per square meter (Figure S6 a), but no effect on RYT measured on thousand kernel weight (Figure S6 b).

268 Biomass yield RYT and grain yield RYT were also both negatively affected by differences in tiller number between
269 genotypes. Tillering is classically associated with competitive ability in cereals (Jennings & Aquino, 1968). Tillering
270 differences between components of the mixture could thus have resulted in a yield loss for the weaker competitor,
271 possibly reducing the overall mixture yield.

272 Quality-related mixing effects were mainly explained by the average properties of the mixtures: RYT on
273 grain protein content was positively affected by aerial angle, root angle, and tiller number CWMs. Such a positive
274 effect on grain protein content could have resulted from a negative effect on grain mass, as expected from the classical
275 trade-off between grain mass and grain protein content in cereals (Simmonds, 1995). Indeed, combining genotypes
276 with wide aerial and belowground statures could have resulted in strong competitive interactions during grain filling,
277 thus reducing grain mass. RYT on thousand kernel weight was negatively affected by both aerial and root angle
278 CWMs (Figure S6 b), giving some support to this hypothesis. Yet, such reduction in grain mass did not alter grain
279 yield, suggesting that mixture grain mass can be optimized independently from mixture productivity, and *vice versa*.

280 In our study, some relationships between trait composition and relative performance of mixtures were
281 puzzling. For example, early biomass CWM and tiller number CWM show opposite effects on grain yield RYT. Early
282 biomass and tiller number are strongly positively correlated (Figure S4) and both of them are related to early vigour
283 and competitiveness. Hence, we might expect similar effects of these two traits on mixture productivity. Such
284 inconsistency suggests that other factors such as phenotypic plasticity could have affected mixture performance.
285 Indeed, since we measured traits in single-variety plots, we might expect different trait values and potentially different
286 trait-performance relationships in mixtures. In cereals, many traits such as tiller number or plant height are known to
287 show plastic response following changes in the biotic or abiotic environment (Dornbusch et al., 2010; Evers, Vos,
288 Andrieu, & Struik, 2006). In herbaceous communities, several results suggest that trait plasticity can increase trait
289 divergence and thus complementarity effect between species (*e.g.* Meilhac, Deschamps, Maire, Flajoulot, & Litrico,
290 2020; Niklaus, Baruffol, He, Ma, & Schmid, 2017). In crops, studies at the interspecific level also suggest that plastic
291 changes in architectural traits can lead to increased light capture and productivity in intercropping systems (Zhu,
292 Werf, Anten, Vos, & Evers, 2015). At the intra-specific level, plasticity has been shown to have both positive and
293 negative effects on mixture performance (Dahlin, Kiær, Bergkvist, Weih, & Ninkovic, 2020).

294 **Implications for the selection and assembly of varietal mixtures**

295 Combining favourable trait values in varietal mixtures represents a major challenge for plant breeding.
296 Indeed, our results suggest that diversity can have both positive and negative effects depending on the trait, and that
297 average trait values can be as important as trait differences to determine mixture performance. This raises the question
298 as to how to select mixtures of genotypes that differ on some traits but remain uniform for others (Litrico & Violle,
299 2015). Functional clustering could be used as a first step to structure the phenotypic variability present in the founding
300 population, and to identify the most beneficial associations. Clustering species into functional groups is a common
301 approach in ecology (*e.g.* Hooper & Dukes, 2004) and our study shows that this approach is also relevant at the
302 intraspecific level. Then, selection practices could draw on breeding methods used in hybrid crops, where selection
303 first takes place in separate groups, *i.e.* heterotic groups, and aims at selecting genes not only for their own value but
304 also for their expected value in combination with genes from other groups (Reif, Hallauer, & Melchinger, 2005).

305 Similarly, selection for high performant mixtures could first take place within each functional group, by promoting
306 trait convergence between groups for some traits and divergent selection for others. Further insights on the
307 contribution of phenotypic plasticity would help to have a better mechanistic understanding and a stronger predictive
308 ability of mixing effects.

309 In this context, agronomic performance could be assessed through multiple facets. Varietal mixtures are often
310 evaluated on their productivity relative to monocultures (Kiær et al., 2009; Reiss & Drinkwater, 2018; Smithson &
311 Lenné, 1996). Depending on the species and on the valued part of the plant, either grain or vegetative biomass yield
312 can be targeted. In cereals such as durum wheat, quality can be as important as productivity to determine the final
313 value of the product (Troccoli et al., 2000). Still, the benefit of mixing varieties on quality is poorly documented (Borg
314 et al., 2018). Moreover, increasing both quantity and quality can be challenging because some quantity and quality
315 indicators can be negatively correlated, as in the case of grain yield and grain protein content (Simmonds, 1995). In
316 line with theoretical expectations (Litrico & Violle, 2015), our results suggest that varietal mixtures could allow
317 escaping such trade-offs. Significant positive mixing effects might be achieved on grain yield, notably through
318 increased spike number per square meter, while slight gains might be achieved on grain protein content, mainly
319 through decreased grain mass. Altogether, our approach confirms the need to extend the dialogue between ecology
320 and agronomy to develop more sustainable agriculture by designing varietal mixtures optimized for multifaceted
321 agronomic performance.

322 **Authors' contributions**

323 GeMo, CV, FF, and HF designed the experiment. AR, GeMo, FC, GuMa, FF, and HF contributed to data collection
324 and trait measurements. GuMa conducted root trait analysis. ME conducted NIRS measurements on leaves, and FC
325 conducted NIRS measurements on grains. GeMo analysed the data and led the writing of the manuscript. Significant
326 inputs from PR helped to interpret and discuss the results. All authors contributed critically to the drafts and gave final
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336 **Data availability statement**

337 Data and R code available via the Zenodo Digital Repository <http://doi.org/10.5281/zenodo.3961626> (Montazeaud et
338 al., 2020)

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465

Table 1: Phenotypic diversity characterized for 19 functional traits across 179 durum wheat genotypes grown in single-variety plots. Minimum, maximum, mean (standard deviation), and coefficient of variation are reported for each trait. Traits are ranked according to decreasing CV values.

	trait	unit	min	max	mean (sd)	CV
Aboveground	Ear. bio.	g.ind ⁻¹	0.28	2.23	0.88 (0.37)	0.42
	Till. nb.	nb till.ind ⁻¹	1.67	7.50	3.49 (0.90)	0.26
	Angle _{aer}	°	11.50	28.50	16.56 (2.83)	0.17
	SLA	m ² .kg ⁻¹	14.88	36.37	20.56 (3.21)	0.16
	LNC	%	2.85	4.78	3.76 (0.39)	0.10
	Height	cm	75.70	129.50	97.94 (9.13)	0.09
Belowground	RLD _{sem}	cm.cm ⁻³	0.07	0.65	0.22 (0.11)	0.48
	RLD _{adv}	cm.cm ⁻³	0.04	0.47	0.17 (0.08)	0.46
	SRL _{sem}	m.g ⁻¹	48.50	188.75	103.48 (24.30)	0.23
	SRL _{adv}	m.g ⁻¹	24.02	65.50	43.74 (8.28)	0.19
	Angle _{root}	°	66.33	158.33	112.42 (18.59)	0.17
	RTD _{sem}	g.cm ⁻³	0.11	0.20	0.15 (0.02)	0.12
	RTD _{adv}	g.cm ⁻³	0.05	0.09	0.07 (0.01)	0.12
	RBI _{sem}	tips.cm ⁻¹	0.94	1.67	1.27 (0.14)	0.11
	Diam _{adv}	mm	0.40	0.74	0.52 (0.06)	0.11
	RBI _{adv}	tips.cm ⁻¹	1.00	1.61	1.27 (0.13)	0.10
	Diam _{sem}	mm	0.21	0.36	0.27 (0.02)	0.09

Phenology	Heading	GDD	1424.00	1696.30	1549.45 (47.10)	0.03
	Maturity	GDD	2219.80	2458.00	2281.75 (43.27)	0.02

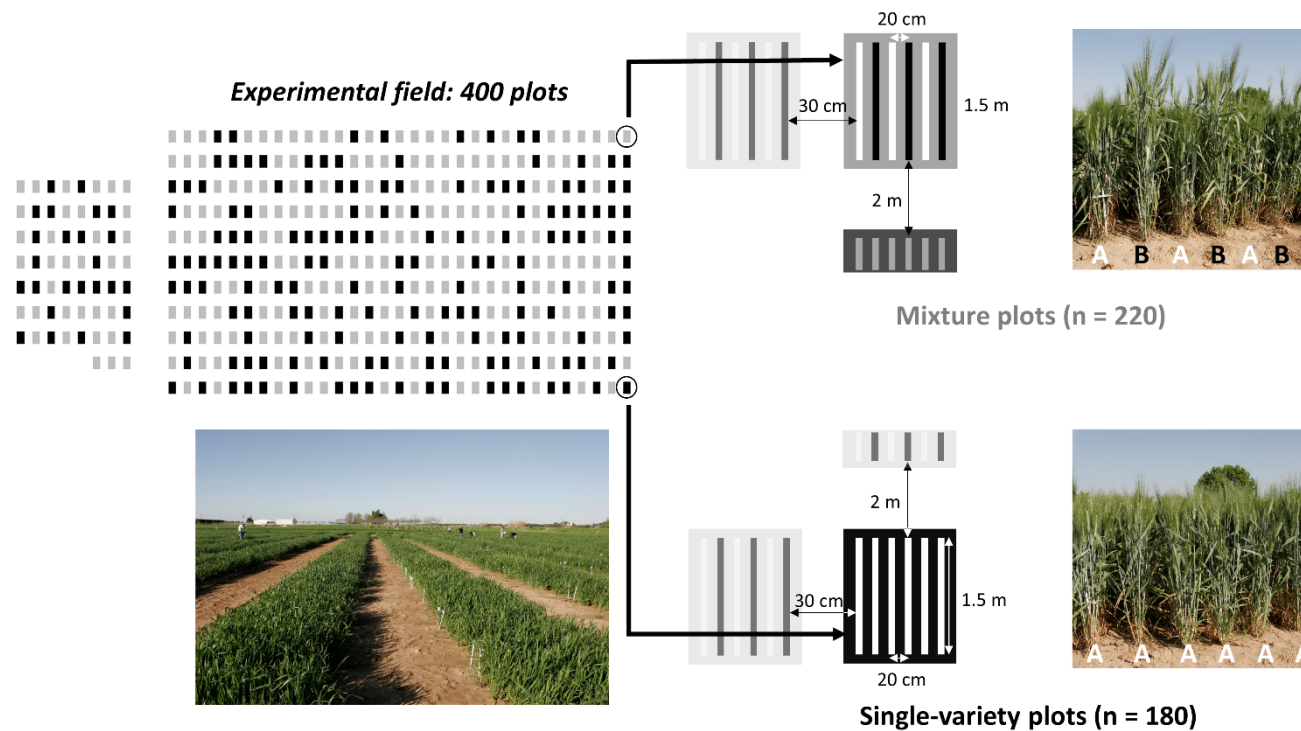


Figure 1: Overview of the experimental design containing 400 plots (180 single-varieties & 220 mixtures). The whole design is represented on the left, with single-variety plots in black, and mixture plots in grey. As shown in the figure, the design had to be splitted in two parts separated by around 10 meters to allow the irrigation system wheel to pass through. Only 376 plots (179 single-varieties & 197 mixtures) were used after data cleaning (see Figure S2). Single-variety and mixture plots were arranged randomly in the field. As depicted on the right, the inter-plot distance was 2 m in the vertical direction and 30 cm in the horizontal direction. Plots were composed of 6 rows of 1.5 m long and distant from 20 cm. In single-variety plots, the same genotype was sown on all 6 rows, whereas two genotypes were sown in alternate rows in mixture plots.

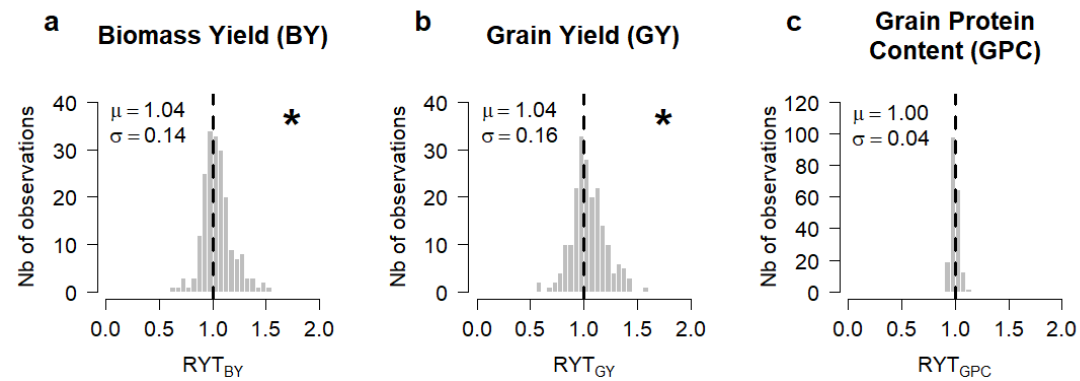


Figure 2: Mixing effect distributions on biomass yield (a), grain yield (b), and grain protein content (c). Mixing effects were quantified with RYT (cf Material and Methods). Means (μ) and standard deviations (σ) are reported. A star symbol indicates a mean RYT significantly different from 1 at $P < 0.05$ (t.test).

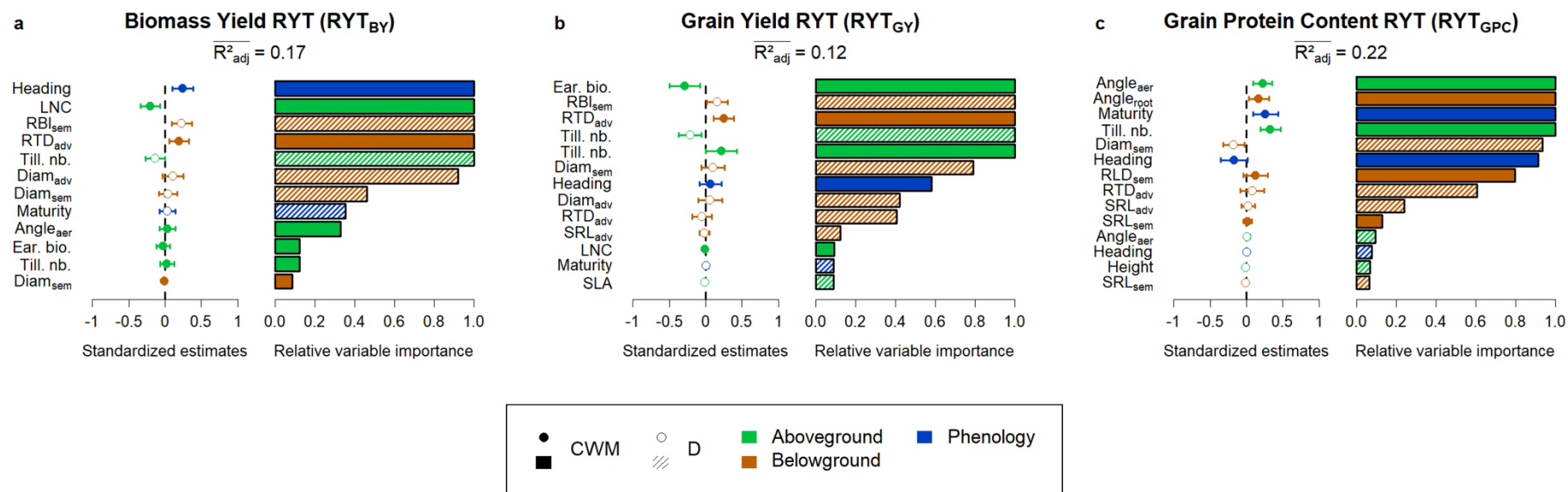


Figure 3: Standardized effects of traits on mixture RYT_s measured on biomass yield (a), grain yield (b), and grain protein content (c). Backward model selection was performed on a full model with RYT as the response variable and all trait CWMs and Ds as explanatory variables. Based on AIC_C , the top-ten models were retained to compute model-averaged estimates reported on the left side of the three panels with their 95% unconditional confidence intervals. Empty symbols represent Ds and filled symbols represent CWMs. The relative importance of the variables are reported on the right side of the three panels and can be interpreted as the probability that the variable appears in the best model. Hatched bars represent Ds and filled bars represent CWMs. Colours refer to the type of traits, with aboveground traits, belowground traits, and phenological traits represented in green, brown, and blue, respectively. Adjusted R-squared averaged across the top-ten models ($\overline{R^2}_{adj}$) are also reported for the three models.

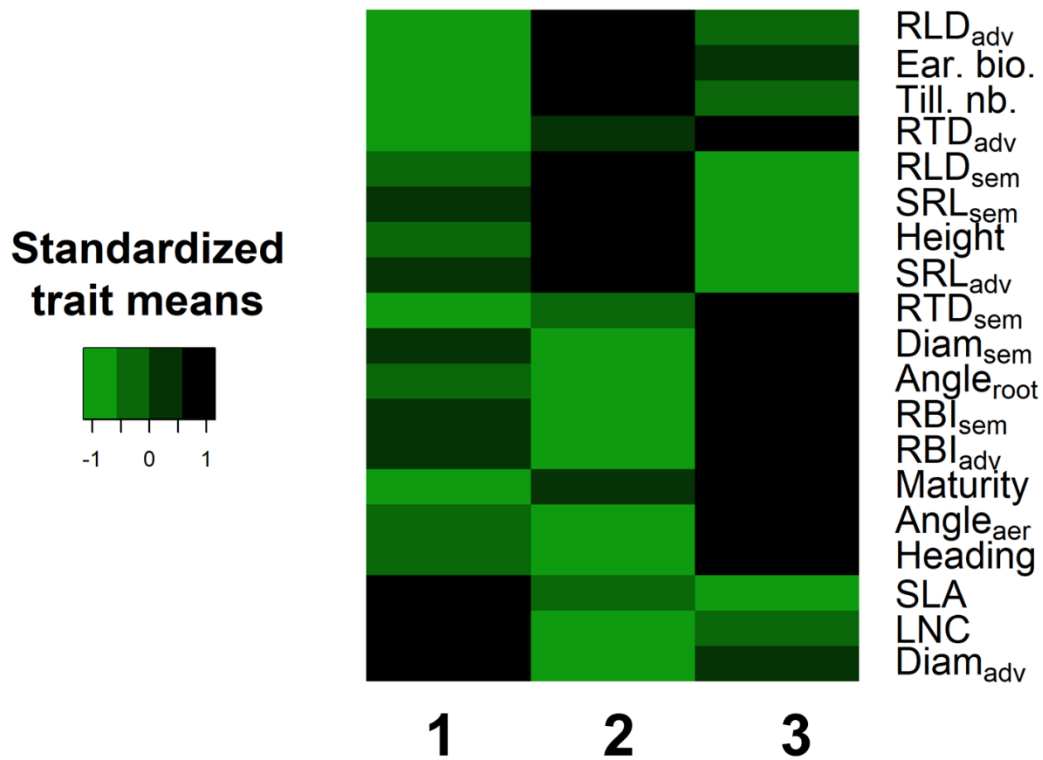


Figure 4: Characterization of the three functional groups based on their trait means measured in single-variety plots. Variables were standardized and clustered so that traits with similar variation on their means over the three groups are clustered together. Values increase from green (lower values) to black (higher values).