Grain Quality as Influenced by the Structural Properties of Weed Communities in Mediterranean Wheat Crops

Eva Hernández Plaza [1,2], Fernando Bastida [3], David J. Gibson [4], Francisco Barro [2], María J. Giménez [2], Yesica Pallavicini [2], Jordi Izquierdo [5] and José L. González-Andújar [2,*]

1   National Institute for Agricultural and Food Research and Technology, Spanish National Research Council (INIA-CSIC), Ctra. La Coruña km 7.5, 28040 Madrid, Spain

2   Institute for Sustainable Agriculture, Spanish National Research Council (IAS-CSIC), Avda Menéndez Pelayo s/n, Campus Alameda del Obispo, Aptdo, 14004 Córdoba, Spain

3   Escuela Técnica Superior de Ingeniería, University of Huelva, Campus de la Rábida, Ctra. Huelva—Palos de la Frontera, s/n, 21071 Palos de la Frontera, Spain

4   School of Biological Sciences, Southern Illinois University Carbondale, Carbondale, IL 62901-6509, USA

5   Department of Agri-food Engineering and Biotechnology, Polytechnic University of Catalonia, Campus Baix Llobregat, C/Esteve Terradas 8, 08860 Castelldefels, Spain

*   Correspondence: andujar@ias.csic.es

Abstract: Weed community structure, including composition, taxonomic and functional diversity, may explain variability in crop quality, adding to the variability accounted by management, climatic and genetic factors. Focusing on Mediterranean rainfed wheat crops, we sampled weed communities from 26 fields in Spain that were either organically or conventionally managed. Weed communities were characterized by their abundance and taxonomic, compositional and trait-based measures. Grain protein concentration and the glutenin to gliadin ratio were used as indicators of wheat grain quality. Linear mixed effects models were used to analyze the relationship between crop quality and weed community variables, while accounting for environmental factors. Nitrogen fertilization, previous crop and precipitation explained a large portion of the variation in wheat grain protein concentration ($R^2_{marginal}$ = 0.39) and composition ($R^2_{marginal}$ = 0.26). Weed community measures had limited effects on grain quality (increasing $R^2_{marginal}$ of models by 1% on average). The weed effects were related to the composition and the functional structure of their communities, but not to their abundance. Environmental conditions promoting higher protein concentration were also selecting for weed species with competitive attributes, whereas the role of weed functional diversity depended on the functional trait and on the resource limiting crop grain quality. Understanding the mechanisms of weed effects on crop quality could aid on designing sustainable weed management practices.

Keywords: community weighted mean; glutenin to gliadin ratio; protein concentration; weed functional diversity; wheat; nitrogen fertilization; species richness

1. Introduction

Crop quality in wheat has been mainly associated with the composition and the protein content of the grain. Grain storage proteins (collectively, prolamins) determine the properties of the wheat flour and its uses. Among these, the gluten proteins (gliadins and glutenins) are especially important because they confer to wheat dough the properties of extensibility, viscosity and elasticity [1]. The variation in protein concentration in wheat has a genetic component and varies between species [2] and among cultivars [3]. However, growing conditions, especially the availability of nitrogen for the crop plant during grain filling, appear to have a larger effect than genotype [4,5]. A positive, linear relationship (with a subsequent level-off) has been described between nitrogen fertilization and wheat grain protein concentration [6,7]. The previous crop and the amount and timing of precipitation events, also influence nitrogen availability. For example, an increase in

grain protein concentration has been associated with growing wheat after legumes as compared to continuous wheat cultivation [8]. On the other hand, high precipitation can reduce nitrogen availability during the growing season decreasing protein content [9], whereas water stress at the end of the cereal cycle was related to an increase in grain protein concentration at the expense of yield in spring barley [5].

Weed communities may compete with the crop for resources affecting crop quality. However, the role of weeds on wheat grain protein concentration and composition has been less explored with the majority of studies looking at the effect of weed abundance. Increasing weed density may have either a positive [9–12], negative [8,12] or neutral effect [8,12] on wheat protein concentration. This variability of responses may depend on the relative importance of the competition between crop and weeds for nitrogen and water in each situation [12]. However, it could be also explained by differences on weed communities in terms of their composition, diversity and functional structure. These aspects of weed communities have been found to explicate the relationship between weed communities and crop yield [13–16] and could be also decisive to explain the role of weeds on wheat quality. In this sense, Ref. [17] found that under high weed density, seed protein concentration of soybean (*Glycine max* L.) was associated with the weed community composition, with protein concentration values varying depending on the species dominating the weed community. Further, [12] showed that the response of wheat grain protein concentration to an increase in weed density depended on functional types of weeds. Hence, apart from total abundance, the main effect of weeds will be based upon the structure of their communities in terms of the species relative abundance and their functional attributes. From experimental plant communities in non-agrarian systems, it is known that increasing species richness, while maintaining high evenness, relates to higher biomass production and soil nitrogen uptake [18]. Therefore, we could expect a negative effect of weed species richness on protein concentration because richer communities are more likely to increase the use of available soil nitrogen. However, in natural communities, like these of weeds, the number of individuals is not generally evenly distributed between species. Moreover, the constituent species are not a random assemblage of the regional pool, but field community composition is the result of abiotic conditions and biotic interactions [19,20]. That is, functional structure can differ highly among weed communities with similar richness or evenness, and the most abundant weed species in a field may not be the most productive but be the ones that have persisted after crop management. In this manner, weed species richness and evenness could partly explain crop grain quality, but the direction of their effect likely depends on the functional identity of the species. Thus, a functional approach, based on trait values, can complement these views. Both the dominant attributes and the distribution of trait values in the community could be informative. Weed communities dominated by highly competitive species may limit crop nitrogen acquisition to a higher extent than those communities in which species show less competitive attributes. On the other hand, higher functional diversity may reduce (or increase) weed pressure on the crop depending on whether it implies the appearance of low (or high) competitive phenotypes.

We explored the relationship between protein concentration and composition in the wheat grain and different aspects of the weed community structure across a broad geographic area and varying growing conditions. To do this, we sampled wheat fields and characterized the structure of the weed communities using different taxonomic diversity and functional structure measures. We also collected data on field management and climatic variables that could potentially affect crop grain quality and weed communities.

We asked: (1) whether weed community structure contributes towards explaining the variability in crop grain protein concentration and composition, (2) whether trait-based measures have a higher explanatory power than weed abundance, species richness or evenness and (3) whether weed communities with competitive phenotypes are associated with lower crop grain protein concentration, and if this effect is buffered by higher weed functional diversity.

2. Materials and Methods

2.1. Field Data Collection and Laboratory Analyses

The study was carried out in 2013–2014 across 26 wheat fields (*Triticum aestivum* L. and *Triticum turgidum* subsp. *durum* Desf.) under a Mediterranean climate in Spain. Fields were either organically or conventionally managed and were located in cereal areas in four Spanish regions: Andalusia (15 fields), Madrid (3 fields), Castilla-La Mancha (4 fields) and Catalonia (4 fields). In each field, ten (1 m × 1 m) plots were established during crop tillering and maintained until harvest. Weeds were sampled at the end of the crop vegetative period (April with dates slightly varying depending on field location). In each plot we recorded the weed species present, and we visually assessed the cover (in cm^2) of each species. For each species, we went individual by individual assessing their cover in cm^2 and we summed the cover across all individuals in each plot. We also measured plant height (cm). We did not collect weed biomass, but we obtained weed biovolume as a proxy of their abundance. The biovolume of each weed species was computed in each plot as the species cover (cm^2) multiplied by its height (cm) and the sum of biovolumes of each species was used to obtain total weed biovolume in the plot. Immediately before crop harvest, we sampled the plots again to obtain crop yield. In each plot we counted the number of wheat stems with ears and cut 30 of them. We determined the dry weight (after 48 h at 65 °C) of the 30 stems, threshed the ears and weighed the grain for each sample. In doing this we obtained an average value for the grain weight of an ear. We calculated crop grain weight in each plot multiplying mean grain weight of each sample by the total number of fertile stems in the plot. In each plot, we obtained two measures of grain quality: the percentage of total dry protein content (protein concentration hereafter) and the glutenin to gliadin ratio. The total protein content was determined using the Kjeldahl standard method at the Laboratorio Agroalimentario de Córdoba (Córdoba, Spain). The procedure for the extraction and determination of glutenin and gliadin content is detailed in supplementary file S1. The glutenin to gliadin ratio was calculated by dividing the amount of glutenins by gliadin content.

For each field we also recorded the crop variety and collected data on two management practices and on one climatic factor that could potentially affect nitrogen availability for crop plants and modulate the effect of weeds on the grain quality (Table 1): fertilization rate (kg N/ha), preceding crop (legume/fallow or sunflower/cereal; categories depending on the nutrient demand of the crop) and precipitation during crop growth season (in mm, from the nearest meteorological stations).

2.2. Weed Community Structure Measures

Data on the cover of each weed species were used to compute five measures that characterized weed community structure in each plot: weed species richness (S; as the number of species present in each plot), weed community evenness (the Pielou index, J), a measure of community composition, as well as the community weighted mean (CWM) and the functional dispersion (FDis) of eight traits. We chose traits that could be related to weed interference with the crop and thus can affect grain protein content: functional type (coded as grass = 1, forb = 0), life form (therophyte = 1, other = 0), growth form (erect, caespitose or climbing = 1, other = 0), maximum height (cm, subsequently we will refer to this trait as height), flowering start (month), flowering duration (number of months), specific leaf area (SLA, mm^2/mg) and seed mass (mg). See Table S1 for details and bibliographic sources. CWM is the average value of a trait in the community (plot) weighted by each species abundance, whereas FDis is the dispersion of phenotypes within the functional space occupied by the whole community. FDis is thus the abundance-weighted mean distance of individual species to the centroid of all species, where weights are their relative abundance. Both measures were computed with the "FD" library [21] under the R environment [22]. A measure of community composition was also used to summarize the whole effect of the different weed phenotypes without focusing on specific traits (see Section 2.3).

Table 1. Fields sampled in the study: location and average value, across the 10 plots per field, of the percentage of wheat grain protein concentration, the glutenin to gliadin ratio, the crop grain weight, the weed biovolume (see text for details) and weed species richness (S). We also include whether the field was managed organically or not (organic), the wheat species and the variety, the fertilization rate (expressed as kg of N/ha), the preceding crop of each field as well as the total precipitation during the crop season (dates varying between fields; see text).

Field Name	Location	% Grain protein concentration	Glutenin/Gliadin	Grain weight (g/m2)	Weed Biovolume (cm3)	S	Organic	Wheat Species	Crop variety	kg N/ha	Preceding Crop	Precipitation (mm)
AGAPA1	Palma del Río, Córdoba	12.75	0.504	456.45	10791	10	no	*T. aestivum*	Artur Nick	134	Fallow	350.8
AGAPA2	Palma del Río, Córdoba	11.2	0.52	295.65	5246.7	11	no	*T. turgidum*	Prospero	134	Leguminous	350.8
CANDÓN1	Candón (Beas), Huelva	9.07	0.739	222.29	10010.2	15	yes	*T. turgidum*	Avispa	1.5	Sunflower	406.1
CANDÓN2	Candón (Beas), Huelva	8.66	0.979	272.7	16013.5	16	yes	*T. turgidum*	Avispa	1.5	Sunflower	406.1
CANDÓN3	Candón (Beas), Huelva	8.6	0.956	227.15	6933.8	13	yes	*T. turgidum*	Avispa	1.5	Sunflower	406.1
CANDÓN4	Candón (Beas), Huelva	8.75	0.823	194.65	13442.8	18	yes	*T. turgidum*	Avispa	1.5	Sunflower	406.1
COLLSUSPINA	Colluspina, Barcelona	10.79	0.729	136.38	201165	6	no	*T. aestivum*	Nogal	129	Winter cereal	233.7
ENCIN1	Alcalá de Henares, Madrid	13.66	0.74	142.62	524.8	3	no	*T. aestivum*	Ovalo	49	Winter cereal	233.2
ENCIN3	Alcalá de Henares, Madrid	18.82	0.435	128.03	6693.9	4	no	*T. aestivum*	Ovalo	40	Leguminous	233.2
ESCACENA	Escacena del Campo, Huelva	14.57	0.536	547.43	215271.6	9	no	*T. aestivum*	Tejada	132	Sunflower	360.9
HIGUERUELA1	Santa Olalla, Toledo	11.75	0.538	139.38	25233.8	12	yes	*T. aestivum*	Pane247	0	Leguminous	295.8
HIGUERUELA2	Santa Olalla, Toledo	13.07	0.447	115.97	171521.5	9	no	*T. aestivum*	Pane247	52	Winter cereal	295.8
HIGUERUELA3	Santa Olalla, Toledo	12.89	0.689	83.13	51637.06	7	no	*T. aestivum*	Pane247	52	Leguminous	295.8
HIGUERUELA5	Santa Olalla, Toledo	12.95	0.699	455.41	6715.8	9	no	*T. aestivum*	Botticeli	103	Winter cereal	295.8

Table 1. *Cont.*

Field Name	Location	% Grain protein concentration	Glutenin/Gliadin	Grain weight (g/m2)	Weed Biovolume (cm3)	S	Organic	Wheat Species	Crop variety	kg N/ha	Preceding Crop	Precipitation (mm)
IAS	Córdoba	15.15	0.431	252.81	230434.8	13	yes	*T. turgidum*	Simeto	147	Leguminous	399
MANZANILLA1	Manzanilla, Huelva	15.68	0.564	211.94	231736.6	8	no	*T. aestivum*	Tejada	163	Sunflower	404.4
MANZANILLA2	Manzanilla, Huelva	14.01	0.57	400.05	36690	7	no	*T. aestivum*	Tejada	163	Sunflower	404.4
MOIA1	Moià, Barcelona	17.3	0.59	460.23	426211.1	3	no	*T. aestivum*	Soissons	129	Winter cereal	233.7
MONCLOVA1	Fuentes de Andalucía, Sevilla	10.59	0.58	267.58	4739.4	10	no	*T. aestivum*	Palesio	133	Sunflower	365.4
MONCLOVA2	Fuentes de Andalucía, Sevilla	13.66	0.653	298.64	2581.935	8	no	*T. turgidum*	Amilcar	129	Sunflower	365.4
MORÓN1	Morón de la Frontera, Sevilla	9.93	0.357	214.96	93310.3	19	yes	*T. aestivum*	Aragon03	0.2	Leguminous	314
MORÓN2	Morón de la Frontera, Sevilla	10.36	0.442	161.28	32331.3	19	yes	*T. aestivum*	Aragon03	0.2	Leguminous	314
PRADERA	Alcalá de Henares, Madrid	16.23	0.468	196.21	56557.6	10	yes	*T. aestivum*	Marius	0	Fallow	233.2
PRATS1	Prats de Lluçanés, Barcelona	12.93	0.854	480.39	358623	10	yes	*T. aestivum*	Soissons	100	Sunflower	350.3
PRATS2	Prats de Lluçanés, Barcelona	12.73	0.766	368.86	437615	7	no	*T. aestivum*	Soissons	100	Winter cereal	350.3
TOMEJIL	Alcalá del Río, Sevilla	15.04	0.641	422.9	49797.1	8	yes	*T. aestivum*	Conil	27	Leguminous	313.2

2.3. Statistical Analyses

All statistical analyses were performed in R [22] using functions from the libraries vegan [23], lme4 [24], MuMIn [25] and jtools [26].

Grain protein concentration, grain weight and weed biovolume were log transformed prior to the analyses.

2.3.1. Community Composition Measures

We performed a principal component analysis (PCA) to obtain an ordination of the plots along axes of maximum variation according to their community composition. PCA was carried out using functions from the vegan library [23]. Prior to the PCA, a Hellinger transformation was applied to the data to avoid the double-zero problem [27]. Further details on this analysis and on the selection procedure used for retaining significant axes are included in supplementary file S2. We retained three axes PCA1, PCA3, PCA5 as measures of weed community composition across plots. PCA1 indicates a gradient regarding sowing date (and concurrently weed emergence). Positive values are characterized by weed species with early emergence (e.g., *Lolium rigidum* or *Anacyclus clavatus*). These species may reach higher abundance where the crop was sown earlier (October–November), as in fields located in Catalonia or the center of Spain compared to the south of the country (November–December). Negative values in this axis are characterized by species that may be more flexible in germination timing as *Papaver rhoeas*, *Anagallis arvensis* or *Sinapis arvensis*. PCA3 can be interpreted as a gradient of water availability in soils, especially during winter, as species with positive values (e.g., *Juncus bufonius* or *Polygonum aviculare*) are typically associated with water-saturated soils during the winter cropping period. PCA5 is related to fertilization use with positive values shown by nitrophilous species while negative values shown by leguminous and short stature species.

2.3.2. Protein Concentration and Glutenin to Gliadin Ratio in Relation to Crop Yield and Growing Conditions

The relationships between grain weight and the grain protein concentration and the glutenin to gliadin ratio were tested with linear mixed effects models, using functions from library lme 4 [24], in which field and wheat variety were included as random factors. We also performed linear mixed effects models to test the relationship between management and climatic factors and both crop quality variables. For all the models we computed the coefficients of determination for mixed-effect models based on [28] and implemented in the library MuMIn [25]: the R^2 marginal (the variance explained by fixed factors; $R^2(m)$ LMM) and the R^2 conditional (variance explained by both fixed and random factors; $R^2(c)$ LMM). Model assumptions of normality of residuals and homogeneity of variance were graphically evaluated.

2.3.3. Protein Concentration and Glutenin to Gliadin Ratio in Relation to Weed Community Structure and Growing Conditions

First, we analyzed the relationship between weed community metrics and variables of crop quality without taking environmental covariates into account. We fitted linear mixed models separately for each weed community structure measure (biovolume, S, J, PCA axes and the CWM and FDis of the eight traits) and for the two response variables. We proceeded in a similar way as described above.

Secondly, we tested these relationships in a given environment. We fitted maximum likelihood models (M) of increasingly complexity and compared them using the Akaike Information Criterion corrected for small sample size (AICc). M0 (environmental model) was the most parsimonious model accounting only for the effects of growing conditions on protein concentration and on the glutenin to gliadin ratio. In each case it included the most important management and climatic variables (Table S2). M1 added the PCA axes to take into account the similarity on weed community composition among the sampled plots. M2 included the variables from M1 plus either weed biovolume, S, J or a functional

weed community measure. Other models of higher complexity, M3 to M9 in the case of protein concentration and M3 to M5 in the case of glutenin to gliadin ratio, were formulated from M2, and they incorporated the possible interactions between the weed community measure and the environmental variables. These models tested whether weed effects on grain quality depended on growing conditions. Field and variety were included in all cases as random factors. Before the analyses we centered all the numerical independent variables, and we assessed their collinearity by computing variance inflation factors (VIF) using a function that can be retrieved from https://stat.ethz.ch/pipermail/r-sig-mixed-models/2018q1/026461.html (accessed on 15 November 2020). In eight models we found VIFs values > 5 in the interaction between the weed community measure and the preceding crop, but our results did not change when running the alternative models without this interaction. In each case, to infer potential causal relationships and for parameter estimation we selected one model following this procedure. First, we identified the model with the lowest AICc. We were conservative and when models were ranked very similar (i.e., ΔAICc < 2) we chose the simplest one. Then we estimated the parameters of the final model using restricted maximum likelihood and we obtained the confidence intervals of each parameter. In cases where a model with interactions had been chosen and the confidence intervals of the interactions included zero, a simpler model was selected, and the estimation of parameters was repeated. Model assumptions were checked by plotting residuals versus fitted values and versus each covariate in the model. Data exploration before the analyses revealed two very high values in the glutenin to gliadin ratio as well as two large observations in the CWM and FDis of seed mass and height and one in the case of CWM and FDis of SLA. The omission of these values in the analyses did not qualitatively change the results but greatly improved the normality of the residuals in the models.

To facilitate a more comprehensive interpretation of the results, we also accounted for the influence of environmental factors on the weed community structure measures. For this, we also fitted linear mixed models in which these metrics were the response variables. Additionally, we performed correlations between the CWM and the FDis of each trait and between PCA axes and S, J and biovolume.

3. Results

Crop grain protein concentration in the plots ranged from 7.7 to 21.0%, whereas the glutenin to gliadin ratio varied from 0.03 to 2.54. Grain protein concentration and the glutenin to gliadin ratio were negatively correlated (rho = -0.37 $p < 0.01$). Weed species richness in the plots, across all the fields, varied from 1 to 26 species/m^2.

3.1. Protein Concentration and Glutenin to Gliadin Ratio in Relation to Crop Yield and Growing Conditions

We found a trade-off between crop grain weight and protein concentration (Chi-square = 4.7, $p = 0.03$; log wheat grain = -0.04 ± 0.02). However, a larger amount of variance was explained by the random factors than by the fixed factor (R^2 LMM (marginal) = 0.009; R^2 LMM (conditional) = 0.83). No relationship was found between crop grain weight and the glutenin to gliadin ratio (Chi-square = 0.22, $p > 0.05$). Grain protein concentration was explained by fertilization, preceding crop and precipitation. Nitrogen fertilization increased grain protein concentration, whereas higher precipitation and having winter wheat or sunflower as previous crop, in comparison to a legume crop or a fallow, reduced its values (Table S2). The glutenin to gliadin ratio was negatively related to fertilization and positively related to having winter wheat or sunflower as previous crop. Precipitation did not affect the ratio and this variable was not retained in the subsequent analyses (Table S2). Weed biovolume was not related to crop grain weight (Chi-square = 1.2, $p > 0.05$).

3.2. Crop Grain Quality in Relation to Weed Community Structure

Weed species richness, PCA1 and PCA3 axes, the CWM of seed mass and the CWM of growth form, were related to the variability in grain protein concentration (Table 2). In all cases the variance explained by these metrics was low (1% to 5%; shown by the marginal R^2 of these models) in relation to the variance accounted by the whole model. Higher species richness was associated with a reduction in grain protein concentration, as shown by the negative estimate (Table 1), though the effect was weak. Increasing the CWM seed mass and having communities with more erect, caespitose or climbing growth forms (higher values of CWM growth form) slightly increased the grain protein concentration (Table 2). The PCA1 axis was negatively related to crop grain protein concentration, PCA3 showed a positive relationship (Table 2), whereas no relationship was found for PCA5. Weed biovolume was not related to wheat grain protein concentration (Chi-square = 1.8, $p > 0.05$). None of the analyzed measures affected the variability in the glutenin to gliadin ratio.

Table 2. Summary of mixed-effects models on the relationship between protein concentration in wheat grains and weed community structure measures. Estimates, confidence intervals (C.I.), p values and R^2 marginal and conditional values of each of the models for which the measure of weed community structure was statistically significant are shown.

	Intercept		Weed Community Measure		R^2	R^2	
	Estimate	C.I	Estimate	C.I	Marginal	Conditional	*p* Value
S	1.13	1.10, 1.18	−0.003	−0.005, −0.0007	0.025	0.81	0.02
CWM seed mass	1.10	1.05, 1.14	0.001	0.0002, 0.002	0.01	0.84	0.01
CWM growth form	1.08	1.03, 1.13	0.035	0.0003, 0.07	0.01	0.82	0.05
PCA1	1.10	1.10, 1.20	−0.12	−0.23, −0.01	0.01	0.84	0.02
PCA3	1.10	1.10, 1.10	0.33	0.14, 0.52	0.05	0.83	<0.001

3.3. Crop Protein Concentration in Relation to Weed Community Structure Considering Growing Conditions

The inclusion of the three PCA axes improved the parsimony of M0 for explaining crop grain protein concentration (AIC values in Table S4a,b). When we compared M1 with models of increasing complexity, we found that the CWM and FDis of height, the CWM of seed mass and the FDis of flowering onset and growth form, and in some cases their interactions with the climatic and management variables contributed to explain protein concentration (Table 3 and Table S3a,b for AIC values). However, the extent of this contribution was in general low, as shown by a null to slight increase (0% to 2%) in the R^2 marginal of these models when compared with that of M1.

The inclusion of environmental factors and considering the PCA axes reduced the importance of species richness in explaining the variability in crop grain protein concentration (Table S3a). Higher CWM height was positively associated with grain protein concentration values (Table 3A). Under higher precipitation values, more diverse weed communities in terms of height (FDis height) were associated with higher grain protein concentration, whereas the opposite occurred for communities more diverse in growth forms or in the dates of flowering start (Figure 1).

Table 3. (A) Estimates, confidence intervals (C.I.) and R^2 marginal and conditional values of the M1 model (management and climatic variables along with PCA axes), and of each of the models in which the inclusion of a community weighted means (CWM) index improved the M1 parsimony for explaining protein concentration in wheat grains. The reference category for preceding crop was sunflower and cereal crops. (B) Estimates, confidence intervals (C.I.) and R^2 marginal and conditional values of models for which the inclusion of FDis functional dispersion index (FDis) improved the parsimony of M1 in explaining protein concentration in wheat grain. The reference category for preceding crop was sunflower and cereal crops.

(A)						
	M1: Management and Climatic Factors + Weed Composition (PCA Axes)		**CWM Height**		**CWM Seed Mass**	
	Estimate	**C.I.**	**Estimate**	**C.I.**	**Estimate**	**C.I.**
Intercept	1.1	1.06, 1.15	1.1	1.06, 1.16	1.1	1.06, 1.15
CWM index			0.0004	1.10^{-4}, 6.10^{-4}	0.0003	-4.10^{-4}, 1.10^{-3}
Fertilization	0.001	7.10^{-5}, 1.10^{-3}	0.001	1.10^{-4}, 2.10^{-3}	0.001	1.10^{-4}, 1.10^{-3}
Precipitation	-0.001	-2.10^{-3}, -5.10^{-4}	-0.001	-2.10^{-3}, -5.10^{-4}	-0.001	-2.10^{-3}, -5.10^{-4}
Preceding crop	-0.04	$-0.1, 0.01$	-0.05	$-0.1, 0.01$	-0.05	$-0.1, 0.01$
PCA1	-0.17	$-0.3, -0.06$	-0.21	$-0.3, -0.1$	-0.14	$-0.3, -0.04$
PCA3	0.23	0.03, 0.4	0.23	0.03, 0.4	0.19	$-0.02, 0.4$
PCA5	0.33	0.1, 0.6	0.28	0.05, 0.5	0.3	0.06, 0.5
CWM index × Precipitation						
CWM index × Fertilization					2.10^{-5}	4.10^{-6}, 3.10^{-5}
CWM index × Preceding crop						
R^2 marginal	0.47		0.48		0.48	
R^2 conditional	0.9		0.9		0.9	

Table 3. *Cont.*

| (A)						
M1: Management and Climatic Factors + Weed Composition (PCA Axes)						
			CWM Height		**CWM Seed Mass**	
	Estimate	C.I.	Estimate	C.I.	Estimate	C.I.
(B)						
	FDis Height		**FDis Growth Form**		**FDis Flowering Start**	
	Estimate	C.I.	Estimate	C.I.	Estimate	C.I.
Intercept	1.1	1.06, 1.15	1.1	1.06, 1.15	1.1	1.06, 1.15
FDis index	−0.03	−0.14, 0.07	−0.03	−0.06, −0.007	−0.03	−0.06, −0.007
Fertilization	0.001	$1.10^{-4}, 1.10^{-3}$	0.001	$6.10^{-5}, 1.10^{-3}$	0.001	$1.10^{-4}, 1.10^{-3}$
Precipitation	−0.001	$-2.10^{-3}, -4.10^{-4}$	−0.001	$-2.10^{-3}, -5.10^{-4}$	−0.001	$-2.10^{-3}, -6.10^{-4}$
Preceding crop	−0.04	−0.1, 0.01	−0.04	−0.1, 0.02	−0.04	−0.1, 0.01
PCA1	−0.14	−0.2, −0.04	−0.18	−0.3, −0.07	−0.17	−0.3, −0.07
PCA3	0.27	0.07, 0.5	0.25	0.05, 0.4	0.22	0.02, 0.4
PCA5	0.33	0.1, 0.6	0.36	0.1, 0.6	0.34	0.1, 0.6
FDis index × Precipitation	0.003	$7.10^{-4}, 4.10^{-3}$	−0.0004	$-7.10^{-4}, -7.10^{-5}$	−0.0005	$-9.10^{-4}, -6.10^{-5}$
FDis index × Fertilization						
FDis index × Preceding crop			0.04	0.005, 0.07		
R^2 marginal	0.47		0.49		0.49	
R^2 conditional	0.89		0.91		0.9	
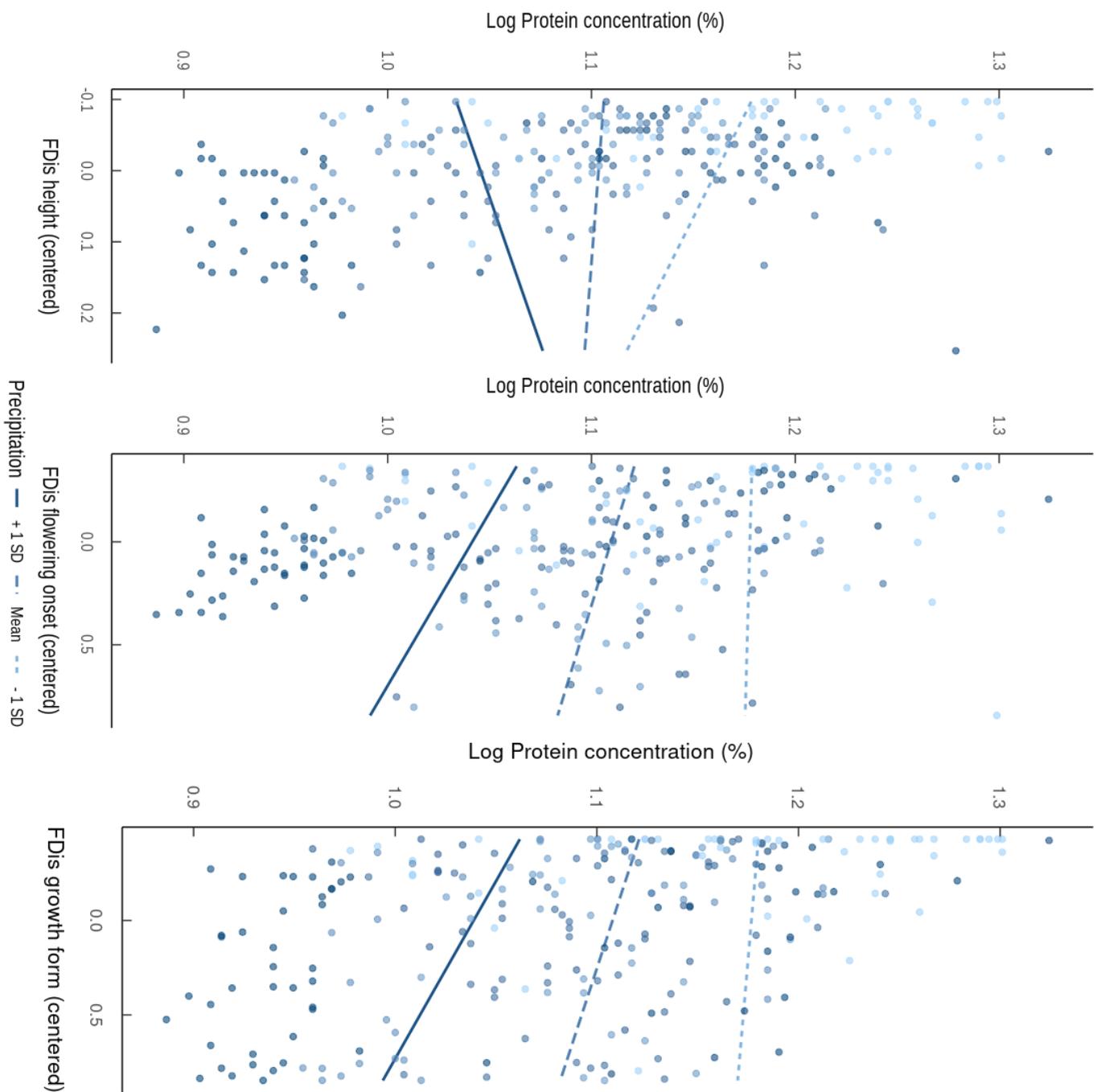
Figure 1. Interaction between FDis height, FDis flowering onset and FDis growth form of weed communities and precipitation during the crop season to explain protein concentration in wheat grains. FDis indices and precipitation were centered before the analysis. Lines show the effect of FDis measures on protein concentration when precipitation takes the mean value and values one standard deviation higher or lower than the mean. Observed data points are shaded in blue depending on the precipitation value registered on the field.

The effect of CWM of seed mass was also dependent on crop management (Table 3). Under higher nitrogen fertilization larger values were positively associated with protein concentration. Finally, when the preceding crop was a fallow or a legume crop increasing the variability in growth form tended to reduce crop grain protein concentration more than when the preceding crop was sunflower or a cereal.

3.4. Glutenin to Gliadin Ratio in Relation to Weed Community Structure Considering Growing Conditions

The inclusion of the weed community composition variable (PCA3 axis in this case) slightly reduced (2.6–2.9 points) the AICc of the M0 in explaining the glutenin to gliadin ratio (Table 4 and Table S4a,b for AIC values). PCA3 was positively related to the glutenin to gliadin ratio. Similarly, diversity of growth forms was positively related to the glutenin to gliadin ratio, and the effect was higher under lower fertilization values (Table 4 and Table S4a,b).

Table 4. Estimates, confidence intervals (C.I.) and R^2 marginal and conditional values of the M1 model (taking into account management and climatic variables along with PCA axis), and of the model for which the inclusion of a weed community structure metric improved the parsimony in explaining the glutenin to gliadin ratio in wheat grains.

	M1: Management and Climatic Factors + Weed Composition (PCA Axes)			
	Weed Composition (PCA Axes)		**FDis Growth Form**	
	Estimate	**C.I.**	**Estimate**	**C.I.**
Intercept	0.48	0.39, 0.56	0.48	0.39, 0.56
Weed community metric			0.05	−0.003, 0.09
Fertilization	−0.001	−0.002, −0.0002	−0.001	−0.002, −0.0001
Preceding crop	0.21	0.09, 0.35	0.21	−0.1, 0.02
PCA3	0.62	0.05, 1.2	0.66	0.08, 1.2
WCM × Fertilization			−0.0008	$-0.002, -5.10^{-5}$
R^2 marginal	0.22		0.25	
R^2 conditional	0.6		0.61	

3.5. Relationships among Weed Community Structure Measures and Their Dependence on Environmental Factors

We found a unimodal relationship between FDis and CWM values in the case of growth form, life form and functional type. For flowering onset, both measures were negatively correlated and in the case of height, seed mass and flowering duration the indices were positively related (Figure S1a,b). PCA axes were also related to the taxonomic diversity measures but not with biovolume. Weed biovolume was negatively related to eH′ and J (Figure S2).

Management factors and precipitation explained the weed community structure. Higher fertilization was associated with conventional fields (*t*-test = 14.7, *p* < 0.001). Plots located on highly fertilized fields had lower weed species richness, more therophyte species and lower FDis regarding flowering duration and functional type (Table S5). PCA1 was also positively related to fertilization. On the other hand, higher weed species richness, evenness and FDis regarding seed mass, height, flowering duration, growth form and functional type were associated with higher precipitation values, whereas the opposite occurred for CWM of functional type and life form and for PCA1. Fields pre-cropped with sunflower or winter cereals had weed communities with longer flowering duration (higher CWM) and higher diversity (FDis) in terms of both this trait and SLA, and were composed of species with lower PCA3 values.

4. Discussion

4.1. The Role of Growing Conditions, Wheat Varieties, and Yields on Wheat Grain Quality

Field and wheat variety, the random factors in our models, accounted for a large part of the variability explained by protein grain concentration and glutenin to gliadin ratio models (as shown by the difference between R^2c and R^2m in the models).

Wheat grain protein concentration responded to management and precipitation in a manner that was consistent with results from previous experimental studies (e.g., [5,7]). Increased nitrogen availability due to inorganic or organic fertilization had a positive effect on grain protein concentration. Model predictions show that shifting nitrogen fertilization from 1.5 kg N/ha to 132 kg N/ha (the first and the third quartiles respectively of the distribution of nitrogen fertilization values in our data set) will increase protein concentration from 9% to 13% when the preceding crop is a sunflower or a cereal, and from 11% to 14% in the case of a legume crop. In addition, the positive effect of fallow and legumes as the previous crop suggests that, in these systems, the inclusion of legume crops in rotations can favor crop grain protein concentration, reducing its dependence on high levels of fertilization. On the other hand, the positive effect of lower precipitation on crop grain protein concentration has a clear limit in these Mediterranean agroecosystems. Here, fields are rainfed and precipitation during the crop season did not exceed 406 mm. Low water availability, can preclude nitrogen acquisition by crop plants, especially during grain filling, compromising crop yields. Management factors and precipitation effects on the glutenin to gliadin ratio were subtler, suggesting that overall, glutenins and gliadins responded in a similar fashion to precipitation, whereas nitrogen availability slightly favored gliadins over glutenins in our case (but see [6]).

On the other hand, the negative, albeit weak, relationship between protein concentration and crop grain weight and the absence of a relationship of crop yield and the glutenin to gliadin ratio reflects that over a range of environmental conditions and wheat varieties, the trade-off between yield and quality is not strong enough to preclude the possibility of achieving optimal quantities of both crop components.

4.2. Wheat Grain Quality and Weed Communities

Weeds had a low but still significant contribution towards explaining wheat grain quality. Weed community structure measures explained more of the variability for wheat grain protein concentration values than for the glutenin to gliadin ratio. Weed community structure measures explained more of the variability on wheat grain protein concentration values than on the glutenin to gliadin ratio. Weed effects were dependent on community composition and functional structure but not on weed abundance. This result emphasizes the value of assessing a variety of aspects of the weed community structure in studies exploring the relationship between weeds and agroecosystem functioning.

Our findings also provide insights into the value of working with non-manipulated plant communities across a broad geographic area as compared to studies where the weed composition is manipulated in experimental plots within a single field (e.g., [17]). Specifically, we found that management and precipitation simultaneously affected weed species and crop quality which underscores that environmental factors underlie the association between this aggregate agroecosystem property and the weed community. In this regard, we observed that the negative effect of weed species richness on crop protein concentration disappeared after accounting for management, climatic variables, and weed community composition. Weed species richness and crop grain protein concentration responded to precipitation and fertilization in an opposite manner. Weed species richness was positively associated with precipitation as expected in rainfed Mediterranean agroecosystems in which rainfall strongly influences seed germination and seedling survival [29]. It was also negatively affected by fertilization, a response consistently shown in non-agrarian plant communities [30]. In our study, some of the highest wheat grain protein concentration values, associated with elevated fertilization inputs, were at the expense of weed richness, thus compromising favorable functions that can be potentially offered by weed vegetation [31,32].

The growing conditions that jointly affected crop quality and the assembly of weed communities were also signaled by the functional structure of weed communities. Specifically, we found that weed communities in which attributes that are competitive in the crop-weed habitat were dominant, including higher average values in growth form (erect,

caespitose or climbing species), height and seed mass, were related to the highest values of wheat grain protein concentration. It appears that the environmental conditions promoting higher protein concentration were also selecting for attributes that allow weed species to surpass or match the crop in its ability to acquire resources. That is, wheat growing conditions functioned as weed assembly filters [33]. For example, being tall or having the ability to grow over the crop canopy are attributes that allow successful competition with the crop for light [34] and at the same time, weed species with these characteristics could have been promoted under competitive circumstances where light is the main resource limiting growth [35].

In accordance with this correspondence between higher grain protein concentration and competitive attributes, the relationship between weed functional diversity and protein concentration was dependent on whether higher weed functional diversity arose from the appearance of low (or high) competitive phenotypes, as well as on the limiting resource [36,37]. The diversity in terms of flowering date and growth form enhanced the negative effect of precipitation on protein concentration, whereas this negative effect was buffered by higher functional diversity in terms of height. The diversity in weed growth forms implied a reduction in the abundance of competitive attributes (erect, caespitose or climbing phenotypes), whereas diversity in flowering dates was related to an increase in the abundance of species that flower earlier than wheat (negative relationship between CWM and FDis), i.e., differing from the crop in the time of resource use towards reproduction. In both traits (growth form and flowering date), higher functional diversity could have diminished competition of the weed community for water. On the contrary, higher functional diversity in terms of height relates to an increase in the abundance of taller phenotypes (positive relationship between FDis and CWM). These species may grow close or above the crop canopy matching the niche occupied by the crop. In this case, weed communities of higher functional diversity could increase competition for light and water, consequently buffering the reduction in crop grain protein concentration due to higher precipitation. By contrast, considering that the most limiting resource is nitrogen, the reduction in competition due to higher variability in growth forms could buffer the negative effect of a cereal or a sunflower as the preceding crop for protein concentration.

On the other hand, the association between community composition measures and crop quality exemplify that high protein concentrations can be achieved with distinct weed communities. The PCA1 and PCA3 axes showed that high protein values can be achieved in communities with different numbers of species (with high richness in PCA1 and low in PCA3; Figure S2) and characterized by diverse weed strategies. For example, positive values of PCA3 were related to higher crop quality and were characterized by species of either small stature or low growth form, and with low biomass such as *Juncus bufonius* or *Polygonum aviculare* as well as by grass and species with a rosette growth form or high stature like *Avena sterilis* or *Picris echioides*. This observation illustrates that there are other important characteristics of the weed genotypes not accounted by distinct traits separately.

Our data do not allow us to test the underlying mechanism by which weed community composition and functional structure relate to crop grain quality. Further studies will be needed to test whether changes in these weed community aspects shift crop-weed competition, ultimately affecting crop grain quality. We suggest that a promising approach could be to test the combined effects of weed functional diversity and resource availability on grain protein concentration, with detailed measurements of the water and nutrient uptake by communities. Weed functional diversity, if reducing the abundance of weed competitive phenotypes, may positively affect crop quality when water availability is scarce and at all levels of nitrogen availability. By contrast, under higher water availability, increasing competition at grain filling may favour grain protein concentration. Under the high stress conditions that can be observed in low-input and semi-arid Mediterranean systems (low rainfall, reduced fertilization) crop yield and grain protein concentration will be limited by water and nitrogen availability. In that case, reducing competition with weeds by increasing weed functional diversity will favour crop protein concentration. However,

it is important to note that weed composition and functional structure are directly related to management and soil and climatic conditions [38]. Reducing agriculture intensification and diversifying cropping systems may be useful to increase weed functional diversity. Further, data collected across broad geographic areas and management regimes will be needed to identify management practices that maximize agricultural multifunctionality in terms of crop performance and biodiversity conservation.

5. Conclusions

Across a broad geographic area, we found that growing conditions determined wheat grain quality and weed community structure. The composition and the functional structure of the weed community also affected crop grain quality but accounted for lower variability than growing conditions in the models.

Regarding management, we found that elevated fertilization levels created the highest wheat grain protein concentration but also corresponded to a loss of weed species richness and to the selection of weed species with competitive attributes. Loss of weed species could compromise the potential of weed vegetation to intercede in several other agroecosystem functions not studied here [31,32]. However, we also found that optimal wheat grain protein concentration values could be achieved with moderate fertilization levels and with the inclusion of legume crops in rotations (positive effect of legume or fallow as a previous crop). These are management recommendations that can be applied to assure both crop quality and weed diversity maintenance. In Mediterranean arable systems, grain legumes are considered as traditional crops but, as in the rest of Europe, their involvement in crop sequences is currently limited [39].

Crop and weed functional diversity could aid in assuring optimal agroecosystem functioning when moving to low input agricultures [14,40]. In our study, we found that high weed functional diversity did not always correspond to higher crop grain quality. This crop quality-weed diversity relationship depended on the weed functional trait and on the management and precipitation conditions. We also highlight the role of weed community composition in explaining grain quality [41]. Composition measures describe weed communities more accurately than taxonomic or functional diversity indexes. By contrast, the information provided by community composition measures is more difficult to compare between studies than values from diversity indexes. Furthermore, a good interpretation of composition measures requires extensive species knowledge [42]. Nonetheless, our results highlight that other weed characteristics not accounted by the traits studied here could also be of value in explaining weed effects on crop grain quality.

In the broad context of the need to improve both global food security and agricultural sustainability [43], we suggest that weed communities and weed management have a critical role [38]. Future studies should aim to quantify the mechanisms underlying weed community effects on crop grain protein concentration and identify management practices that can maximize crop performance and biodiversity conservation.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13010049/s1, Figure S1: Relationship between community weighted means and functional dispersion values of four traits; Figure S2: Relationship between weed biovolume (Biovol.), weed taxonomic diversity indices (S, richness; eH′, the exponent of Shannon diversity index and J, Pielou index) and axes of a principal component analyses used to characterize weed community composition across plots; Table S1: Trait values for species recorded along the 26 sampled fields; Table S2: Summary of the mixed effects models on the relationship between management and climatic variables and protein concentration and the glutenin to gliadin ratio in wheat grains; Table S3: AICc values for mixed effects models fitted between protein concentration in wheat grains and measures of weed community structure; Table S4: AICc values for mixed effects models fitted between glutenin to gliadin ratio in wheat grains and measures of weed community structure; Table S5: Relationship between growing conditions and weed community structure measures. File S1: Extraction and determination of the amounts of glutenin and gliadin

fractions; File S2: PCA analyses of weed community composition. References [44–67] are cited in Supplementary Materials.

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