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## Disentangling the effect of nitrogen input and weed control on crop—weed competition suggests a potential agronomic trap in conventional farming

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### ABSTRACT

Weeds are commonly assumed to induce yield loss because of resource competition. However, there is growing empirical evidence that the picture is much more complex, because fertilizer inputs, by modifying the level of resources and weeding by removing some weed species, affect the outcome of crop-weed interaction. We assess how two important crop production inputs - nitrogen (N) fertilization and weed-control - affect a fundamental non-chemical means of weed management, the outcome of crop-weed competition, in real field conditions in 56 winter cereal fields, of which 23 were organically farmed. We used a factorial design with two levels (absence/ presence) of nitrogen input and weed control inputs, but in our case, the "control" (i.e., presence level) for both practices was the usual practice of the farmer (which therefore varied). We found that crop aboveground biomass and grain yield were positively related to the amount of  $N_{total}$  (N soil plus N fertilizer), while weed species assemblages were negatively affected, showing lower species richness and weed abundance (i.e., number of plants). We also detected a contrast between farming systems: conventional fields (CF), managed with higher amount of total N and weed control, showed higher crop biomass and grain yield, and lower weed abundance compared to organically farmed fields (OF). Importantly, the findings showed that the outcome of the competition between crops and weeds was largely in favor of the crop plants in CF fields, even in the absence of weed control. In OF fields, the outcome of the competition between weeds and crop plants was still largely in favor of the crop, but at a lesser extent than in CF fields. The patterns were similar in unfertilized plots, though weed control in CF fields was more effective at low amounts of N, suggesting that more intense weed control is required in N-rich fields to maintain crop production. Overall, we argue that these results may underlie an agronomic trap: while an increased supply of nitrogen generally increases crop yield, it also benefits to weeds, requiring more efficient weed control.

### 1. Introduction

A major priority in the agroecological transition concerns pesticide reduction, since pesticides negatively affect agroecosystem biodiversity (Geiger et al., 2010). But despite numerous government incentives, pesticide use – and especially herbicides – has not significantly decreased over the last ten years (Möhring et al., 2020), hence impeding any major recovery of biodiversity in agroecosystems. Indeed, weeds can also play an important role in maintaining ecosystem services such as pollination and biological control (Bretagnolle and Gaba, 2015; Marshall et al., 2003). One reason for persistent herbicide use is that weeds have long been viewed as a limiting factor in crop production.

They are considered as major pests as weeds can reduce wheat yields by 23 % (potential loss) or 7 % (actual loss), depending on weed species (Oerke, 2006). Thus weeds are intensively managed by farmers with a combination of agricultural techniques, including tillage and, above all, herbicide application (Tilman et al., 2002). The latter has environmental costs on ground and surface water (Kudsk and Streibig, 2003), biodiversity (Geiger et al., 2010) and human health (Wilson and Tisdell, 2001). Therefore, reducing the use and risk of herbicides is a complex issue, currently stifling the agroecological transition, i.e., moving towards farming systems that enhance natural regulations (i.e. natural pest control) and ensure for food security, farmers welfare and biodiversity conservation (Altieri, 1995).

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Weed regulation through crop competition may be a sustainable alternative option to herbicides (Sardana et al., 2017), but relies on better knowledge of crop-weed competition. For long studies have focused on reducing the impact of weeds on crop production, i.e., the effects of crop-weed competition on the crop (Zimdahl, 2004). In arable fields, crop plants are dominant competitors, are enhanced by nitrogen (N) supply – which improves their competitive ability –, establish early in the season, and are sown at very high density (Gaba et al., 2014). Studies that have investigated crop-weed competition from the crop viewpoint have mostly used pairwise crop-weed species interactions involving generally only one weed species (Kristensen et al., 2008; Olsen et al., 2005; but see Gibson et al., 2008 and Gaba et al., 2018 for exceptions). However, in reality, crop plants in arable fields interact with multiple weed species, and the competitive interactions between the crop and weeds are diffuse and vary with the competitive ability of the weed species (Blackshaw et al., 2004; Blackshaw and Brandt, 2008). Furthermore, few studies were conducted in real fields, monitoring weed flora and using experimental designs allowing to assess crop-weed competition (Colbach et al., 2020), and even fewer were conducted with farmers in their real farmed conditions (Gaba and Bretagnolle, 2020). However, results from experimental research units are likely to differ from those from real farms since they do not account for variability in farmers' decision-making, local pedo-climatic conditions or landscape

Crop-weed competition is affected by farming practices, such as weed control (including both herbicide application or mechanical weeding) or the increase of N availability, since weed control and nitrogen fertilizers affect weed diversity (Fried et al., 2008; José-María et al., 2010; Moss et al., 2004). High level of N supply may favor weeds over crop plants since fertilizers promote nitrophilous weeds with strong competitive ability (Berger et al., 2007; Iqbal and Wright, 1997; Moreau et al., 2014). However, there is no consensus on how nitrogen input affects the crop-weed competition. Several studies revealed a disadvantage of weeds in N-rich environments (Blackshaw and Brandt, 2008; Di Tomaso, 1995; Iqbal and Wright, 1997; Van Delden et al., 2002) while others showed that excessive N input may translate into higher potential yield loss (Moss et al., 2004), i.e., weeds acquiring N at higher rate than the crop (Blackshaw et al., 2003; Harbur and Owen, 2004). The rate, the timing and application method of N input can affect crop competitive ability, hence the potential for crop plants to regulate weeds (Abouziena et al., 2007; Cathcart and Swanton, 2003; Primot et al.,

Weed control, either chemical or mechanical, removes weed plants or injures weed seedlings, and is thus expected to favor crop plants (Chauhan and Opeña, 2012; Melander et al., 2005). However, weed control may enhance the abundance of competitive weeds by filtering species (Fried et al., 2012; Gaba et al., 2016) hence increasing the magnitude of the competition of weeds on crop plants. Finally, the efficacy of weed control may vary with N input. The relative competition ability of oilseed rape against weeds was shown to be improved by herbicide application especially at higher N levels (Wang et al., 2019). Better understanding how weed control and N fertilization affect the outcome of crop-weed interactions can help farmers enhance crop competition as a means of non-chemical weed management. Studying competition in real field conditions is however complex, and requires to tease apart potential confounding factors.

In this study, we experimentally explored crop-weed competition and how it is affected by the presence and absence of fertilizer input and weed control in 56 farmed winter-cereal fields selected along a gradient of management intensity, based on the amount of nitrogen provided by farmers and their weed control intensity (i.e., number of herbicide applications and/or of mechanical weeding). Our design included organically and conventionally farmed fields to cover a wide gradient of management intensity and to understand the differential effects of chemical and mechanical weed control on crop-weed interaction. In each field, we implemented a factorial experimental design, combining

the presence or absence of nitrogen input during crop growth with the presence or absence of weed control. In each experimental plot, we quantified the separate and combined effects of weed control and fertilizer input on the competitive outcome between the dominant species (the crop) and the weed species assemblage (i.e., all the species that were found in a plot), as well as its consequences on yields. We first explored changes in crop biomass, weed diversity, weed abundance and weed biomass along the gradient of the amount of N both in the presence and absence of weed control at two key periods of the cropping season: crop flowering and crop harvest. Second, by using different indices to measure competitiveness, either on the side of weeds or crop plants, we quantified relationships between the competitive outcome and the N resource interacting with weeding pressure. Finally, in order to disentangle the effect of N input from the effect of weed control, we focused on the unfertilized plots and analysed changes in crop biomass, weed diversity, abundance and biomass, as well as weed-crop interaction along a gradient of the amount of N in soil, in the presence or absence of weed control.

### 2. Material and methods

### 2.1. Study site and experimental design

The study was performed in 2013 and 2014 in winter cereal fields in the Long-Term Social-Ecological Research (LTSER) 'Zone Atelier Plaine & Val de Sèvre' located in the south of the Deux-Sèvres district (46°23′N,0°41′W) in the Nouvelle-Aquitaine region in France (Bretagnolle et al., 2018). This agricultural landscape is dominated by intensive cereal production, as well as winter oilseed rape, maize and sunflower. Crops grown are mainly winter cereals (38.2 %) with 34.8 % wheat and 3.5 % barley, winter oilseed rape (8.3 %), corn (9.6 %), sunflower (10.4 %), but also meadows and alfalfa fields (12.3 %) and about 10 % of woods and built-up. We chose to conduct the study in real farming conditions to evaluate the studied effects over a wide range of weed species assemblages and farm management strategies. The fields and farms were selected to provide a representative range along a gradient of management intensity depending on farming practices that included N fertilizer application and weed control (see also Gaba et al., 2018). The experiment was conducted in 56 farmed fields (16 in 2013 and 40 in 2014), of which 23 were farmed organically. Four winter cereals with similar agronomic characteristics were used by farmers i.e., winter wheat (42 of which ten were farmed organically), barley (one in conventional farming and four in organic farming), spelt (four in organic farming) and triticale (four in organic farming). Within each field, in addition to the farming intensity gradient, we set up a two-factor experimental design to manipulate the presence or absence of nitrogen input and the presence or absence of weed control (i.e., herbicide sprayed in CF fields, and mechanical weeding in OF fields). The farmers themselves implemented the treatments, following their standard practices. Therefore, in plots receiving N or weed control in a given field, the amount of N applied and the type and intensity of weed control were that used by the farmer in the rest of the field (Gaba and Bretagnolle, 2020). Otherwise, in plots not receiving N or weed control, farmers cut the spreader/sprayer, or lift the harrow on the corresponding plots. The experimental area varied between 150 and 200 m<sup>2</sup> and was divided in four areas corresponding to each experimental modality alone and in interaction (Fig. 1d). In 2013, the experimental plots were in the first 5 m of the field, and in 2014 in the center of the field (for further details see Catarino et al., 2019; Gaba et al., 2018; Gaba and Bretagnolle, 2020). The experiment started each year when the crops were sown and ended at crop harvest. At the end of the experiment, we interviewed the farmers to collect data on their agricultural practices: the amount and type of agrochemicals (fertilizers and pesticides) applied and the number and type of soil operations. Inorganic nitrogen is rapidly available to plants, hence the quantity of nitrogen used was directly calculated according to the fertilizer composition and the respective quantity applied.

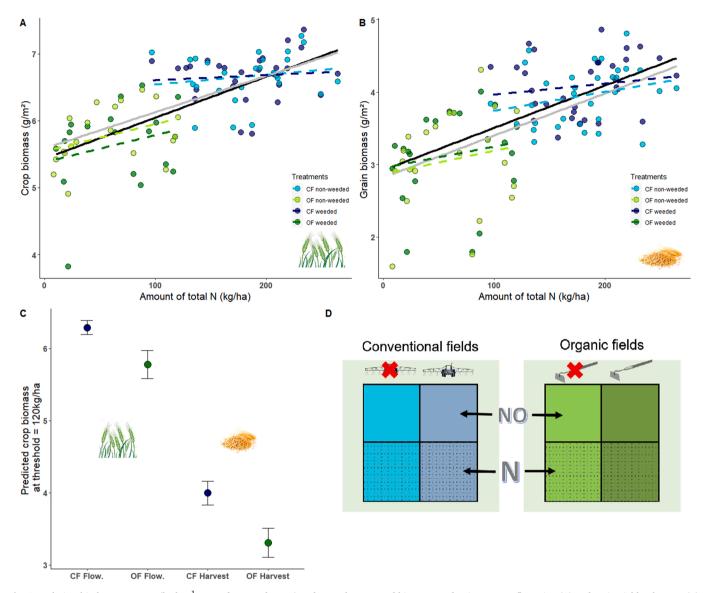


Fig. 1. Relationship between  $N_{total}$  (kg.ha<sup>-1</sup>; sum of  $N_{soil}$  and  $N_{input}$ ) and crop aboveground biomass production at crop flowering (A) and grain yield at harvest (B). Data was collected in the presence of fertilizer. Circles show raw data. Light or dark green indicates data collected in OF fields in the absence (light) or presence (dark) of weed control. Light or dark blue indicates data collected in CF fields in the absence (light) or presence (dark) of weed control. The lines represent the predicted values of the linear models (solid when significant, dashed otherwise). The black and gray lines show the predicted relationships without accounting for the farming system in the presence and absence of weed control, respectively. Because of nestedness, we cannot separate whether the effect comes from the intercept or the covariance. The significance level of the effect of N within the farming system was thus obtained by separate models (one per farming system). (C) Predicted values of crop biomass and grain yield in CF (blue) and OF (green) fields for the threshold value of 120 kg.ha<sup>-1</sup> of N fertilizer. SEs are shown. (D) Schematic representation of the experiment with the four treatments presented.

Organic fertilizer sources were of various forms of organic fertilizers: organic liquid or solid manure, litter, compost, animal by-products. Since organic nitrogen has to go through mineralization in order to be converted into its inorganic forms, the quantity of nitrogen mineralized in the organic fertilizers was calculated following Jeuffroy and Recous (1999). The soil characteristics (% of clay, sand and silt; content of organic matter) and the amount of nitrogen content in the soil (N soil) were estimated from soil samples (mixture of three soil cores 15-20 cm in depth) collected at the end of January in each experimental plot. Analyses were carried out by the accredited INRAE Soil Analysis Laboratory (Arras, France: COFRAC accredited). We defined available N as the sum of N present in the soil in late January (as measured in soil samples) plus N input provided by the farmer during crop growth (after January). Because crop plants were sown late October, our estimate of N present in the soil in January therefore accounted for the uptake of crop plants between October and January, though it is limited (i.e., crop plants being at tillering stage).

### 2.2. Weed and crop sampling

In each of the four experimental areas, two 1  $\rm m^2$  sampling plots were placed 2 m from the edge to avoid border effect. At crop flowering (end of May) and at harvest (early July), weed species identity and abundance were recorded in one of the sampling plots (Fig. S1; see also details in Gaba et al., 2018). At crop flowering, weed and crop aboveground biomass were estimated by harvesting 0.36  $\rm m^2$  in one of the two sampling plots at crop flowering. At harvest, weed aboveground biomass and crop grain were sampled in the entire 1  $\rm m^2$  plot in the remaining sampling plot. Both dead and living weed plants were collected. Plant samples were oven-dried at 80  $^{\circ}$ C for 48 h and weighed for biomass estimation.

### 2.3. Statistical analysis

In a first step, we examined the effects of N<sub>total</sub> (N<sub>input</sub> plus N<sub>soil</sub>), as a continuous variable, on crop and weed plants using linear models, in presence or absence of weed control treatment (one model per modality). We first tested these effects on crop aboveground biomass and grain biomass, respectively collected at crop flowering and at harvest in fertilized plots. We accounted for the type of farming system (OF vs CF) by including N<sub>total</sub> statistically nested within the farming system in the models. As we found N<sub>total</sub> in OF to be much lower compared to that N<sub>total</sub> in CF (see 'Results'), we compared the estimated crop aboveground production and grain yield at a value of 120 kg.ha<sup>-1</sup> corresponding to a value observed in both farming systems using the estimates of the model parameters. This value being selected arbitrary, we also estimated crop aboveground and grain yield at four other values to check for the robustness of our results (see Fig. S2). Then we examined how weed species richness, abundance and biomass varied with the amount of N<sub>total</sub>, in the presence or absence of weed control, again using data collected in fertilized weedy or weeded plots at flowering and harvesting periods. The same model was used for crops and weeds.

In a second step, to disentangle the effect of weed control from those of N fertilization on crop and weed plants, we tested the impact of weed control in unfertilized plots at crop flowering and at harvest, on crop aboveground biomass (or grain yield at harvest), weed species richness, weed abundance and weed aboveground biomass. We used two linear models, one for each weed control treatment (presence of absence), with the soil N content ( $N_{\text{soil}}$ , without any N input) alone and nested within the farming system.

The third step of our analyses consisted in exploring how Weed Control Effect on the crop ( $WCE_{crop}$ ) or Weed Control Effect on weeds ( $WCE_{weed}$ ) vary with the amount of  $N_{soil}$  or  $N_{total}$ . To measure the effect of weed control, we used an index that is computed as the percentage difference, in the presence or absence of weed control, in crop aboveground biomass or in weed aboveground biomass, abundance or richness. The percentage differences were computed symmetrically for the crop and weeds to illustrate the expected effect of weed control, i.e. positive for crops and negative for weeds:

Fortherrop, 
$$WCE_{crop} = \frac{X_{WC1}^{i} - X_{WC0}^{i}}{X_{WC1}^{i} + X_{WC0}^{i}}$$
 (1)

Forweeds, 
$$WCE_{weed} = \frac{X_{WC0}^{i} - X_{WC1}^{i}}{X_{WC0}^{i} + X_{WC1}^{i}}$$
 (2)

where  $X_{WC1}^i$  and  $X_{WC0}^i$  are the crop or weeds response variables in the presence ( $WC_1$ ) or absence ( $WC_0$ ) of weed control. The values range from -100–100%, with *positive values* indicating a positive effect of weed control in crop and *negatives values* indicating an efficient decrease of weeds (either in terms of richness, abundance or biomass depending on the response variable). Response variables for the crop included biomass or grain, while for weeds it included biomass, abundance or richness. In the five cases, the same model structure was used. The amount of N was either  $N_{total}$  or  $N_{soil}$ , depending on whether data were collected in fertilized or unfertilized plots.

Finally, after analysing the crop and weeds separately, we replicated our analytical framework to investigate crop—weed interaction in relation to N amount ( $N_{total}$ , or  $N_{soil}$  only) and weed control. We used a metric of competition, the Competitive Balance Index (CBI), inspired of the relative interaction index (RII) recommended to explore competition (Armas et al., 2004). This metric is better suited to examining how the competition outcome between weed and crop plants varies with the amount of N, and is computed as the contribution of crop aboveground biomass over the total amount of plot aboveground biomass (from crop and weed plants):

$$CBI = \frac{B_{crop} - B_{weed}}{B_{crop} + B_{weed}} \tag{3}$$

This competition index, CBI, was computed in the presence and absence of nitrogen, using biomass data from fertilized plots when exploring the effect of  $N_{total}$ , or unfertilized plots when exploring the effect of  $N_{soil}$ , and in presence and absence of weed control using biomass data from non-weeded and weeded plots. The variation of CBI indexes was assessed using the same model as for WCE indexes.

All analyses were conducted for two time periods, crop flowering (in May) and harvest time (late June/early July). We checked for the prerequisites of homogeneity of variances, normality of the residuals, and collinearity of all models. Crop biomass, weed abundance and biomass were log-transformed. All statistical analyses were performed with R software (R Core Team, 2020).

### 3. Results

### 3.1. Crop response to nitrogen input

The amount of  $N_{total}$  (i.e.,  $N_{soil}$  plus  $N_{input}$ ) varied strongly across fields (from 8.3 to 264.6 kg.ha<sup>-1</sup>), which resulted in strikingly different resource levels for crops and weed species assemblages between fields (Fig. 1).  $N_{total}$  was significantly lower in OF fields compared to CF fields (mean $\pm$ sd: OF = 63.2  $\pm$  38.9 kg.ha<sup>-1</sup> and CF = 180.4  $\pm$  39.5 kg.ha<sup>-1</sup>; Wilcoxon test: W=3008, p-value<0.0001).

At crop flowering, in fertilized plots, crop aboveground biomass was significantly and positively related to the Ntotal (Fig. 1a, Table 1). Similar results were observed at harvest, with an overall positive effect of the Ntotal on grain yield (Fig. 1b). As expected, at both periods, crop and grain biomass were significantly higher in CF fields compared to OF fields (Table 1, Fig. 1a-b), though this could result either from difference in farming system or a difference in N input (in the statistical model, N input was nested within farming system, thus the relative effect of two factors could not be statistically teased apart). By setting N<sub>total</sub> at 120 kg. ha<sup>-1</sup> for both farming systems (a value of input that existed for both farming systems, Fig. 1a-b), and estimating crop and grain biomass at this N threshold for each farming system based on their individual equation derived from Fig. 1, we found that at both crop flowering and harvest, there was a significant difference between farming systems. CF exceeded by about 20 % OF system yield at 120 kg.ha<sup>-1</sup> N (Fig. 1c). As a difference between farming systems was clearly observed despite N<sub>input</sub> being set at an equal value (for a robustness analysis in regard to the threshold value, see Fig. S2), we looked for possible differential effects of weeds and/or weed control between the two farming systems.

### 3.2. Weed response to nitrogen input

In fertilized plots, the amount of  $N_{total}$  had a negative effect on weed species assemblages at both crop flowering and harvest (Fig. 2). In particular, at crop flowering, weed species richness and weed abundance significantly decreased with  $N_{total}$  (Fig. 2b-c; Table 1), with richness being three times lower in fields with a high amount of  $N_{total}$  compared to those with the lowest  $N_{total}$  although the effect of  $N_{total}$  was only significant at crop flowering (Fig. 2a, Fig. 2d; Table 1) and actually only in the case of  $N_{total}$  compared to the case of  $N_{total}$  fields were applied (Fig. 2a). On average, weed species richness was slightly higher in  $N_{total}$  fields (Fig. 2b), but the largest difference between farming systems was observed for weed abundance in weeded plots, which was about twice as high in  $N_{total}$  fields as in  $N_{total}$  (Figs. 2c and 2f, Table 1).

### 3.3. Crop and Weed responses to weed control and its impacts on crop-weed competition

Weed control had no significant effect either on crop above ground biomass production at crop flowering (Figs. 3a, 3e; 639.9  $\pm$  372.6 g/m² in weeded plots and 625.8  $\pm$  327.2 g/m² in non-weeded plots; paired Wilcoxon test: V = 468, p-value = 0.64) or on grain yield (Fig. S3a, S3e;

Table 1
Summary of the linear models. Effects of available nitrogen (N) on biomass of crops and weeds, and weed species richness and abundance at two periods were explored in fertilized weedy and weeded plots. Significant effects are in bold.

	At flowering						At harvest						
	with Weed Control			without Weed Control			with Weed Control			without Weed Control			
	F value	Df	Pr (>F)	F value	Df	Pr (>F)	F value	Df	Pr (>F)	F value	Df	Pr (>F)	
(A) Crop													
N total	39.24	1	> 0.0001	52.90	1	> 0.0001	37.01	1	> 0.0001	35.12	1	> 0.0001	
N total: Farming system	4.42	1	0.0412	2.75	1	0.1045	4.26	1	0.0441	2.77	1	0.1022	
Residuals		44			44			51			51		
(B) Weed aboveground biomass													
N total	8.02	1	0.0070	1.14	1	0.2926	2.72	1	0.1056	1.43	1	0.2375	
N total: Farming system	0.34	1	0.5645	0.09	1	0.7629	3.05	1	0.0870	0.81	1	0.3732	
Residuals		44			44			48			51		
(C) Weed species richness													
N total	19.17	1	> 0.0001	11.53	1	0.0014	14.06	1	0.0005	5.83	1	0.0195	
N total: Farming system	1.96	1	0.1682	0.09	1	0.7660	0.36	1	0.5491	0.05	1	0.8264	
Residuals		47			48			48			50		
(D) Weed abundance													
N total	15.33	1	0.0003	16.20	1	0.0002	11.25	1	0.0016	8.26	1	0.0059	
N total: Farming system	2.27	1	0.1385	3.84	1	0.0560	8.40	1	0.0057	8.47	1	0.0054	
Residuals		45			48			46			50		

 $42.97\pm25.27~g/m^2$  in weeded plots and  $48.18\pm28.51~g/m^2$  in nonweeded plots; V = 491, p-value = 0.44). Conversely, we detected significant negative effect of weed control on weed species richness (Fig. 3c; average in fertilized non-weeded plots:  $8.4\pm3.6~species/m^2$  and in fertilized weeded plots:  $6.7\pm3.9~species/m^2$ ; V = 221, p-value = 0.011), weed aboveground biomass (Fig. 3b; average in fertilized nonweeded plots:  $88.02\pm105.91~g/m^2$  and in fertilized weeded plots:  $45.16\pm71.48~g/m^2$ ; V = 162, p-value <0.0001), and weed abundance (Fig. 3d; average in fertilized non-weeded plots:  $74.99\pm86.2~plants/m^2$  and in fertilized weeded plots:  $63.22\pm80.21~plants/m^2$ ; V = 309.5, p-value = 0.0118) at crop flowering.

The effect of weed control on both the crop and weeds further varied with the amount of N. In CF, the effect of weed control on crop biomass (evaluated with WCE $_{\rm crop}$ ) decreased significantly with  $N_{\rm total}$  (Fig. 3a). In addition, weed control effect (evaluated with WCE $_{\rm weed}$ ) on weed aboveground biomass significantly increased with  $N_{\rm total}$  (Fig. 3b). However, the trend was opposed between OF and CF fields, i.e., the effect of weed control on weed aboveground biomass increase with  $N_{\rm total}$  in OF fields and decrease with  $N_{\rm total}$  in CF fields (Fig. 3b). The opposite overall pattern was observed with  $N_{\rm soil}$ , i.e., WCE $_{\rm weed}$  significantly decreased with  $N_{\rm soil}$ , especially in CF fields (Fig. 3f), suggesting a decrease in weed control efficacy with increasing N.

At crop flowering, most of the biomass was produced by crop plants (high positive CBI values), indicating that the competitive balance was largely in favor of crop plants in fields (Fig. 4a). The high dominance of crop plants was noticeable when N<sub>total</sub> was higher than 120 kg.ha<sup>-1</sup>, but was more variable below this threshold value (Fig. 4). In the fields with a lower N<sub>total</sub>, weed aboveground biomass accounted for some 20 % of the total aboveground biomass production, whereas in the former it was always lower than 10 %. This pattern was almost similar in weeded (Fig. 4a) and non-weeded (Fig. 4b) plots. Indeed, when comparing CBI values computed in weeded and non-weeded plots, very few differences could be observed confirming the absence of weed control effect (Fig. 4a, b). Such pattern was even more pronounced at crop harvest (Fig. 4c-d). The magnitude of the weed control effect was hardly noticeable because weed biomass was generally low in non-weeded plots, especially compared to the crop biomass. CBI also increased significantly with N, either N<sub>total</sub> or N<sub>soil</sub> (Fig. 4), indicating that N supply increases crop biomass at the expense of weed biomass, though this effect decreases with increasing N, and is negligible at very high N levels. Similar patterns were found at harvest (Fig. 4c-d, Fig. 4g-h).

In summary, our results reveal contrasted patterns below and above  $120~kg.ha^{-1}$  of  $N_{total}$ , i.e. between OF and CF fields, since fields with an amount of  $N_{total}$  lower than this threshold value were generally OF fields. In OF fields, weed abundance was higher, the competitive balance between weed and crop plants was in favor of the crop and weed control was less efficient than in CF fields. Moreover, opposite patterns of the effect of weed control with N were observed between CF and OF both with  $N_{soil}$  and  $N_{input}.$  These patterns further suggest that the effect of weed control increased at higher N in OF, while its decreases in CF fields. However, we were not able to disentangle the effect of different types of weed control (mechanical weeding vs herbicides) from the effect of N amount.

### 3.4. Effects of $N_{soil}$ and weed control in unfertilized plots

Weed control intensity (estimated by the treatment frequency index of herbicides, or TFI, or the number of mechanical weeding operations) was unrelated to  $N_{soil}$  (Spearman correlation test:  $r_s = -0.024$ , p-value = 0.864), in contrast to  $N_{\text{total}}$ , the latter being significantly, highly and positively related to weed control intensity (r<sub>s</sub> = 0.834, p-value < 0.0001). We therefore explored the effect of weed control on weed and crop plants as well as the outcome of competition in the absence of N fertilization. In contrast to N<sub>total</sub>, we observed no significant differences in N<sub>soil</sub> (amount of N in the soil at the end of January) between OF and CF fields (Wilcoxon test: W = 1668, p-value = 0.38). In such unfertilized plots, grain yield increased significantly with the amount of N<sub>soil</sub> (Fig. 5b, Table 2), as it did in fertilized plots, although crop biomass, one month earlier, did not (Fig. 5a). In regard to weeds, N<sub>soil</sub> had a similar effect as N<sub>total</sub> on weed species richness and weed abundance, which both decreased significantly in OF and CF fields at flowering (Fig. 5d-e) while a significant decrease was only observed for weed species richness at harvest (Fig. 5g-h; Table 2). In contrast, no effect was detected on weed aboveground biomass (Fig. 5c, Fig. 5f). This suggests that at crop flowering the species or individuals in the fields with a higher amount of N<sub>soil</sub> produce more biomass per individual plant.

Similarly, in unfertilized plots, weed control had almost no effect on the crop, and little effect on weed plants, except for biomass (Table 2; Fig. 3e-h), as was found in fertilized plots. The only difference concerned weed aboveground biomass (Fig. 3f), which significantly decrease with increasing N, an opposite trend to that observed in fertilized plots (i.e., positive relationship; Fig. 3b). In OF fields, the effect of weed control on

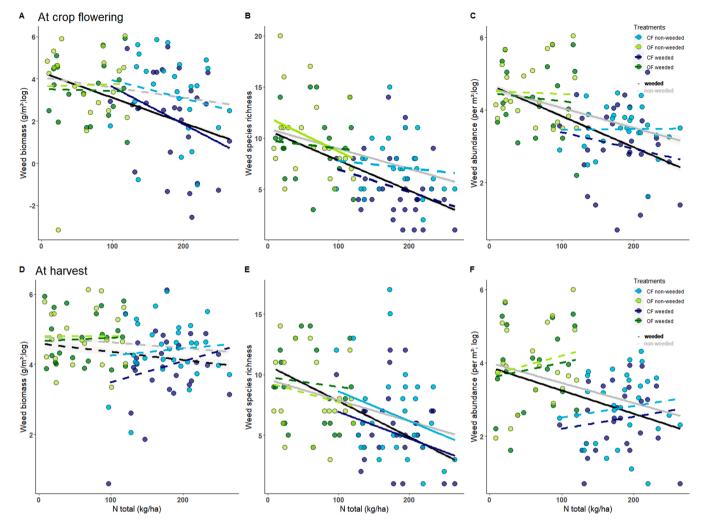


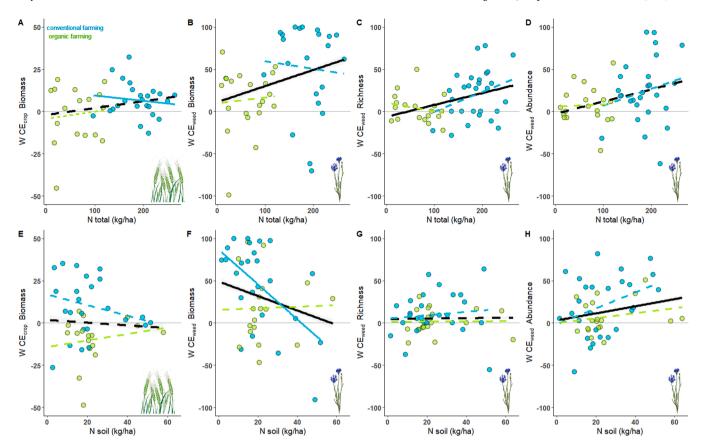
Fig. 2. Relationship between  $N_{total}$  (kg.ha<sup>-1</sup>; sum of  $N_{soil}$  and  $N_{input}$ ) and weed biomass (A;D), weed species richness (B;E) and weed abundance (C;F) at crop flowering (A-C) and at harvest (D-F). Data was collected in the presence of N fertilizer. Circles show raw data. Light or dark green indicates data collected in OF fields in the absence (light) or presence (dark) of weed control. Light or dark blue indicates data collected in CF fields in the absence (light) or presence (dark) of weed control. The lines represent the predicted values of the linear models (solid when significant, dashed otherwise). The significance level of the effect of N within the farming system was thus obtained by separate models (one per farming system). The black and gray lines show the predicted relationships without accounting for the farming system in the presence and absence of weed control, respectively.

crop biomass decreased with N soil, highlighting the efficacy of mechanical weeding in fields with a lower amount of  $N_{soil}$  (Fig. 3e).

Overall, weed control appeared more efficient in CF fields compared to OF fields, both for weeds and the crop (Fig. 3). This translated into the competitive balance, which was always largely in favor of the crop in CF fields compared to OF fields (Fig. 4e). In addition, the competitive balance in weeded and non-weeded plots were very similar, and crop advantage increased with the amount of  $N_{\rm soil}$  (Fig. 4e-f), suggesting that an increase of N availability provided a higher benefit to crop plants, which in turn produced more biomass at the expense of weeds. In both CF and OF fields, the increase of  $N_{\rm soil}$  differentially benefitted crop plants, regardless of weed control. Weed control in CF fields was, however, more efficient on crop biomass in fields with lower  $N_{\rm soil}$  (Fig. 3a). This pattern was not observed in OF fields, suggesting a qualitative difference between weed control in CF (chemical weeding) and OF fields (mechanical weeding).

### 4. Discussion

The aim of this study was to explore, experimentally, the importance of competition between weeds and winter cereal crop plants, and how nitrogen and weed control affect such competition in real farming conditions. Using an 'existing' gradient of nitrogen as provided by farmers, in addition to the experimental removal of weed control and N fertilization in organic and conventional farming fields, we were able to tease apart the effect of nitrogen and weed control on the competitive outcome between crop and weed plants, and the consequences on yield. We found that N supply (i.e., referring to N<sub>total</sub> in our study) had a prominent effect, increasing yields but decreasing weeds. We also found that although weed control had some effect on weeds, it had no effect on crop yield and on the competitive outcome between weeds and crop plants, because, all experimental treatments combined, weeds only contributed to around 10-20 % of the total plant biomass in conventional fields. However, by comparing fertilized and unfertilized plots, as well as organically and conventionally farmed fields, we found that N supply interacted with weed control: first, mechanical weeding appeared less efficient compared to herbicides, and second, while weed control did not affect yield at low N levels, it was apparently needed at high N levels to avoid a decrease in crop biomass. In the latter situation (i.e., high N levels), without herbicide application, weed biomass remained stable whatever the N applied level, while herbicide application suppressed a larger part of weed biomass with increasing N application (Fig. 2a). The relative efficacy of weed control in CF however decreased with N, being it Nsoil or Ntotal (Fig. 3a-e).



**Fig. 3.** The effect of weed control on the crop (WCE<sub>crop</sub>) and the weeds (WCE<sub>weed</sub>) in fertilized (A-D) and unfertilized (E-H) plots at crop flowering. WCE<sub>crop</sub> is computed on crop biomass (A; E), while WCE<sub>weed</sub> is computed on weed biomass (B; F), richness (C; G) and abundance (D; H). The OF and CF fields are shown in green and blue respectively. The lines represent the predicted values of the linear models (solid when significant, dashed otherwise). The black lines show the predicted relationships without accounting for the farming system. The significance level of the effect of N within the farming system was thus obtained by separate models (one per farming system). (A-E) Positive effect of weed control on crop plants (WCE<sub>crop</sub>) indicates that crop aboveground biomass is higher in the presence than in the absence of weed control. (B-D;F-H) Positive effect of weed control on weed plants (WCE<sub>weed</sub>) indicates that weed aboveground biomass (richness or abundance) is higher in the absence (non-weeded plots) than in the presence of weed control (weeded plots).

### 4.1. Crop-weed relationships in relation to N supply

In fertilized and weeded plots, the results provide strong evidence that with more nitrogen the crop largely outcompetes weed plants, and that the crop-weed competitive balance favors crop plants. Increasing the resource supply (i.e. N supply) increases crop biomass at the expense of weed biomass. These findings align with previous results showing the high competitive ability of cereals over weeds (Gaba et al., 2018; Lutman et al., 2013; Stefan et al., 2021; van der Meulen and Chauhan, 2017). For instance, Gaba et al. (2018) found that in the absence of crops, weed biomass increased with N supply, but in the presence of crops and given the latter's competitive advantage, the expected increase in weed biomass was suppressed. In our studied fertilized plots, weed biomass represented less than 15 % of the total plant biomass (both crop and weeds) produced at crop flowering and at harvest in CF fields, whatever the N amount. In situations of lower N supply, i.e. in OF systems or in CF/OF unfertilized plots, the competitive balance was not so uneven, although still largely in favor of the crop both at crop flowering and harvest. The pattern was virtually unchanged without weed control since the competitive balance was similar between weeded and non-weeded fertilized plots in both farming systems, except at highest levels of N input. In unfertilized plots, weed control had no effect on competitive balance, still strongly biased in favor of crop plants in CF fields, with only slight variation between weeded (82.8 %  $\pm$  22.3 %) and non-weeded (77.7  $\% \pm 21.0$  %) plots. Although biomass (and also abundance) of weeds would be expected to increase with N input, the reverse was found in CF, both at crop flowering and harvest, indicating that the presence of the crop alone can reverse expected patterns, although the magnitude of this effect was lower in high N conditions. In all situations, OF or CF, increasing N (whether  $N_{total}$  or  $N_{soil}$ ) resulted in lowering weed species richness. Increasing the N supply therefore enhanced the competitiveness of crop plants against weeds in almost all contexts, confirming the higher ability of cereal crop plants to take up N over weed plants (Andrew et al., 2015; Blackshaw et al., 2002; Blackshaw and Brandt, 2008; Mohler, 2001; Ruisi et al., 2015).

### 4.2. Difference in weed abundance and biomass in conventional and organic farming

While there were no significant differences in weed species richness, weed abundance and biomass were twice as high in OF fields compared to CF fields. This may be related to the different types of fertilizer between the two farming systems, only compost and organic manure in OF, and mainly inorganic manure in CF. However, the effect of the type of fertilizer in our study cannot be confirmed. Previous studies have revealed contrasting effects of organic fertilizer on weeds (Rotchés-Ribalta et al., 2016; Rotchés-Ribalta et al., 2020), showing negative (Dyck, 1995), positive (Blackshaw, 2005) or neutral effects (Cordeau et al., 2021) on weed biomass when comparing organic and mineral fertilizers. Weed control strategy also differs between CF and OF, and mechanical weeding appears to be less effective than herbicides as revealed by our comparison of the two farming systems in unfertilized plots. Our results further suggest differences in the ability of the crop plants to suppress weeds between CF and OF. In our fields, OF and CF differed in their

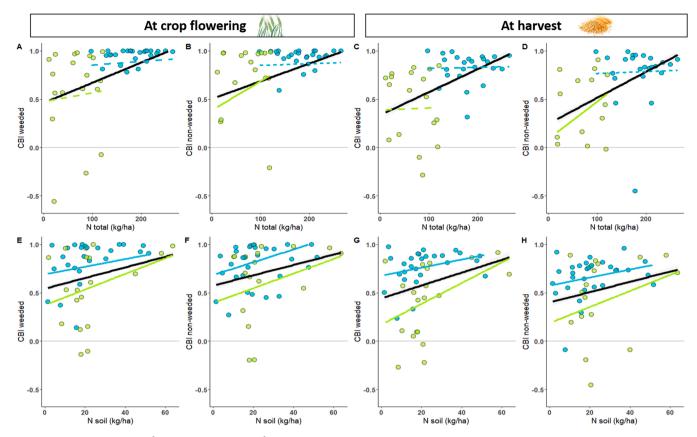


Fig. 4. The effect of N<sub>total</sub> (kg.ha<sup>-1</sup>; A-D) and N soil (kg.ha<sup>-1</sup>; E-H) on the competitive balance (CBI) between crop and weed plants in weeded (A,C,E,G) and non-weeded (B,D,F,H) plots. Data was collected at crop flowering (first two columns) and at harvest (third and fourth columns). The OF and CF fields are shown in green and blue respectively. The lines represent the predicted values of the linear models (solid when significant, dashed otherwise). The black lines show the predicted relationships without accounting for the farming system in the presence or absence of weed control, respectively. The significance level of the effect of N within the farming system was thus obtained by separate models (one per farming system). Positive CBI weeded and CBI non-weeded values indicate that crop plants contribute more to biomass production than weeds, while negative values indicate that weed plants contribute more than crop plants.

cereal species or cultivars, which are known to vary in their ability to take up N and in their competitiveness (Andrew et al., 2015; Storkey et al., 2021). Crops in organic agricultural systems are generally less sensitive to weed competition compared to corresponding conventional systems (Ryan et al., 2009), and organic farmers sow cereals at lower density with larger row spacing to facilitate mechanical weeding. Higher weed abundance and biomass in OF fields may therefore be the result of lower crop competition on weeds compared to CF fields, mainly due to lower competition for light because of larger row spacing. This mechanism may also explain the lack of an effect of weed control on production in OF fields, even though weed biomass is high.

### 4.3. Weed control effect and efficacy

Overall, weed control had a small effect, whether on crop yield, weed species assemblage, or crop—weed competition. Weed control decreased weed biomass by some 20 % in CF and decreased weed species richness in both farming systems, independently of availability of N, but did not affect statistically weed abundance in any system, either at crop flowering or harvest. A comparison of the effect of weed control between organic and conventional fields suggested that there is a qualitative difference in weed control efficacy between these two farming systems, with chemical weeding being more effective than mechanical weeding in controlling weeds (Pannacci and Tei, 2014). However, this qualitative difference was expressed only at high N input. In situations of limited N (i.e., unfertilized plots when only  $N_{\rm soil}$  was available), there was no effect of weed control on weed species assemblage (abundance, richness or biomass) (Fig. 4c-d-e). This underlines an interactive effect between N

input and weed control method. These results, obtained in real farming conditions, extend previous experimental studies by revealing lower herbicide efficacy at low levels of nitrogen (Kim et al., 2006; Singh et al., 2015; Sønderskov et al., 2012; Wang et al., 2019).

Given the minor effect of weed control on weed species assemblage, one would expect little effect as well on the competitive outcome between weeds and crop plants. Our two measures of competition between weeds and crop plants confirmed this prediction. At low N input (unfertilized plots), in both farming systems, weed control had no effect on the competitive balance (CBI). However, with increasing N input, we found that the crop significantly benefitted from weeding (Fig. 4e-h). This can be explained by the decrease of the magnitude of the regulation of weeds by crop competition that was observed in high N fields. Indeed, in CF fields, we found a positive relationship between weed control intensity (estimated by TFI) and N input. Increased weed control in N-rich CF fields resulted in lower weed biomass, but this did not translate into a significant gain of crop biomass.

### 4.4. Nitrogen input and herbicide use, an agronomic trap in conventional farming?

We detected a strong positive relationship between the amount of N input and of herbicides applied in CF fields. While a high amount of N was related to higher yields, the presence of weed control was not significantly related to an increase in yield, although yield was on average 10% higher in the presence of weed control. This gain, however, decreased with the amount of N input and, hence, with the amount of herbicide applied (these being positively correlated), the latter being

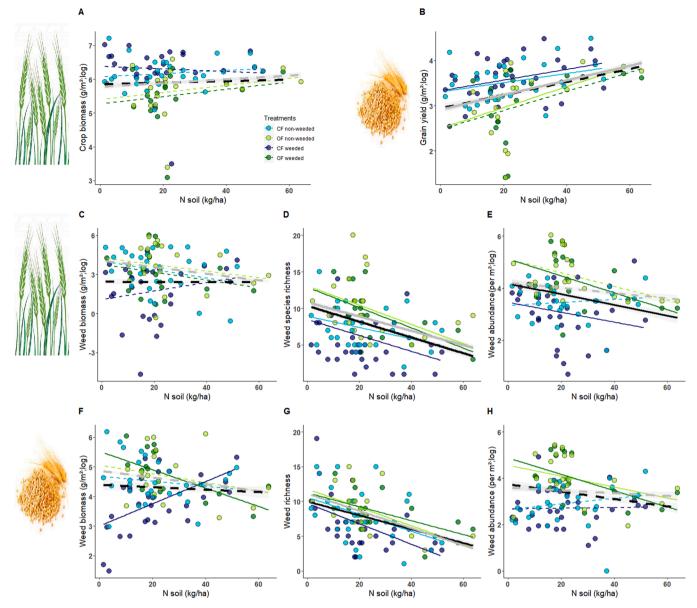


Fig. 5. Relationship between N soil and crop aboveground biomass production at crop flowering (A) and grain yield at harvest (B), as well as with weed biomass (C; F), weed species richness (D;G) and weed abundance (E;H) at crop flowering (second row) and at harvest (third row). Data was collected in the absence of N fertilizer. Circles show raw data. Light and dark green indicate data collected in OF fields in the absence (light) or presence (dark) of weed control. Light and dark blue indicate data collected in CF fields in the absence (light) or presence (dark) of weed control. The lines represent the predicted values of the linear models. The significance level of the effect of N within the farming system was thus obtained by separate models (one per farming system). The black and gray lines show the predicted relationships without accounting for the farming system in the presence and absence of weed control, respectively.

relatively less efficient at high N input. Indeed, the negative relationship between the weed control effect (WCE) on crop biomass and the amount of  $N_{\rm total}$  in CF fields (Fig. 3a), despite an apparent higher increase in crop biomass with increasing  $N_{\rm total}$  (Fig. 1a), also suggests that the efficacy of herbicides decreased with increasing N. This suggests that higher crop production in the presence of high N input may only be achieved with an increase in the amount of herbicide applied, a pattern that suggests a decisional cascade in which increasing N supply to increase crop yield, also benefits to weeds whose management required a more intense weed control to maintain the yield at the same level. This can be seen as an agronomic trap requiring a high reliance on agronomic inputs.

By suggesting such agronomic trap in conventional farming, our experimental results point to a possible pathway (i.e., a combined reduction of N input and weed control) for sustainable weed management in the meantime, in line with growing empirical evidence that reducing weed control in the short term does not necessary relate to

higher yield loss and may even result in higher income for farmers (Catarino et al., 2019; Gaba et al., 2016; Lechenet et al., 2017). Our study confirms a more complex picture than straightforward crop—weed competition. Indeed, diverse crop and weed species assemblage may show no negative effects (Epperlein et al., 2014; Gaba et al., 2016), or even a positive relationship between crop and weed diversity or biomass (Adeux et al., 2019; Ryan et al., 2009). Further studies are needed to confirm our results especially in other contexts with crop types with a lower potential to cope with weeds than winter cereals. The study over a longer term of reduced weed control is also of high importance to determine whether crop competition is sufficient to avoid a significant increase of propagules in the seed bank. Overall, our results offer insights that open new avenues for designing more sustainable weed management strategies to foster the agroecological transition.

Table 2
Summary of the linear models. Effects of nitrogen (N) in the soil on biomass of crops and weeds, and weed species richness and abundance at two periods were explored in unfertilized weeded and non-weeded plots. Significant effects are in bold.

	At flowering						At harvest						
	with Weed Control			without Weed Control			with Weed Control			without Weed Control			
	F value	Df	Pr (>F)	F value	Df	Pr (>F)	F value	Df	Pr (>F)	F value	Df	Pr (>F)	
(A) Crop													
N soil	0.0007	1	0.9790	0.6421	1	0.4271	3.9755	1	0.0520	5.7192	1	0.0208	
N soil: Farming system	5.9338	1	0.0193	6.0945	1	0.0173	7.3942	1	0.0091	4.1701	1	0.0468	
Residuals		41			46			47			47		
(B) Weed aboveground biomass													
N soil	0.2999	1	0.5868	2.8768	1	0.0966	1.6312	1	0.2082	1.6478	1	0.2056	
N soil: Farming system	1.4047	1	0.2425	0.7933	1	0.3777	0.5823	1	0.4495	0.4237	1	0.5183	
Residuals		43			46			44			47		
(C) Weed species richness													
N soil	11.777	1	0.0013	7.1584	1	0.0103	13.443	1	0.0006	15.5687	1	0.0003	
N soil: Farming system	10.485	1	0.0022	4.3143	1	0.0434	8.552	1	0.0053	0.6257	1	0.4328	
Residuals		46			46			46			48		
(D) Weed abundance													
N soil	3.8861	1	0.0547	0.2157	1	0.6446	0.4030	1	0.5287	0.0004	1	0.9842	
N soil: Farming system	12.2224	1	0.0011	5.3846	1	0.0248	8.3029	1	0.0060	4.8554	1	0.0324	
Residuals		46			46			46			48		

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### CRediT authorship contribution statement

VB and SG coordinated the research project and designed the experiment. VB supervised the experiment. SG, OM and AB analysed the data. SG and VB wrote the first draft of the manuscript. All authors contributed to the writing of the final version of the manuscript,

### **Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Data availability

Data will be made available on request.

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### Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.agee.2022.108232.

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