Effect of long-term tillage and residue managements on weed flora and its impact on winter wheat development

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Abstract

 In Hesbaye region (Belgium) with a loamy soil and under temperate climatic condition, winter wheat is a key component of agricultural rotations. As part of these rotations, soil management is a known driver of soil fertility and carbon storage. However, it could also influence the weed flora. In this study, the long-term effect of four soil management on the expressed and potential weed flora was examined. Soil management levers were i) the export (OUT) or restitution (IN) of crop residues and ii) the burial of residues by conventional tillage (mouldboard ploughing 25 cm depth, CT) or reduced tillage (cultivator ploughing 10cm depth, RT). The weed seedbank and expressed flora in winter wheat were characterized. Weed diversity was assessed using the Richness and the Shannon diversity index. Then, the impact of flora and management on yield were investigated. Tillage management showed little impact on weed diversity with only a slight increase in diversity in reduced tillage. However, reduced tillage resulted in a higher weed seedling density and a higher weed density than conventional tillage, which indirectly led to yield losses. Exporting residues had no clear effect on weeds. In conclusion, within cropping systems based on the cultivation of wheat, reduced tillage can pose problems for the long-term management of the weed flora, and great attention has to be paid to its management.

Keywords Crop Residue, Tillage, Reduced Tillage, Weed flora, Wheat

1. Introduction

 Agricultural soils management is known to have an impact on carbon storage and potentially could help mitigate the rise in atmospheric CO2 concentration (Martin et al., 2021). The management of crop residues, which can be exported (e.g. for animal fodder or bioenergy production) or incorporated into the field using reduced or conventional tillage, can therefore play a role in carbon storage (Autret et al., 2016; Hiel et al., 2018). Beside impacting the soil carbon content, soil management can have impacts on soil geochemical dynamics (Blanco-Canqui and Lal, 2009; Hiel et al., 2018) and on soil microbial communities (Degrune et al., 2017, 2016; Spedding et al., 2004). Furthermore, soil 40 management can also have an impact on weed flora (Nichols et al., 2015).

 The effect of tillage alone (without residue incorporation or exportation) on weeds is widely documented, although different trends are sometimes observed between studies on both the flora expressed and the seedbank (Nichols et al., 2015; Plaza et al., 2011; Santín-Montanyá et al., 2016). These differences are mainly explained by complex interactions with other factors such as: differences in the duration of the experiment, the history of the field, and the species present (Nichols et al., 2015). However, it is commonly reported that reducing tillage increases weed density and favours grass populations (Nichols et al., 2015; Schnee et al., 2023; Travlos et al., 2018; Trichard et al., 2013). On the other hand, residue restitution can influence weed dynamics by changing nutrient dynamics, soil temperature or soil moisture (Liebman and Mohler, 2001; Nichols et al., 2015). Yet, it is not very clear whether the burial of retained crop residues by tillage favours weed development or not (Nichols et al., 2015). Furthermore, the resulting composition and harmfulness of the weed flora in the long term are poorly documented (Nichols et al., 2015). However, the mulch effect of residues has a proven effect on reducing weed germination if the quantity is sufficient. If the quantity is insufficient, the effect may be the opposite (Chauhan et al., 2012; Nichols et al., 2015). Plaza et al., (2011) highlighted the importance of long-term trials to shed light on the effect of agricultural practices on weed diversity. Furthermore, long-term of tillage and residue management could directly impact crop yield while also exerting an indirect influence on weed flora. To highlight the direct and indirect relationships between different variables , Structural equation modelling (SEM) has gained traction within ecological studies (Majdi et al., 2014; Puech et al., 2015). Moreover, recent research, such as the case study conducted by Quinio et al. (2017), has successfully employed path analysis to investigate the impact of farming practices on weeds and winter wheat production.

 The aim of this paper was therefore to characterize the long-term effect of residue and tillage management on weed pressure and crop productivity after 14 years of cultivation. The focus was put on a winter wheat cropping season, as this crop exhibit an important phenotypic plasticity and as it occupies ~45% of the Walloon arable lands. Monitoring of i) the weed seedbank and ii) the in-season expressed weed flora were performed. Finally, iii) it was determined whether differences in flora composition and levels of infestation could impact winter wheat yield potential.

2. Material and Methods

2.1. Site description and experimental design

 The long-term trial is established since 2008 on the experimental farm of Gembloux Agro-Bio Tech, University of Liège, in Belgium (50°33'49.6''N, 4°42'45.0''E). The climate in this region is oceanic temperate (Climate Cfd in the Köppen-Geiger classification) with an average annual rainfall of 793.4 mm, an annual average temperature of 9.6 °C and an average solar radiation 74 of 825J cm⁻² day⁻¹. The soil type is classified as Cutanic Luvisol (FAO_WRB classification) with a silt loam texture (18-22% of clay, 70-80% of silt, and 5-10% of sand). The experiment was designed as a Latin square disposal with four replications. Each plot measured 15 m wide and 40 m long. Crop rotation since the beginning of experimentation in 2008 is present in the Table 1. Since 2015, the rotation has remained the same, with a winter wheat crop present every other year (maize, winter wheat, sugar beet, winter wheat).

 The trial compared two different factors for managing soil and crop residues: (i) the restitution (IN) or the exportation (OUT) of crop residues, and (ii) the intensity of tillage: conventional tillage (CT) or reduced tillage (RT). The combination of these two factors results in four different crop residue managements: CT-IN, CT-OUT, RT-IN and RT-OUT.

Regarding the exportation of crop residue, stubble and chaff were always kept on site,

 but the rest of residue (straw and what's left of leaves) were exported (OUT) or maintained (IN). Tillage is carried out to a depth of 25cm in CT and 7-10cm in RT. RT and CT treatments were both breaked with a Stubble breaker after the harvest. In CT, ploughing was carried out a few days before sowing winter wheat. Finally, seedbed preparation was identical in RT and CT (using a stubble cultivator). For more information on the trial see the article of Hiel et al. (2018). Details of all winter wheat cultivation operations in 2021 and 2022 (the year in which the measurements were taken for this paper) are shown in [Table 2](#page-7-0).

92 *Table 1: Crop rotation between 2008 and 2022 and weeding history applied to trial between 2008 and 2022. HRAC group are* 93 *the herbicide mode of action group made by Herbicide Resistance Action Committee*

Year	crop	Date	Weeding	Ingredients	Modes of action	HRAC group
2008- 2009	rapeseed	$09-03-$ 08	Application of Roundup [6.43 L ha $\frac{1}{2}$ prior sowing	Glyphosate, potassium salt	Inhibition of enolpyruvyl shikimate phosphate synthase	G
		$10-13-$ 08	Application of Butisan $[1.7 L ha-1]$	Metazachlor	Inhibition of very long-chain fatty acid synthesis	K ₃
2009- 2010	winter wheat	$04 - 14 -$ 10	Application of Atlantis WG [0.30 kg ha^{-1}], Milan [1.25 L ha^{-1}], Primus [0.05 L ha^{-1}] and Vegetop [1] L ha ⁻¹]	Mesosulfuron-methyl- sodium, iodosulfuron- methyl-sodium, mefenpyr-diethyl, bifenox, pyraflufen- ethyl, florasulam, esterified rapeseed oil	Inhibition of acetolactate synthase, inhibition of protoporphyrinogen oxidase	B, E
2010- 2011	winter wheat	$04-13-$ 11	Application of Othello $[1.2 L ha^{-1}]$ and Legacy [0.2 L ha- 1]	Diflufenican, iodosulfuron-methyl- sodium, mesosulfuron- methyl-sodium, mefenpyr-diethyl, MCPA	Inhibition of phytoene desaturase, inhibition of acetolactate synthase, auxin mimics	F1, B, O
$2011 -$ 2012	winter wheat	$03-28-$ 12	Application of Othello $[1.2 L \text{ ha}^{-1}]$ and Legacy [0.4 L ha- 1]	Diflufenican, iodosulfuron-methyl- sodium, mesosulfuron- methyl-sodium, mefenpyr-diethyl, MCPA	Inhibition of phytoene desaturase, inhibition of acetolactate synthase, auxin mimics	F1, B, O
2012- 2013	cover crop (mustard)	$03-18-$ 13	Application of TAIFUN 360 [2.59 L ha^{-1}]	Glyphosate, isopropylamine salt	Inhibition of enolpyruvyl shikimate phosphate synthase	\overline{G}

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96 *Table 2: Winter wheat cultivation operations in 2021-2022*

 The history of the various herbicide applications since 2008 is presented in [Table 1.](#page-3-0) Cover crops were generally terminated by applying glyphosate. Herbicides were applied at spring during within winter wheat cropping seasons and applied between one and three times, depending upon the success of the weed control. Maize crop was managed with a single post-emergence application of herbicide. Lastly, the FAR weed control itinerary (usually applied in Belgium) was applied during the sugar beet seasons, which consists of repeated low-dose passes of a mixture of foliar herbicide (phenmedipham), an activator (ethofumesate) and a residual herbicide (e.g. metamitron). Rapeseed and faba bean were cultivated only once since establishment of the experiment and herbicides were applied following business-as-usual management. More details about ingredients, modes of action and HRAC groups (HRAC, 2024) are presented in [Table 1.](#page-3-0)

107 *2.2. Field data collection*

108 **A. Weed seedbank**

 To determine the impact of residue management on weed density and diversity, weed seedbank samples were systematically collected on the17th January, 2022. A 'W' sampling pattern was employed, with five composite samples derived from four soil cores each (diameter=2cm) per plot. The 4 soil sub- samples were collected at each corner of a 50 x 50 cm quadrat. Sampling was conducted at two different depths: 0-10cm (maximum working depth in RT) and 10-25cm (maximum working depth in CT). In total, 160 samples (4 treatments*4 replications* 5 samples/plot * 2 depths) underwent analysis using the emergence method. The composite samples were stored for 15 days in a cold room at 5°C in order to break the dormancy of some specific seed species (Mahé et al., 2021). The composite soil samples were

 sieved and then spread on trays, over potting soil (1cm) and argex balls (2cm). The samples were themselves spread with a maximum depth of 2cm to allow germination of all seeds (Mahé et al., 2021). A PVC tube was inserted at the corner of the tray for regular irrigation. In addition, micro-sprinkler irrigation was carried out every week to prevent the surface layer of soil samples from drying out. Weed seedlings were identified and counted every 2-3 weeks. Once identified at the species levels (or genus when it was not possible to identify at species level), the weeds were removed. Species are named using both the latin name and the EPPO code ("EPPO Global Database," n.d.) The emergence was monitored between 02 February 2022 and 30 November 2022. The first phase of monitoring (until 11 September) was carried out in a germination room with 574 lux light and a temperature between 17 and 20°C. Between 08/04/2022 and 22/04/2022 the samples were not irrigated to force drought. On 22/04 the samples were crumbled by hand before irrigation was applied again. This period of dryness followed by crumbled is intended to stimulate germination (Mahé et al., 2021). From 12 September to the end of November, the weed seedbank was installed in an unheated greenhouse to enhance autumnal germination.

B. In-season crop and weed sampling

 In order to characterise the weed flora expressed during winter wheat cropping season (sowing in autumn 2021) and its impact on yield, samples were taken during the 2022 winter wheat growing season. Weed density by species was measured at the time of wheat tillering and at flowering stages within 5 quadrats of 50 cm * 50 cm per plot. In addition, at wheat flowering, weed biomass by species and crop biomass were measured within the same quadrat as weed density.

 Finally, at wheat maturity, the yield was measured in 5 quadrats of 50 cm * 50 cm per plot. Each quadrat was sampled within a 2 m radius of the quadrat within which data were collected at wheat tillering and flowering. At maturity, components of yield (stem biomass, spike biomass and number of spikes per m²) were measured directly from samples. The average grain biomass per spike was derived as follows:

141 Grain biomass per spike =
$$
\frac{\text{spike biomass.m}^{-2}}{\text{number of spikes.m}^{-2}} \text{Eq. 1}
$$

142 All the biomass samples were dried at 60°C in an oven until the biomass remained unchanged.

Biomass were measured at the nearest 0.01g

2.3. Weed diversity index

 Species richness (number of species per quadrat) and Shannon-Weiner index were computed from weed-related data. Indices were computed on weed seedbank observations and were calculated for in- season field data, at tillering and flowering of winter wheat. Shannon-Weiner index, which measure the α -diversity was calculated by samples (seedbank) or quadrats (in-season) as follow:

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$$
H = 1 - \sum_{i=1}^{S} p_i * \ln(p_i) \text{ Eq. 2}
$$

150 Where p_i is the relative proportion of individuals of species *i* in a community of *S* species and *S* is the total number of species.

2.4.Statistical analysis

 Statistical analysis were perform using R statistical Software (V4.3.1; R Core Team, 2021). Response variables (weed density, weed biomass, species richness, Shannon index, yield…) were modelled with the *glmmTMB* package. Model diagnostics were verified with the *DHARMa* package. Response variable were expressed as a function of crop residue exportation, tillage intensity and their interaction. For seedbank related-data, depth of sampling was also studied. In this case response variable were expressed as a function of crop residue exportation, tillage intensity, depth of sampling and their integrated triple interaction. Rows and column of the Latin square design were always included as random intercept. Distribution was selected to meet the conditions. In addition, the model with the lowest Akaike's Information Criterion (AIC) was chosen. All selected models are presented in supplementary Table A.1. ANOVA were performed on these models to assess the significance of fixed effects. Finally, an Estimated marginal means analysis was performed using the emmeans package.

 Correlation between weed density, density of the two most prevalent weed species at flowering and yield components were calculated with Spearman correlation due to violation of parametric assumptions.

 A path analysis (covariance structural analysis) was performed with the *lavaan* package in order to illustrate the relationships of direct and indirect effects between the variables impacting yield. The

 model was constructed based on standardised variables (i.e., centred mean and scaled by standard deviation). Path models are built upon both latent variables (LV) and manifest variables (MV). The first LV, "Weed pressure", initially used the same MVs as Quinio et al. (2017) , i.e. richness, Shannon index and abundance (except that abundance is expressed here in terms of biomass rather than individuals). The second LV, "Soil management", comprises MV "Ploughing" (conventional or reduced tillage) and MV "Residue exportation" (residue exported or maintained). The third latent variable refers to the productivity; as proposed by Quinio et al., (2017) it was composed solely of the yield. Two MVs related to yield components (number of spikes per m² and average biomass of grains per spike) were added to the model.

178 The quality of the model was assessed using five indicators. First, the chi-square test (χ^2) was calculated. A p-value >0.05 indicates an acceptable model fit. Secondly the comparative fit index (CFI) and the Tucker-Lewis index (TLI) should respectively have a value above 0.90 and 0.95. Finally, the Root Means Square Error Approximation (RMSEA) and the Standardized Root Mean Square Residual (SRMR) with value below 0.08 generally indicate a well-fitting model.

 Based upon preliminary results, a second model was built. Only the MV related to weed abundance indicator (expressed in biomass) was eventually kept to feed the LV related to weed pressure. The other two indicators were proven to not contribute to build a quality model. Additionally, the MV related to residue fate was removed from the LV soil management. This variable was not providing any additional insight to the model. In fact, in this trial, the lack of significant impacts of residue exportation

188 on yield had been demonstrated in earlier studies

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190 *Figure 1: structural equation model for the relationship between productivity, yield components, weed pressure and soil* 191 *management. Latent variables are in an oval colored in gray and manifest variable are in rectangle. A direct path is* 192 *represented by a single arrow that directly connects two traits (e.g., Residue management and weed pressure). the dotted* 193 *rectangles correspond to the variables which were tested in the initial path analysis but which were not kept in order to respect* the conditions of the path analysis.

195 **3. Results**

196 *3.1. Weed seedbank*

197 The seedbank revealed a total of 18 different species [\(Table 3\)](#page-12-0). The dominant species in the

198 seedbank were *Matricaria chamomilla* L. (MATCH) and *Alopecurus myosuroides* Huds. (ALOMY),

- 199 and represented respectively 73.6% and 18.7% of the seedling density. *Polygunum aviculare* L. ranked
- 200 third and represented only 4.4% of all seedling density.

 ANOVA revealed a significant interaction of sampling depth, tillage and residue exportation on seedling density. ANOVA results are provided in Table A.2 in the supplementary material. Results were separately analysed by sampling depth. In the 10-25 horizon, no significant difference in seedling density was observed between the different residue management methods. However, on the 0-10 horizon, weed density was lowest in CT-IN and highest in RT-IN.

 Concerning the seedling density of the two most abundant species in the seedbank (MATCH and ALOMY), they both showed a significant interaction between sampling depth and tillage (see Table A.2). At depths of 10-25 no significant difference in seedling density was observed, whereas at 0-10 the weed seedling density was higher in RT than in CT.

Figure 2: Total weed seedling density m⁻² as a function of sampling depth and soil management. Treatments with the same **214** *coloured letters are not significantly different. Letters correspond to the interaction eff*

 coloured letters are not significantly different. Letters correspond to the interaction effect of total weed seedling density between the different soil management. "0-10" and "10-25" are respectively the sampled soil depths of 0-10cm and 10-25cm.

RT=reduced tillage, CT= conventional tillage, IN = residue restitution, OUT= residue exportation.

The average species richness (sample scale) was significantly higher in RT compared to CT, with

an average of one species more in favour of RT (3 and 2 respectively). The trend was identical for the

Shannon index, with an average value of 0.55 in RT and 0.27 in CT [\(Figure 3\)](#page-15-0). No significant difference

was observed with the factor related to the exportation of residues.

 Figure 3: Biodiversity index (Shannon index above and species richness below) based on Weed Seedbank on the left and on weed counting in-season (in winter wheat) on the right as a function of crop residue management. Treatments with the same letters are not significantly different. RT= reduced tillage, CT= conventional tillage. IN= restitution of residues, OUT= exportation of residues.

3.2. In-season weed community expression

 The weed flora at the end of winter was mainly composed of *Alopecurus myosuroides* Huds. (ALOMY) and *Matricaria chamomilla* L. (MATCH) (see in supplementary Table A.3 for all species present). The timing of the weed survey (at wheat tillering - before herbicide application 231 - and at wheat flowering - after herbicide) had no impact on total weed density or on ALOMY density. However, a 56% reduction was observed in MATCH between the two surveys (pvalue=0.01213). CT reduced weed abundance (pvalue <0.0001) measured at tillering by 78% compared to RT. Similar trend was found for the two main weeds (ALOMY and MATCH), with an average reduction of 69% and 87% respectively [\(Figure 4\)](#page-16-0). However, no significant effect of residue exportation (IN vs OUT) was observed.

 At wheat flowering, weed biomass was significantly higher in RT than in CT, with an average 238 biomass of 24 g m⁻² and 12.2 g m⁻² respectively [\(Figure 4\)](#page-16-0). While the trend was identical for MATCH (pvalue=0.001475), there was no significant effect of tillage on ALOMY biomass (pvalue= 1.8074).

 Figure 4: Weed density at wheat tillering and at wheat flowering (top graph) and biomass of weeds at wheat flowering (bottom graph). Treatments with the same letters are not significantly different. Letters correspond to the effect of tillage on weed density or biomass. RT= reduced tillage, CT= conventional tillage. IN= restitution of residues, OUT= exportation of $residues.$

 No effect of weed survey, tillage and residue exportation on the Shannon index was observed [\(Figure 3\)](#page-15-0). However, tillage had an effect on the average number of species (sample scale), with an average of 3 species in RT and 2 species in CT.

3.3. Impacts of weeds on crop growth and yield components

 Total weeds biomass exhibited a negative correlation with yield with a value of -0.58. When 252 comparing the yield of quadrats with the highest $(50g.m⁻²)$ and lowest $(0g.m⁻²)$ weed biomass, 28% loss

 Upon examining the yield components, total weed biomass and ALOMY biomass exhibit negative correlations with spike density (resp. -0.5 and -044), and the biomass of grains per spike (resp. -0.33 and -0.34) [\(Table 4\)](#page-17-0). The correlation with weed biomass was furthermore a bit higher for the spike biomass than with the spike density. The weakest correlations were reported with the biomass per spike 261 and with the biomass of grains per spike. The same trends were observed for ALOMY, but no significant correlations were observed with MATCH.

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266 *3.4. Path Analysis*

 When an ANOVA was carried out on yield as a function of tillage and export residue the effect of tillage is significant (pvalue =0.04326). However, when weed biomass was added as an explanatory 269 variable to predict yield, no effect of tillage was observed (pvalue= 0.306) whereas the effect of weed biomass was significant (pvalue=0.00525). This led us to consider an integreated approach trougth the path analysis. The final model [\(Figure 1](#page-12-1) without the dotted rectangles) met all the statistical conditions to perform a relevant path analysis (pvalue>0.05, CFI=0.997, TLI=0.990, RMSEA= 0.081, SRMR=

273 0.006). Soil management (here only represented by the tillage practice) had a path coefficient σ_{direct} that 274 is exclusively significant with weed pressure ($\sigma_{\text{direct}} = -0.38$). Soil management did not exhibit any significant direct coefficient with yield components. On the contrary, weed pressure (expressed here through the manifest variable of the abundance measured in terms of biomass) was the only significant 277 factor impacting yield components. A grater path coefficient was found for spike density ($\sigma_{\text{direct}} = -0.44$) 278 compared to the biomass of grains per spike $(-\sigma_{\text{direct}} = -0.33)$. Spike density was the most impactful 279 component on productivity with a $\sigma_{\text{direct}} = 0.84$, while the biomass of grain per spike has a σ_{direct} equalling 0.39 [\(Figure 5\)](#page-18-0).

 The indirect effect of weed pressure on productivity were mainly expressed by the effect on the 282 number of spikes ($\sigma_{\text{indirect}} = -0.37$) and to a lesser extent through the biomass of grains per spike (σ_{indirect}) =-0.13). The global indirect effect on weed pressure productivity is -0.50. Finally, and consequently to those results, the indirect significant influence of tillage was expressed through weed suppression. The 285 indirect path coefficient on spike density equalled 0.17 and the $\sigma_{indirect}$ on grain biomass per spike equalled 0.13, for a global indirect path coefficient on productivity equalling 0.29.

 Figure 5: Path coefficients of the final model for the relationship between productivity, yield component, weed pressure and soil management (only Tillage practices). Latent variables are in an oval colored in gray and manifest variable are in rectangle. Path coefficients (σ) were computed from regressions (red arrow = negative and green arrow = positive). A direct path is 291 *represented by a single arrow that directly connects two traits (e.g., soil management and Weed pressure) whereas an indirect* path occurs when the path between two variables is separated by other(s) variable(s) *path occurs when the path between two variables is separated by other(s) variable(s) (e.g., Productivity and Weed pressure).*

 Insignificant paths (pvalue>0.05) are indicated by "ns", statistical significance of the path coefficient at p-value≤0.05 is indicated by "*".

- **4. Discussion**
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4.1. Impact of long-term soil management in weed diversity

 Weed diversity was relatively low in all treatments. Only two species (ALOMY and MATCH) dominated both the seedbank and the expressed weed flora. A slight increase in both Shannon diversity and species richness (on average one more species) was observed in the seedbank in RT compared to NT. The same trend was found in the flora expressed during the winter wheat cropping season, despite no clear pattern in Shannon diversity was found. The results are in line with those of long-term trial documented in the literature. Within the seedbank, Sosnoskie et al. (2006) showed a slightly higher specie richness in RT compared to CT, with ca. 2 species more. However, they reported no difference in the Shannon index between RT and CT, while in the present study, a significant, yet low, difference was reported. The results regarding the expressed flora were in line with those of the long-term trial by Plaza et al. (2011), where no differences in terms of Shannon diversity were observed and the same trend of a slight increase in species richness (+1 species on average) in RT. It was hypothesized by the authors that RT could allow a slight increase in the number of species due to a greater diversity of ecological niches and germination opportunities. Complementary, results gain in the present study suggest that residue exportation had no reported long-term impact on weed diversity.

 The prevalence of two dominant weed species, particularly associated with cereal crops, can be attributed to the rotational strategy employed. The rotation emphasizes the recurrent cultivation of winter crops, initiated in early autumn during the trial, contributing to the establishment of a distinctive flora (Nichols et al., 2015; Storkey and Neve, 2018). The effect of crop rotation is indeed known to be a much more powerful driver of weed flora composition than tillage (Fried et al., 2008).

4.2. Impact of soil management on weed density

 As previously observed in the literature, RT increases the seedling density of seedbank on the upper soil layer compared with CT. However, the quantity of weed seedling within soil depth 10-25 cm was found to not be statistically different between CT and RT, which result is in line with other studies (Cardina et al., 2002; Schnee et al., 2023). This effect was confirmed independently for the two

dominant weeds (ALOMY and MATCH).

 The expressed weed density measured before the first weeding operation was higher in RT, in agreement with several long-term studies (Plaza et al., 2011; Santín-Montanyá et al., 2016, 2013). However, in the current study, no major effect of residue exportation was observed on the weed seedbank and the expressed weed density during the wheat cropping season. A potential explanation could be associated to the dilution of surface residue, which only occurs within the 0-10 cm soil profile in reduced tillage and would contribute to explain such results. Indeed, it is likely that the mulch effect impacting the density of germinating weeds, as observed by Anderson (1999) under no-tillage system, was not expressed in this case. Furthermore, this might be reinforced by the fact that the preceding crop (sugar beet) returns only a small quantity of residue on the field. The actual effect of residues on the expressed weed flora is more likely to be observed after a crop leaving a larger quantity of residue (such as wheat or maize).

 Chemical weed control did not result in a reduction of total weed density during the season. Moreover, when examining the species individually, it became evident that the herbicide exhibited no discernible impact on the ALOMY population. Following complementary laboratory analysis (data not shown), it was determined that this ALOMY population demonstrated resistance to the spring herbicides used during winter wheat cultivation (resistance to Acetolactate Synthase and Acetyl CoA Carboxylase). The emergence of this resistance may be attributed to the recurrent use of identical active ingredients in winter wheat (Zeller et al., 2021). Indeed, since 2008 the mode of action of Inhibition of Acetolactate Synthase has always been applied in winter wheat and Acetyl CoA Carboxylase was applied in 2014, 2020 and 2022 [\(Table 1\)](#page-3-0). Conventional tillage (CT) proved to be an efficient method for managing the ALOMY population in comparison to reduced tillage (RT). Zeller et al. (2021) demonstrated that ALOMY was reduced by 70 to 80% when rotational ploughing was implemented. Weed biomass, on average, was higher in RT than in CT, indicating that the greater number of weeds at tillering led to an increased total weed biomass. However, there was no discernible significant impact on ALOMY biomass among the tillage and residue exportation methods. One might have thought that the non-significant effect was due to a higher biomass per ALOMY in CT than in RT, but no significant effect

of biomass per ALOMY was observed (see Table A.2).

4.3.Impact of tillage, residue exportation and weeds development on yield.

 When performing ANOVA between yield and soil management (tillage and residue exportation), significant impact was reported for soil tillage. A higher yield were reported under CT and was in line with the European literature (Van den Putte et al., 2010). On the other hand, the ANCOVA between the yield, soil component and the biomass of weed as an explanatory variable (see Table A.2), the analysis revealed no effect of tillage. Instead the sole influential factor was the weed biomass. In this trial, the effect of tillage appears to be indirect, as highlighted by the path analysis.

 Weed pressure was found to be also significantly linked with yield (using both ANOVA and path analysis). This highlights the importance of regulating weed flora in the event of weed infestations. Looking more in depth to the impact of the different species, MATCH, although present, was not significantly correlated with yield losses. However, ALOMY was found to explain the majority of the 160 loss (-28% between an area without naturally ALOMY and an area with 50g of ALOMY m^{-2} ^s, Fig.B.2). ALOMY is a species that is phylogenetically close to wheat and shares similarities in its development, with the same germination period and a slightly shorter cycle, which means there is a great deal of competition for resources (Adeux et al., 2019). No direct effect between crop residue management and yield was reported by the path analysis. However, results gained in this study suggest that tillage expressed its impacts mostly through the control it puts on weeds which themselves had direct significant impacts on yield components.

 Among the yield components studied, the one that explained yield the best was spike density, in agreement with the literature (Lenoir et al., 2023; Slafer et al., 2014). This component exhibited the highest path analysis coefficient with weed pressure. It was confirmed in this study that the competition induced by weeds leads to a loss of yield, mainly by reducing the wheat's capacity to produce spikes, as suggested in previous studies (Adeux et al., 2019; Welsh et al., 1999). This confirms that competition can act early in the season (Welsh et al., 1999; Zimdahl, 2007) and can lead to a greater tillers recession when wheat competes with weeds for light and nutrients in the environment. It would therefore be interesting to monitor tillers dynamic earlier in the season to confirm this hypothesis. Finally, weed induced competition was found to cause yield losses, to a lesser extent, by affecting grain filling (monitored here through the grain biomass per spike). Adeux et al. (2019) showed in their experiment that a weed community composed almost exclusively of ALOMY had no effect on the 1,000-kernel weight but did have an effect on the number of grains per spike, suggesting that the competition generated by ALOMY takes place until wheat flowering. The indirect effect of tillage management on yield through weed competition could explain the earlier observation reported by Hiel et al. (2018) on the same experimental site, who did not systematically observed an impact of soil management over the year but who reported a -3.4% cumulative yield decrease between 2010 and 2015.

- **5. Conclusion**
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 The long-term effect of tillage and residue management, by exporting or maintaining residues on site and incorporating them or not through tillage, showed no effect of residues exportation on yield and weeds. The lack of link between weed flora (diversity and abundance) monitored through the seedbank or during the cropping season of winter wheat showed that, in a rotation based on wheat, residue exportation was of little importance in the context of this study. The lack of effect of maintaining residues could be explained by a dilution of crop residues in the upper soil profile that still occurs in some reduced tillage (RT) systems (such as the one implemented here), preventing the mulch effect to occur. Reduced tillage was found to have no major impact on weed diversity (richness was a little bit higher compared to conventional tillage) but resulted mostly in an increase of weed density. While this increase is in line with results reported in other long-term trials, it was most likely exacerbated in this case by the frequent return of autumn crops to the rotation. In a system based on wheat, RT might facilitate the development of ALOMY, a very competitive species that is detrimental to yield. This management technique might favour the appearance of resistance -as observed in this trial, especially in winter wheat-based cropping systems. Above all, it highlights the problem of long-term sustainable management of the weed flora. Reduced tillage management technique might indirectly lead to higher yield losses through poor control of the weed flora in systems based on wheat cultivation. In this regard, while RT is promoted for its potential to maintain or enhance soil health over the long term, it would be

- interesting to compare the sustainability of weed management within different soils and cropping
- systems management, including systems with a higher proportion of spring crops.

6. Declaration of interest

The authors declare no conflicts of interest.

7. Acknowledgement

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