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

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16 Abstract

In Hesbaye region (Belgium) with a loamy soil and under temperate climatic condition, winter wheat is 17 18 a key component of agricultural rotations. As part of these rotations, soil management is a known driver 19 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 20 management levers were i) the export (OUT) or restitution (IN) of crop residues and ii) the burial of 21 residues by conventional tillage (mouldboard ploughing 25 cm depth, CT) or reduced tillage (cultivator 22 23 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 24 flora and management on yield were investigated. Tillage management showed little impact on weed 25 26 diversity with only a slight increase in diversity in reduced tillage. However, reduced tillage resulted in 27 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 28 29 based on the cultivation of wheat, reduced tillage can pose problems for the long-term management of 30 the weed flora, and great attention has to be paid to its management.

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

32 **1. Introduction**

Agricultural soils management is known to have an impact on carbon storage and potentially 33 could help mitigate the rise in atmospheric CO2 concentration (Martin et al., 2021). The management 34 of crop residues, which can be exported (e.g. for animal fodder or bioenergy production) or incorporated 35 36 into the field using reduced or conventional tillage, can therefore play a role in carbon storage (Autret 37 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 38 39 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).

41 The effect of tillage alone (without residue incorporation or exportation) on weeds is widely 42 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). 43 These differences are mainly explained by complex interactions with other factors such as: differences 44 45 in the duration of the experiment, the history of the field, and the species present (Nichols et al., 2015). 46 However, it is commonly reported that reducing tillage increases weed density and favours grass 47 populations (Nichols et al., 2015; Schnee et al., 2023; Travlos et al., 2018; Trichard et al., 2013). On 48 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 49 50 whether the burial of retained crop residues by tillage favours weed development or not (Nichols et al., 51 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 52 53 reducing weed germination if the quantity is sufficient. If the quantity is insufficient, the effect may be 54 the opposite (Chauhan et al., 2012; Nichols et al., 2015). Plaza et al., (2011) highlighted the importance 55 of long-term trials to shed light on the effect of agricultural practices on weed diversity. Furthermore, 56 long-term of tillage and residue management could directly impact crop yield while also exerting an 57 indirect influence on weed flora. To highlight the direct and indirect relationships between different 58 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 59

al. (2017), has successfully employed path analysis to investigate the impact of farming practices onweeds 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.

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2. Material and Methods

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2.1. Site description and experimental design

70 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 71 oceanic temperate (Climate Cfd in the Köppen-Geiger classification) with an average annual 72 rainfall of 793.4 mm, an annual average temperature of 9.6 °C and an average solar radiation 73 of 825J cm⁻² day⁻¹. The soil type is classified as Cutanic Luvisol (FAO_WRB classification) 74 with a silt loam texture (18-22% of clay, 70-80% of silt, and 5-10% of sand). The experiment 75 was designed as a Latin square disposal with four replications. Each plot measured 15 m wide 76 and 40 m long. Crop rotation since the beginning of experimentation in 2008 is present in the 77 Table 1. Since 2015, the rotation has remained the same, with a winter wheat crop present every 78 other year (maize, winter wheat, sugar beet, winter wheat). 79

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.

84 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.

Table 1: Crop rotation between 2008 and 2022 and weeding history applied to trial between 2008 and 2022. HRAC group are
 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 ⁻ ¹] 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	K3
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 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	G

2013	faba bean	04-08- 13 06-10-	pre-emergence weeding with application of Lingo $[1.4 L ha^{-1}]$ and Stomp 400 SC $[1.8 L ha^{-1}]$ manually only on	Clomazone, linuron, pendimethaline Glyphosate	Inhibition of deoxy- D-xylulose phosphate synthase, inhibition of photosynthesis at PS II, inhibition of microtubule assembly Inhibition of	F4, C1 C2, K1
		13	thistle with application of GLYFOS [5.67 L ha ⁻¹]		enolpyruvyl shikimate phosphate synthase	G
		08-28- 13	Application of Diquanet SL	Diquat dibromide	PS 1 electron diversion	D
2013- 2014	winter wheat	04-01- 14	Application of Atlantis [0.3 kg ha ⁻¹], Hussar Ultra [0.1 L ha ⁻¹] and Actirob B [1 L ha ⁻¹]	Mesosulfuron-methyl- sodium, iodosulfuron- methyl-sodium, mefenpyr- diethyl,esterified rapeseed oil	Inhibition of acetolactate synthase	В
		04-25- 14	Application of Axial [1.47 L ha ⁻¹]	Pinoxaden, cloquintocet-mexyl	Inhibition of acetyl CoA carboxylase	А
		05-16- 14	Application of Allie [30.55 g ha ⁻¹]	Metsulfuron-methyl	Inhibition of acetolactate synthase	В
2014- 2015	cover crop (oats and peas)	03-17- 15	Application ofGLYPHOGAN [4.16 L ha ⁻¹]	Glyphosate	Inhibition of enolpyruvyl shikimate phosphate synthase	G
2015	maize	05-28- 15	Application of Andes [1.6 L ha ⁻¹], Callisto [0.71 L ha ⁻¹] and Samson extra 6 [0.42 L ha ⁻¹]	Flufenacet, terbuthylazine	Inhibition of very long-chain fatty acid synthesis, inhibition of photosynthesis at PS II	K3, C1 C2
2015- 2016	winter wheat	03-22-16	Application of ATLANTIS WG [0.30 kg ha ⁻¹], Capri duo [252.04 g ha ⁻¹] and ACTIROB B [1.00 L ha ⁻¹]	Iodosulfuron-methyl- sodium,mesosulfuron- methyl-sodium, mefenpyr-diethyl, florasulam, pyroxsulam, cloquintocet-mexyl (esterified rapeseed oil)	Inhibition of Acetolactate Synthase	В
		08-26- 16	Application [after harvest] of CLINIC [2.50 L ha ⁻¹] and ROSATE 360 SL [1.67 L ha ⁻¹]	Glyphosate, isopropylamine salt	Inhibition of enolpyruvyl shikimate phosphate synthase	G
2016- 2017	cover crop (mustard and phacelia)	03-14- 17	Application of GLYFOS [0.81 ha ⁻¹], ROSATE 360 SL [1.21 L ha ⁻¹] and GLYFALL PLUS [1.82L ha ⁻¹]	Glyphosate, isopropylamine salt	Inhibition of enolpyruvyl shikimate phosphate synthase	G

2017	sugar beet	04-21- 17	Application of DIANAL 160 [0.38 L ha ⁻¹], MEDIFAM SE [0.28 L ha ⁻¹], ACTIROB B [0.48 L ha ⁻¹], METATRON SC [0.50 L ha ⁻¹] and ETHOMAT 500 [0.15 L ha ⁻¹]	Phenmedipham, esterified rapeseed oil, metamitron, ethofumesate	Inhibition of photosynthesis at PS II, inhibition of very long-chain fatty acid synthesis	C1 C2, K3
		05-03- 17	Application of SAFARI [19.87 g ha ⁻¹], DIANAL 160 [1.00 L ha ⁻¹], ETHOMAT 500 [0.30 L ha ⁻¹], VEGETOP [0.54 L ha ⁻¹], METATRON SC [0.42 L ha ⁻¹] and Beetix 700sc [0.06 L ha ⁻¹]	Triflusulfuron-methyl, phenmedipham, ethofumesate, esterified rapeseed oil, metamitron	Inhibition of acetolactate synthase, inhibition of photosynthesis at PS II, inhibition of very long-chain fatty acid synthesis	B, C1 C2, K3,
		05-10-17	Application of DIANAL 160 [1.00 L ha^{-1}], ETHOMAT 500 [0.30 L ha^{-1}], Beetix 700sc [0.75 L ha^{-1}], SAFARI [19.87 g ha^{-1}] and VEGETOP [0.54 L ha^{-1}]	Phenmedipham, ethofumesate, triflusulfuron-methyl, esterified rapeseed oil, metamitron	Inhibition of photosynthesis at PS II, inhibition of very long-chain fatty acid synthesis, inhibition of acetolactate synthase	C1 C2, K3, B
		05-25- 17	Application of DIANAL 160 [0.06 L ha^{-1}], BETADES [1.95 L ha^{-1}], ETHOMAT 500 [0.13 L ha^{-1}], ETHOFOL 500 SC [0.17 L ha^{-1}], FRONTIER ELITE [0.40 L ha^{-1}] and CENTIUM 36 CS [0.05 L ha^{-1}]	Phenmedipham, desmedipham, ethofumesate, dimethenamid-p, clomazone	Inhibition of photosynthesis at PS II, inhibition of very long-chain fatty acid synthesis	C1 C2, K3
		05-31- 17	Application of MATRIGON [0.49 L ha ⁻¹] and FUSILADE MAX [1.64 L ha ⁻¹]	Clopyralid, monoethanolamine salt, fluazifop-P-butyl	Auxin mimics	0
2017- 2018	winter wheat	04-10- 18	Application of Othello [1.03 L ha ⁻ - 1] and VEGETOP [0.59 L ha ⁻ -1]	Diflufenican, iodosulfuron-methyl- sodium, mesosulfuron- methyl-sodium, mefenpyr-diethyl, esterified rapeseed oil	Inhibition of phytoene desaturase, inhibition of acetolactate synthase	F1, B

		08-21- 18	Application after harvest of GLYFALL PLUS [4.86 L ha^-1]	Glyphosate, isopropylamine sal	Inhibition of enolpyruvyl shikimate phosphate synthase	G
2018- 2019	cover crop (mustard and phacelia)	03-30- 19	Application of CLINIC UP [6.00 L ha^-1]	Glyphosate, isopropylamine salt	Inhibition of enolpyruvyl shikimate phosphate synthase	G
2019	maize	06-21- 19	Application of CALLISTO [0.70 L ha^-1], SAMSON EXTRA 60 OD [0.50 L ha^-1] and ASPECT T [1.62 L ha^-1]	Mesotrione, nicosulfuron, flufenacet, terbuthylazine	Inhibition of hydroxyphenyl pyruvate dioxygenase, inhibition of acetolactate synthase, inhibition of very long-chain fatty acid synthesis, inhibition of photosynthesis at PS II	F2, B, K3, C1 C2
2019- 2020	winter wheat	04-08- 20	Application of SIGMA STAR [0.30 kg ha^-1] and ACTIROB B [1.00 L ha^-1]	Iodosulfuron-methyl- sodium, mesosulfuron- methyl-sodium, thiencarbazone-methyl- sodium, mefenpyr- diethyl, esterified rapeseed oil	Inhibition of acetolactate synthase	В
		05-08- 20	Application of Axial [1.19 L ha^-1]	Pinoxaden, cloquintocet-mexyl	Inhibition of acetyl CoA carboxylase	А
2020- 2021	cover crop (mustard and phacelia)	03-02- 21	Application of GLYFALL PLUS [3.00 L ha^-1]	Glyphosate, isopropylamine salt	Inhibition of enolpyruvyl shikimate phosphate synthase	G
2021	sugar beet	05-20-21	Application of DIANAL 160 [0.99 L ha^-1], ETHOMAT 500 [0.40 L ha^-1], Allitron 700 Sc [0.79 L ha^-1], SAFARI [14.90 g ha^-1] and VEGETOP [0.70 L ha^-1]	Phenmedipham, ethofumesate, metamitron, triflusulfuron-methyl, esterified rapeseed oil	Inhibition of photosynthesis at PS II, inhibition of very long-chain fatty acid synthesis, inhibition of acetolactate synthase	C1 C2, K3, B
		05-30- 21	Application of DIANAL 160 [1.74 L ha^-1], ETHOMAT 500 [0.40 L ha^-1], Goltix Queen [0.99 L ha^-1], SAFARI [19.87 g ha^-1] and VEGETOP [0.50 L ha^-1]	Phenmedipham, ethofumesate, metamitron, quinmerac, triflusulfuron-methyl, esterified rapeseed oil,	Inhibition of photosynthesis at PS II, inhibition of very long-chain fatty acid synthesis, inhibition of acetolactate synthase, auxin mimics	C1 C2, K3, B, O

		06-05- 21	Application of DIANAL 160 [0.99 L ha^-1], ETHOMAT 500 [0.30 L ha^-1], Goltix Queen [0.99 L ha^-1], SAFARI [19.87 g ha^-1] and VEGETOP [0.70 L ha^-1]	Phenmedipham, ethofumesate, metamitron, quinmerac, triflusulfuron-methyl, esterified rapeseed oil	Inhibition of photosynthesis at PS II, inhibition of very long-chain fatty acid synthesis, inhibition of acetolactate synthase, auxin mimics	C1 C2, K3, B, O
		06-15- 21	Application of MATRIGON [1.50 L ha^-1] and VEGETOP [1 L ha^- 1]	Clopyralid, monoethanolamine salt, esterified rapeseed oil	Auxin mimics	0
		06-19- 21	Application of CENTIUM 360 CS [0.07 L ha^-1] and FRONTIER ELITE [0.79 L ha^-1]	Clomazone, dimethenamid-p	Inhibition of deoxy- D-xylulose phosphate synthase, inhibition of very long-chain fatty acid synthesis	F4, K3
2021- 2022	winter wheat	03-10- 22	Application of Sigma Star [0.33 kg ha [^] - 1]and Actirob B [1 L ha [^] -1]	Iodosulfuron-methyl- sodium, mesosulfuron- methyl-sodium, thiencarbazone-methyl- sodium, mefenpyr- diethyl, esterified rapeseed oil	Inhibition of acetolactate synthase	В
		04-27- 22	Application of Axial [1.2 l ha^-1], Biathlon Duo [0.060kg ha^-1] and Actirob B [0.8 L ha^- 1]	Pinoxaden, cloquintocet-mexyl, florasulam, tritosulfuron, esterified rapeseed oil	Inhibition of acetyl CoA carboxylase, Inhibition of acetolactate synthase	A, B

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

	Depth			CT-		RT-
Operation	(cm)	Additional information	CT-IN	OUT	RT-IN	OUT
ploughing	25	with mouldboard plough	Х	Х		
seedbed preparation		with stubble cultivator (Lemken				
	10	Smaragd 9/300)	Х	Х	Х	Х
sowing		wheat variety is Camesino (275				
		grain.m ⁻²), the tractor was equipped				
		with a dual cultivator (Jadin) in front				
		and rotary harrow and wedge ring				
		roller combined with seed drill				
	7	(Amazone)	Х	Х	х	Х
weeding		application of Sigma Star (0.33				
		kg.ha ⁻¹)and Actirob B (1 L.ha ⁻¹)	Х	Х	Х	Х

nitrogen fertilisation	liquid nitrogen (39%), 60 kg.ha ⁻¹ of				
	nitrogen	Х	х	Х	х
nitrogen fertilisation	liquid nitrogen (39%), 50 kg.ha ⁻¹ of				
	nitrogen	Х	х	Х	Х
weeding	application of Axial (1.2 l.ha ⁻¹),				
	Biathlon Duo (0.060kg.ha ⁻¹ and				
	Actirob B (0.8 L.ha ⁻¹)	Х	х	х	х
growth regulator	application of Cycofix (1L.ha ⁻¹)	Х	Х	Х	Х
fungicide	application of Balaya (1.51.ha ⁻¹)	Х	Х	Х	Х
nitrogen fertilisation	solid nitrogen calcium ammonium				
	nitrate (27% N), 60 kg.ha ⁻¹ of				
	nitrogen, 20kg.ha ⁻¹ CaO	Х	х	х	х
harvest	harvest of winter wheat	Х	Х	Х	Х
residue exportation	exportation of straw bale out of the				
_	field		x		x

97 The history of the various herbicide applications since 2008 is presented in Table 1. Cover crops were generally terminated by applying glyphosate. Herbicides were applied at spring during within winter 98 99 wheat cropping seasons and applied between one and three times, depending upon the success of the 100 weed control. Maize crop was managed with a single post-emergence application of herbicide. Lastly, 101 the FAR weed control itinerary (usually applied in Belgium) was applied during the sugar beet seasons, 102 which consists of repeated low-dose passes of a mixture of foliar herbicide (phenmedipham), an 103 activator (ethofumesate) and a residual herbicide (e.g. metamitron). Rapeseed and faba bean were 104 cultivated only once since establishment of the experiment and herbicides were applied following 105 business-as-usual management. More details about ingredients, modes of action and HRAC groups 106 (HRAC, 2024) are presented in Table 1.

107

2.2. Field data collection

108 A. Weed seedbank

109 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, 110 with five composite samples derived from four soil cores each (diameter=2cm) per plot. The 4 soil sub-111 112 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 113 total, 160 samples (4 treatments*4 replications* 5 samples/plot * 2 depths) underwent analysis using the 114 115 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 116

sieved and then spread on trays, over potting soil (1cm) and argex balls (2cm). The samples were 117 themselves spread with a maximum depth of 2cm to allow germination of all seeds (Mahé et al., 2021). 118 A PVC tube was inserted at the corner of the tray for regular irrigation. In addition, micro-sprinkler 119 120 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 121 when it was not possible to identify at species level), the weeds were removed. Species are named using 122 123 both the latin name and the EPPO code ("EPPO Global Database," n.d.) The emergence was monitored 124 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. 125 Between 08/04/2022 and 22/04/2022 the samples were not irrigated to force drought. On 22/04 the 126 127 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 128 November, the weed seedbank was installed in an unheated greenhouse to enhance autumnal 129 130 germination.

131

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

142

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

143 Biomass were measured at the nearest 0.01g

144 2.3. Weed diversity index

145 Species richness (number of species per quadrat) and Shannon-Weiner index were computed from 146 weed-related data. Indices were computed on weed seedbank observations and were calculated for in-147 season field data, at tillering and flowering of winter wheat. Shannon-Weiner index, which measure the 148 α -diversity was calculated by samples (seedbank) or quadrats (in-season) as follow:

149
$$H = 1 - \sum_{i=1}^{S} p_i * \ln(p_i)$$
 Eq.

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.

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2.4.Statistical analysis

Statistical analysis were perform using R statistical Software (V4.3.1; R Core Team, 2021). 153 154 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. 155 Response variable were expressed as a function of crop residue exportation, tillage intensity and their 156 interaction. For seedbank related-data, depth of sampling was also studied. In this case response variable 157 were expressed as a function of crop residue exportation, tillage intensity, depth of sampling and their 158 integrated triple interaction. Rows and column of the Latin square design were always included as 159 random intercept. Distribution was selected to meet the conditions. In addition, the model with the 160 lowest Akaike's Information Criterion (AIC) was chosen. All selected models are presented in 161 162 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. 163

164 Correlation between weed density, density of the two most prevalent weed species at flowering 165 and yield components were calculated with Spearman correlation due to violation of parametric 166 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 169 deviation). Path models are built upon both latent variables (LV) and manifest variables (MV). The first 170 171 LV, "Weed pressure", initially used the same MVs as Quinio et al. (2017), i.e. richness, Shannon index 172 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 173 MV "Residue exportation" (residue exported or maintained). The third latent variable refers to the 174 175 productivity; as proposed by Quinio et al., (2017) it was composed solely of the yield. Two MVs related 176 to yield components (number of spikes per m² and average biomass of grains per spike) were added to the model. 177

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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Figure 1: structural equation model for the relationship between productivity, yield components, weed pressure and soil management. Latent variables are in an oval colored in gray and manifest variable are in rectangle. A direct path is represented by a single arrow that directly connects two traits (e.g., Residue management and weed pressure). the dotted 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). The dominant species in the

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

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

201	Table 3: N	lumber of species	present in the	seedbank trial	and there weed	seedling density pr	roportion
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Species EPPO code	Species Latin names	number of individuals counted	% of total seedling counted
MATCH	Matricaria chamomilla L.	556	73.5
ALOMY	Alopecurus myosuroides L.	141	18.7
POLAV	Polygonum aviculare L.	33	4.4
CHEAL	Chenopodium album L.	5	0.7
SONAS	Sonchus asper (L.) Hill	5	0.7
CAPBP	Capsella bursa-pastoris (L.) Medick	2	0.3

CIRAR	Cirsium arvense (L.) Scop.	2	0.3
AETCY	Aethusa cynapium L.	2	0.3
ATXHA	Atriplex prostrata Boucher ex DC.	1	0.1
BROMO	Bromus hordeaceus L.	1	0.1
PAPRH	Papaver rhoes L.	1	0.1
EPIAD	Epilobium tetragonum L.	1	0.1
GALAP	Galium aparine L.	1	0.1
STEME	Stellaria media (L.) Vill.	1	0.1
VIOAR	Viola arvensis Murray	1	0.1
TARSS	Taraxacum sp.	1	0.1
VERHE	Veronica Hederifolia L.	1	0.1
ERICA	Erigeron canadensis L.	1	0.1

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 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.

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

217 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

219 Shannon index, with an average value of 0.55 in RT and 0.27 in CT (Figure 3). No significant difference

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

221



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.

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3.2. In-season weed community expression

The weed flora at the end of winter was mainly composed of Alopecurus myosuroides Huds. 228 (ALOMY) and Matricaria chamomilla L. (MATCH) (see in supplementary Table A.3 for all 229 species present). The timing of the weed survey (at wheat tillering - before herbicide application 230 - and at wheat flowering - after herbicide) had no impact on total weed density or on ALOMY 231 density. However, a 56% reduction was observed in MATCH between the two surveys 232 (pvalue=0.01213). CT reduced weed abundance (pvalue <0.0001) measured at tillering by 78% 233 234 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). However, no significant effect of 235 236 residue exportation (IN vs OUT) was observed.

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

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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.

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No effect of weed survey, tillage and residue exportation on the Shannon index was observed
(Figure 3). 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.

250 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 comparing the yield of quadrats with the highest $(50g.m^{-2})$ and lowest $(0g.m^{-2})$ weed biomass, 28% loss

253	of yield was recorded (see in supplementary Fig.B.1). Regarding the compartments of the plant, the
254	greatest correlation with total weed biomass was found with spike biomass (-0.57), then total biomass
255	(-0.55) and finally stem biomass (-0.47). At flowering, the impact of weeds on total crop biomass was
256	already noticeable (correlation of -0.36).

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). 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 and with the biomass of grains per spike. The same trends were observed for ALOMY, but no significant correlations were observed with MATCH.

263 Table 4: Significant correlation between yield components and total weed biomass and ALC	ЭМҮ.
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Yield component	Weed biomass	ALOMY biomass	MATCH biomass
	Cicilias	0101111055	
Yield	-0.58	-0.54	
Spike biomass	-0.57	-0.52	
Total biomass	-0.55	-0.52	
Spike density	-0.5	-0.44	
Stem Biomass	-0.47	-0.46	
Biomass at flowering	-0.36	-0.35	
Biomass grain per			
spike	-0.33	-0.34	
biomass per spike	-0.32	-0.35	

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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 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 trough the path analysis. The final model (Figure 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 ($\sigma_{direct} = -0.38$). Soil management did not exhibit any significant direct coefficient with yield components. On the contrary, weed pressure (expressed here 275 276 through the manifest variable of the abundance measured in terms of biomass) was the only significant factor impacting yield components. A grater path coefficient was found for spike density ($\sigma_{direct} = -0.44$) 277 compared to the biomass of grains per spike (- σ_{direct} =-0.33). Spike density was the most impactful 278 279 component on productivity with a $\sigma_{direct} = 0.84$, while the biomass of grain per spike has a σ_{direct} equalling 280 0.39 (Figure 5).

The indirect effect of weed pressure on productivity were mainly expressed by the effect on the number of spikes ($\sigma_{indirect} = -0.37$) and to a lesser extent through the biomass of grains per spike ($\sigma_{indirect}$ =-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 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.



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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
 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) (e.g., Productivity and Weed pressure).

293 Insignificant paths (pvalue>0.05) are indicated by "ns", statistical significance of the path coefficient at p-value≤0.05 is 294 indicated by "*".

- **4. Discussion**
- 296

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

297 Weed diversity was relatively low in all treatments. Only two species (ALOMY and MATCH) 298 dominated both the seedbank and the expressed weed flora. A slight increase in both Shannon diversity 299 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 300 301 no clear pattern in Shannon diversity was found. The results are in line with those of long-term trial 302 documented in the literature. Within the seedbank, Sosnoskie et al. (2006) showed a slightly higher 303 specie richness in RT compared to CT, with ca. 2 species more. However, they reported no difference 304 in the Shannon index between RT and CT, while in the present study, a significant, yet low, difference 305 was reported. The results regarding the expressed flora were in line with those of the long-term trial by 306 Plaza et al. (2011), where no differences in terms of Shannon diversity were observed and the same 307 trend of a slight increase in species richness (+1 species on average) in RT. It was hypothesized by the 308 authors that RT could allow a slight increase in the number of species due to a greater diversity of 309 ecological niches and germination opportunities. Complementary, results gain in the present study 310 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).

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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

321 dominant weeds (ALOMY and MATCH).

322 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). 323 However, in the current study, no major effect of residue exportation was observed on the weed 324 325 seedbank and the expressed weed density during the wheat cropping season. A potential explanation 326 could be associated to the dilution of surface residue, which only occurs within the 0-10 cm soil profile 327 in reduced tillage and would contribute to explain such results. Indeed, it is likely that the mulch effect 328 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 329 330 (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 331 as wheat or maize). 332

333 Chemical weed control did not result in a reduction of total weed density during the season. 334 Moreover, when examining the species individually, it became evident that the herbicide exhibited no 335 discernible impact on the ALOMY population. Following complementary laboratory analysis (data not 336 shown), it was determined that this ALOMY population demonstrated resistance to the spring herbicides 337 used during winter wheat cultivation (resistance to Acetolactate Synthase and Acetyl CoA Carboxylase). 338 The emergence of this resistance may be attributed to the recurrent use of identical active ingredients in 339 winter wheat (Zeller et al., 2021). Indeed, since 2008 the mode of action of Inhibition of Acetolactate 340 Synthase has always been applied in winter wheat and Acetyl CoA Carboxylase was applied in 2014, 2020 and 2022 (Table 1). Conventional tillage (CT) proved to be an efficient method for managing the 341 342 ALOMY population in comparison to reduced tillage (RT). Zeller et al. (2021) demonstrated that 343 ALOMY was reduced by 70 to 80% when rotational ploughing was implemented. Weed biomass, on 344 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 345 biomass among the tillage and residue exportation methods. One might have thought that the non-346 347 significant effect was due to a higher biomass per ALOMY in CT than in RT, but no significant effect

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

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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.

356 Weed pressure was found to be also significantly linked with yield (using both ANOVA and path 357 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 358 359 significantly correlated with yield losses. However, ALOMY was found to explain the majority of the loss (-28% between an area without naturally ALOMY and an area with 50g of ALOMY m^{-2 s}, Fig.B.2). 360 361 ALOMY is a species that is phylogenetically close to wheat and shares similarities in its development, 362 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 363 364 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 365 significant impacts on yield components. 366

367 Among the yield components studied, the one that explained yield the best was spike density, in 368 agreement with the literature (Lenoir et al., 2023; Slafer et al., 2014). This component exhibited the 369 highest path analysis coefficient with weed pressure. It was confirmed in this study that the competition 370 induced by weeds leads to a loss of yield, mainly by reducing the wheat's capacity to produce spikes, as 371 suggested in previous studies (Adeux et al., 2019; Welsh et al., 1999). This confirms that competition 372 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 373 374 interesting to monitor tillers dynamic earlier in the season to confirm this hypothesis. Finally, weed375 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 376 377 that a weed community composed almost exclusively of ALOMY had no effect on the 1,000-kernel 378 weight but did have an effect on the number of grains per spike, suggesting that the competition 379 generated by ALOMY takes place until wheat flowering. The indirect effect of tillage management on 380 yield through weed competition could explain the earlier observation reported by Hiel et al. (2018) on 381 the same experimental site, who did not systematically observed an impact of soil management over the 382 year but who reported a -3.4% cumulative yield decrease between 2010 and 2015.

- **5.** Conclusion
- 384

385 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 386 weeds. The lack of link between weed flora (diversity and abundance) monitored through the seedbank 387 388 or during the cropping season of winter wheat showed that, in a rotation based on wheat, residue 389 exportation was of little importance in the context of this study. The lack of effect of maintaining 390 residues could be explained by a dilution of crop residues in the upper soil profile that still occurs in 391 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 392 393 higher compared to conventional tillage) but resulted mostly in an increase of weed density. While this 394 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 395 396 facilitate the development of ALOMY, a very competitive species that is detrimental to yield. This 397 management technique might favour the appearance of resistance -as observed in this trial, especially in 398 winter wheat-based cropping systems. Above all, it highlights the problem of long-term sustainable 399 management of the weed flora. Reduced tillage management technique might indirectly lead to higher 400 yield losses through poor control of the weed flora in systems based on wheat cultivation. In this regard, 401 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 402
- 403 systems management, including systems with a higher proportion of spring crops.

6. Declaration of interest 404

The authors declare no conflicts of interest. 405

406

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