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Weed Biomass Responses to Tillage Systems in a Long-Term Mediterranean Cereal–Legume Experiment

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Abstract

In Mediterranean dryland agroecosystems, conservation tillage is increasingly adopted, yet its long-term effects on weed biomass within cereal–legume rotations remain poorly quantified. This study evaluated the effects of conventional tillage (CT), minimum tillage (MT), and no-tillage (NT) on aboveground weed biomass over a seven year period (2011–2017) within a long-term (established in 1985) cereal–legume rotation experiment in central Spain. Weed biomass was sampled annually prior to herbicide application and analyzed using linear mixed-effects models to assess the effects of crop type, tillage system, year, and their interactions. A total of 36 weed species were recorded, with annual broadleaf species accounting for most of the biomass. Mean weed biomass was greater in legume phases than in wheat, and tillage system significantly affected biomass. Minimum tillage resulted in greater weed biomass than CT or NT, particularly in legume crops. A significant crop type × tillage interaction indicated that tillage effects on weed biomass were crop-dependent, with stronger differences among tillage systems in legumes than in wheat. These results demonstrate that weed biomass responses to tillage cannot be generalized across crops, highlighting the importance of considering crop–tillage combinations when designing weed management strategies in Mediterranean cereal–legume rotations.

Keywords: annual broadleaf weeds; conservation tillage; integrated weed management; minimum tillage; no-tillage; Mediterranean dryland

1. Introduction

Weed management remains a critical component of sustainable agricultural practices, particularly in agroecosystems where resource availability is limited and climatic variability is high. Weeds compete with crops for essential resources such as light, water, and nutrients, leading to reduced yields, impaired crop quality, and significant economic losses worldwide [1]. In addition to direct competition, weeds may act as hosts for pests and pathogens, further exacerbating their negative impact on cropping systems. Traditionally, tillage has been employed as a primary method for weed control by physically destroying emerged weeds, modifying soil structure, redistributing weed seeds within the soil profile, and influencing seed dormancy and germination conditions. However, the choice of tillage system, conventional, minimum, or no-tillage, plays a decisive role in shaping soil physical and biological properties, erosion rates, and long-term weed population dynamics.

In the Mediterranean basin, conventional tillage (CT) has been practiced for centuries as a key agronomic tool for weed control, particularly in rainfed systems characterized by irregular precipitation and frequent drought events. CT allows the removal of early weed cohorts that emerge prior to crop sowing and facilitates seedbed preparation [2,3]. This practice strongly influences the vertical distribution of weed seeds in the soil profile [4,5], often burying seeds to depths where germination is reduced or delayed. Consequently, CT affects weed demography, abundance, and species richness [6–8], as well as weed community composition and functional diversity [9–11]. Despite its effectiveness in short-term weed suppression, CT is increasingly questioned due to its association with soil degradation, organic matter loss, and increased susceptibility to erosion.

More recently, the introduction of conservation agriculture (CA) techniques, based on minimum soil disturbance and often combined with crop residue retention, has progressively replaced CT in many regions as a strategy to enhance agricultural sustainability without compromising land productivity [12,13]. Conservation agriculture practices such as no-tillage (NT) and minimum tillage (MT) modify soil biological activity, nutrient cycling, and water infiltration, thereby promoting increased water and nutrient use efficiency [14,15]. Nevertheless, these systems have also been widely reported to increase weed density and biomass, particularly during the transition period from conventional to conservation practices [3,16]. Changes in weed flora under CA are often characterized by a greater prevalence of perennial species and annual grasses adapted to surface seed placement and reduced soil disturbance.

In Spain, cereal–legume rotations have historically been a cornerstone of Mediterranean dryland farming systems, contributing to soil fertility maintenance and yield stability under low-input conditions. However, during recent decades, these traditional rotations have largely been replaced by simplified systems dominated by continuous cereals or cereal–fallow sequences [17]. This shift has been driven by agricultural mechanization, reduced labor availability, market pressures, and the widespread adoption of herbicide-based weed control strategies. Although simplified rotations may enhance short-term productivity and operational efficiency, they are frequently associated with declining soil fertility, reduced agroecosystem resilience, and an increased risk of herbicide-resistant and difficult-to-control weed species in Spanish drylands. Restoring cereal–legume rotations is increasingly recognized as a key strategy for advancing sustainable agriculture, as these systems provide biologically fixed nitrogen, disrupt weed, pest, and disease cycles, improve soil structure, and reduce dependence on synthetic fertilizers and herbicides [18]. However, the interaction between crop rotation and tillage system remains complex. Several studies have reported that conservation tillage may increase the density or biomass of perennial weeds and certain annual grasses [19,20]. For example, in a 23-year field experiment, Hernández-Plaza et al. [6] observed greater weed species richness under minimal tillage compared to conventional plowing, while Demjanová et al. [21] reported significantly lower weed biomass under CT than under reduced tillage systems. Conversely, recent evidence from semi-arid cereal systems in central Spain indicates that weed control efficacy and community dynamics are strongly influenced by the combined effects of cropping system and tillage intensity, with no-tillage systems sometimes suppressing weeds more effectively than organic or reduced-input approaches when integrated within diversified rotations [22].

In this context, long-term field experiments are essential to disentangle the cumulative effects of tillage practices and crop rotation on weed biomass and community dynamics under Mediterranean conditions. In this study, we present the results of a 7-year experiment examining the impact of conventional tillage (CT), minimum tillage (MT), and no-tillage (NT) systems on weed biomass within a long-term cereal–legume rotation. Specifically, our objectives were to (1) assess the long-term effects of different tillage systems on weed biomass, and (2) evaluate the interaction between tillage practices and crop type. By addressing these objectives, this study aims to contribute to the development of integrated weed management strategies that balance effective weed control with soil conservation and long-term system sustainability in Mediterranean dryland agriculture.

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2. Results and Discussion

A total of 36 weed species were identified during the experimental period, reflecting a moderately diverse weed community typical of Mediterranean rainfed cropping systems (Table 1). The composition of this flora is commonly found in cereal and legume fields in Spain [6,23,24]. The weed flora was strongly dominated by annual broadleaf (dicotyledonous) species, which accounted for the majority of the total aboveground weed biomass. This dominance reflects both the climatic conditions of the study area, characterized by cool, moist winters followed by dry summers, and the long-term management practices applied in the system. Among all species recorded, *Descurainia sophia* (L.) Webb ex Prantl, *Papaver rhoeas* (L.), and *Veronica hederifolia* (L.) were consistently the most abundant and widely distributed across all tillage systems, suggesting a high degree of ecological adaptability and competitive ability under contrasting soil disturbance regimes. Only two grass species were identified during the study period, namely *Hordeum murinum* (L.) and *Lolium rigidum* (Gaudin) (Table 1), and their contribution to total weed biomass was comparatively low. The limited presence of grass weeds contrasts with findings from other Mediterranean studies where annual grasses, particularly *L. rigidum*, often dominate under reduced or no-tillage systems. While previous studies primarily describe the weed flora characteristic of Mediterranean cereal-based systems at the regional scale, the present study emphasizes overall species composition pooled across tillage systems, allowing for an integrated assessment of weed community structure under long-term rotational management .

Several Mediterranean studies have reported that reduced tillage favors annual dicot species adapted to shallow seed burial and surface seedbanks, traits that enhance rapid emergence and early-season competitiveness [21,25]. Our results are consistent with this general pattern, as dominant species such as *D. sophia* and *P. rhoeas* are well adapted to germination from the upper soil layers and can take advantage of reduced soil disturbance. However, in contrast to Dorado and López-Fando [26], who reported that conservation tillage favored *L. rigidum* and *Anthemis arvensis* (L.), these species were not abundant in our experiment. This discrepancy likely reflects the specific crop rotation and long-term weed management history of the El Encín experimental site, where repeated cereal–legume rotations combined with sustained herbicide programs may have exerted strong selection pressure favoring winter annual broadleaf species over annual grasses.

Additionally, differences in soil texture, fertility, and moisture dynamics, as well as microclimatic conditions and initial seedbank composition, may further explain the contrasting weed assemblages observed among studies conducted under conservation tillage. These factors highlight the importance of site-specific conditions in shaping weed community responses to tillage systems and underscore the need for long-term experiments to capture cumulative management effects on weed species composition in Mediterranean agroecosystems.

Table 1. Weed species recorded in the experimental plots during the study years, classified by group, dicot (D) or grass (G).

Weed species	group
Amaranthus blitoides S. Watson	D
Anacyclus clavatus (Desf.) Pers.	D
Asperugo procumbens L.	D
Atriplex patula L.	D
Bassia scoparia (L.) Voss.	D
Bombycilaena erecta (L.) Smolj.	D
Capsella bursa-pastoris (L.) Medik.	D
Cardaria draba (L.) Desv.	D
Cnicus benedictus L.	D

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Chenopodium album L.	D
Convolvulus arvensis L.	D
Conyza canadensis (L.) Cronq.	D
Descurainia sophia (L.) Webb. Ex Prantl.	D
Epilobium brachycarpum C. Presl	D
Fumaria officinalis L.	D
Fumaria parviflora Lam.	D
Galium tricornutum Dandy	D
Heliotropium europaeum L.	D
Hordeum murinum L.	G
Hypecoum imberbe Sm.	D
Lactuca serriola L.	D
Lamium amplexicaule L.	D
Lolium rigidum Gaudin	G
Malva sylvestris L.	D
Papaver rhoeas L.	D
Papaver hybridum L.	D
Polygonum aviculare L.	D
Portulaca oleracea L.	D
Roemeria hybrida (L.) DC.	D
Salsola kali L.	D
Sisymbrium irio L.	D
Sonchus asper (L.) Hill	D
Sonchus oleraceus L.	D
Trigonella polyceratia L.	D
Urtica urens L.	D
Veronica hederifolia L.	D

Mean total aboveground weed biomass differed markedly between crop types, averaging 15.40 ± 54.29 g m^{-2} in wheat and 30.45 ± 46.34 g m^{-2} in the legume phase (mean ± SD). From an agronomic and management perspective, these results indicate that crop choice within the rotation has a direct and measurable effect on weed pressure, which is highly relevant for on-farm weed management decisions. The consistently higher weed biomass observed in legume crops suggests that these phases constitute critical periods for weed proliferation and seedbank replenishment, requiring increased management attention by farmers. Previous studies have shown that grain legumes are generally less competitive with weeds due to slower early growth, lower biomass accumulation, and more open canopies that allow greater light penetration to the soil surface [27,28]. For farmers implementing integrated weed management (IWM), this reduced competitiveness implies that legumes alone cannot be relied upon for weed suppression. Instead, legume phases should be strategically supported with complementary practices such as increased seeding rates, narrower row spacing, early mechanical control where feasible, or carefully timed herbicide applications to prevent early weed establishment. Consistent with these findings, Dorado et al. [22] demonstrated that pea cultivation in reduced-input or organic rotations led to higher weed biomass than barley, while continuous no-till barley combined with herbicide use achieved the highest weed suppression. For growers, this highlights the practical value of integrating competitive cereal crops into rotations as a suppressive phase that can help reduce overall weed pressure. Cereal phases may be used intentionally to "reset" weed populations following legume crops by limiting weed growth and reducing seed production. Research comparing grass- and legume-based systems has consistently shown lower weed biomass during grass-dominated phases due to faster establishment, greater early biomass production, and improved ground cover [29,30]. In practical terms, farmers can exploit these traits by prioritizing well-adapted, vigorous cereal cultivars, optimizing sowing dates, and ensuring adequate fertilization to enhance early crop competitiveness. Although many of these studies focus on cover crops, the same principles apply to cereal–legume rotations in Mediterranean dryland systems. Overall, these results underline the importance of viewing crop rotation design as an active weed management tool rather than solely a soil fertility strategy. Effective IWM should combine competitive cereal phases, appropriate tillage practices, and diversified control tactics tailored to the weaker competitive ability of legume crops [31]. By adopting such an integrated approach, farmers can reduce long-term weed pressure, decrease reliance on herbicides, and improve the sustainability and resilience of cereal–legume cropping systems under Mediterranean conditions. Cereal crops play a key role in this strategy due to higher seeding rates, greater plant densities, and strong tillering capacity further enhance early ground cover and light interception, which are key determinants of crop competitive ability against weeds. By rapidly occupying available space and capturing aboveground resources, grass crops effectively limit weed emergence and growth during the critical early stages of crop establishment. This competitive advantage is particularly relevant in Mediterranean environments, where early-season resource pre-emption can strongly influence final weed biomass.

In addition to their strong aboveground competitiveness, grasses contribute to weed suppression through residue-mediated effects. The higher carbon-to-nitrogen (C:N) ratio of grass residues results in slower decomposition rates and longer persistence of crop residues on the soil surface. This sustained soil cover can physically impede weed emergence, buffer soil temperature fluctuations, and reduce light penetration to the soil surface, all of which are known to suppress weed germination and early growth. Moreover, residue retention can indirectly affect weed communities by modifying soil moisture dynamics and microbial activity, further reinforcing the suppressive effect of grass-based cropping systems.

Conversely, legumes influence weed dynamics through different mechanisms. While their ability to fix atmospheric nitrogen provides substantial agronomic benefits to subsequent crops, the nitrogen released during residue decomposition or root turnover can also become available to weeds, potentially enhancing weed growth, biomass accumulation, and seed production [25,32]. This nutrient-mediated stimulation of weeds may partially offset the rotational benefits of legumes if not properly managed, particularly in systems with reduced tillage where residues remain on the soil surface. Consequently, these contrasting functional traits of grasses and legumes highlight the importance of integrating crop functional diversity into rotation design as a deliberate strategy to balance soil fertility benefits with effective long-term weed suppression.

ANOVA results (Table 2) showed that crop type had a highly significant effect on weed biomass ($F_{1,73} = 33.18$, $p < 0.0001$), clearly indicating that the choice between wheat and legume crops strongly influenced weed biomass levels across the study period. On average, legume crops supported substantially higher weed biomass than wheat, highlighting the lower competitive ability of legumes and their greater vulnerability to weed infestation under Mediterranean dryland conditions. This result confirms that crop identity is a dominant driver of weed biomass within cereal–legume rotations and should be explicitly considered when designing weed management strategies. The effect of year, nested within crop type, was also statistically significant ($F_{5,73} = 2.88$, $p = 0.02$), indicating considerable interannual variability in weed biomass. This variability likely reflects year-to-year differences in rainfall amount and distribution, temperature patterns, and the timing of precipitation events, which are known to strongly influence weed emergence and growth in semi-arid environments [33]. These findings emphasize the importance of long-term experiments for capturing the cumulative and variable nature of weed responses to management practices, as short-term studies may fail to account for climatic variability inherent to Mediterranean systems. Tillage system had a strong and highly significant effect on weed biomass ($F_{2,73}$ = 14.93, $p < 0.0001$), underscoring the critical role of soil disturbance intensity in shaping weed dynamics. Differences among conventional, minimum, and no-tillage systems reflect contrasting effects on weed seed burial, seedling mortality, and soil surface conditions that regulate weed emergence and survival. These results reinforce the concept that tillage is a powerful management lever within integrated weed management frameworks. Importantly, the interaction between crop type and tillage system was statistically significant ($F_{2,73}$ = 3.87, p = 0.02), indicating that the effect of tillage on weed biomass was not uniform across crop types. Specifically, weed biomass under minimum tillage was substantially higher in legume crops than in wheat, suggesting that reduced soil disturbance combined with the lower competitive ability of legumes creates favorable conditions for weed establishment and growth. In contrast, differences among tillage systems were less pronounced in wheat, particularly under no-tillage, where strong crop competitiveness and residue-mediated suppression appeared to buffer the effects of reduced disturbance on weed biomass.

These interaction effects highlight the context-dependent nature of weed management outcomes and demonstrate that tillage practices cannot be evaluated in isolation from crop type. From a management perspective, the results suggest that minimum tillage may require additional or more carefully timed weed control measures when applied to legume crops, whereas wheat grown under no-tillage can achieve relatively stable weed suppression. Overall, the significant crop × tillage interaction underscores the need for integrated, system-specific weed management strategies that jointly consider crop competitiveness, tillage intensity, and climatic variability.

Table 2. Analysis of variance (ANOVA) table for the linear model testing effects of crop type (wheat or legume), year, tillage system (CT, MT, NT) and the interaction crop type x tillage system on aboveground total weed biomass.

Source	df	F	p-value
Crop type (C)	1	33.18	>0.0001
Year (Crop type)	5	2.88	0.0197
Tillage (T)	2	14.93	>0.0001
C x T	2	3.87	0.0253
Residual	73		

Among the tillage systems, minimum tillage (MT) consistently resulted in the highest levels of weed biomass in both legume and wheat crops, with mean values of 40.77 ± 50.47 g m^{-2} and 18.55 ± 61.21 g m^{-2}, respectively, clearly exceeding those observed under conventional tillage (CT) and no-tillage (NT) systems (Table 3). This pattern indicates that MT created particularly favorable conditions for weed establishment and growth, regardless of crop type, although the magnitude of the effect was notably greater in legume crops. The large standard deviations observed further suggest a heterogeneous spatial distribution of weeds under MT, likely reflecting localized differences in seedbank density and microenvironmental conditions.

These findings are consistent with those reported by Santín-Montanyá et al. [34], who observed that conservation tillage systems increased weed density and species richness in cereal-based systems in central Spain. In their study, certain indicator species, such as *Cardaria draba* (L.) and *Silene vulgaris* (Moench.) Garcke, were closely associated with tillage intensity and interannual rainfall variability, highlighting the interaction between soil disturbance and climatic conditions in shaping weed communities. The present findings support this interpretation and suggest that MT may enhance weed biomass by maintaining a large proportion of viable seeds in the upper soil layers while simultaneously providing favorable conditions for germination and early growth.

Similar trends have been documented in previous studies [35,36], which reported that MT systems, particularly those comparable to chisel ploughing, tend to promote higher weed emergence due to shallow seed burial and limited seed mortality. Under MT, weed seeds are often redistributed within the topsoil rather than being buried at depths that inhibit germination, as occurs under CT, or left undisturbed on the soil surface, as in NT systems. This intermediate level of disturbance can therefore maximize weed emergence by combining sufficient soil-seed contact with favorable moisture and temperature conditions.

From a management perspective, these results suggest that MT may represent a critical risk scenario for weed proliferation in Mediterranean dryland systems if not complemented with additional weed control measures. While MT is often adopted to reduce soil erosion and conserve moisture, its tendency to increase weed biomass highlights the need for integrated weed management strategies, such as enhanced crop competitiveness, residue management, or targeted herbicide applications, particularly in legume phases. In contrast, NT systems, which maintained lower weed biomass levels, appear to offer greater potential for long-term weed suppression when combined with competitive crops and consistent management practices.

Table 3. Average biomass (± standard deviation) (g/m2) in the three-tillage systems.

Tillage system	Legume	Wheat
Minimum tillage	40.77±50.47	18.55±61.21
Conventional tillage	26.23±36.72	14.03±61.21
No-tillage	24.34±50.47	13.62±36.72

Overall, our results indicate that minimum tillage (MT) systems require a greater reliance on integrated weed management than either conventional tillage (CT) or no-tillage (NT). Minimum tillage promotes weed emergence by inducing shallow soil disturbance that favors germination from the upper soil layers, while lacking both the deep seed burial effect characteristic of CT and the residue-mediated suppression typically observed under NT. This vulnerability is particularly pronounced in legume crops, where lower inherent crop competitiveness further amplifies weed biomass under MT conditions. Consequently, MT systems cannot rely on tillage alone for weed suppression and must be supported by complementary management tactics. The need for such integration is consistent with Kurstjens [37] and with recent evidence showing that tillage systems commonly grouped under conservation agriculture, including minimum tillage, can differentially affect weed functional traits, emergence patterns, and community assembly. These differential responses increase the importance of combining cultural, mechanical, and chemical control methods to achieve effective and resilient weed management [38,39].

In summary, weed biomass in Mediterranean cereal–legume rotations was strongly influenced by both crop type and tillage system, with minimum tillage generally associated with higher weed biomass, particularly during legume phases. This pattern likely reflects a combination of mechanisms reported in previous studies, including reduced seed burial, more favorable germination conditions near the soil surface, and lower crop competitive ability in legumes, although these processes were not directly quantified in the present study. Ultimately, decisions regarding tillage-based weed management strategies should account not only for their effects on weed biomass but also for broader agronomic, environmental, and economic considerations, as highlighted in previous research [22,40]. This reinforces the need for integrated, system-specific approaches [39] aimed at balancing effective weed suppression with soil conservation goals and long-term farm profitability.

3. Materials and Methods

3.1. Experimental Site and Design

This study was carried out at El Encin Experimental Station (40º 29′N; 3º 22′W, Alcala de Henares, Madrid, Spain, 610 a.s.l.). It was established in 1985 to determine the long-term effects of conventional vs. conservation tillage techniques in a cereal–legume rotation system typical of central Spain. Characterized by the Mediterranean semi-arid climate with mild, humid winters and dry, hot summers. Mean annual precipitation is 445 mm and the mean annual temperature is 13.8 ºC. Both the tillage treatments (CT, MT, NT) and the cereal–legume rotation have been applied consistently

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since the experiment was established. The soil is an Alfisol-Xeralf [41] with a loam texture, a pH of 7.8, and 1.2% organic matter.

Three tillage systems were compared: conventional tillage (CT; moldboard plowing with multiple passes before planting), minimum tillage (MT; chisel plowing or cultivator use with reduced soil disturbance) and no tillage (NT; direct planting into the residue of the previous crop without soil disturbance, except by the planter). In this experiment, minimum tillage referred to reduced soil disturbance using a chisel plow or cultivator, but no specific residue retention percentage was targeted. Tillage intensity, including the degree of soil disturbance and surface residue cover, was not quantitatively measured in this study. Tillage treatments were defined operationally based on the implements used and the long-term management history of the experiment. The cropping system followed a winter wheat (*Triticum aestivum* L.) and legume rotation. Winter wheat was grown in 2011, 2013, 2015, and 2017, while the legume phase—either vetch (*Vicia sativa* L.) or pea (*Pisum sativum* L.), cultivated for forage—was grown in 2012, 2014, and 2016. During the wheat years, planting dates ranged from October 30 to December 19. Fertilizers were applied at sowing (28 kg N, 37 kg P2O5, 26 kg K2O ha-1) and at mid-tillering (53 kg N ha−1). Weed control was conducted using a post-emergence herbicide treatment (0.2 kg a.i. ha−1 ioxynil + 0.2 kg a.i. ha−1 bromoxynil + 1.012 kg a.i. ha−1 mecoprop) at the tillering stage following standard management practices, herbicide efficacy was not quantitatively assessed. Leguminous crops were planted between November 6th and January 19th. Fertilizers were applied at planting time (14 kg N, 14 kg P2O5, and 14 kg K2O ha−1 for vetch and 19 kg N, 38 kg P2O5, and 71 kg K2O ha−1 for pea). For the NT treatment, straw and stubble from the previous wheat crop were destroyed by chopping in advance to sowing the leguminous crop.

The experiment followed a complete randomized block design with four replicates. Tillage treatments were randomly assigned to plots (20 m x 40 m) within each block.

3.2. Sampling and Data Collection

Aboveground weed biomass was sampled annually between 2011 and 2017, 26 years after the experiment was established by clipping all weed vegetation at the soil surface within each sampling quadrat. Each year, ten quadrats (30 × 33 cm) per plot were harvested between mid-February and mid-April, corresponding to the mid-tillering stage of wheat and the stem elongation stage of the legume crop. Quadrats were placed along an M-shaped transect at approximately 15 m intervals, at least 3 m from plot borders. Sampling occurred prior to herbicide application.

Samples were oven-dried at 80 °C for 48 h until constant mass and then weighed. Biomass of dicotyledonous and grass weed species was analyzed separately. Weed species were identified in the field at the time of sampling. Each species collected from a quadrat was dried separately to obtain species-level biomass values; however, only total biomass was used in statistical analyses. Only aboveground biomass was recorded; weed density was not assessed in this study.

3.3. Statistical Analysis

All analyses were conducted in the R statistical environment, version 4.5.0 [42]. Total aboveground weed biomass was analyzed using linear mixed-effects models to examine the various components of the experimental design. First, a repeated-measures model was fitted with year, tillage system, and their interaction as fixed effects, and block as a random effect, to evaluate temporal variation and overall tillage responses. Covariance structures tested for the repeated year effect included no correlation, first-order autoregressive [AR(1)], compound symmetry, unstructured, and heterogeneous variance structures, with the Bayesian Information Criterion (BIC) used to identify the best-fitting model.

Second, to assess whether tillage effects differ between crop phases within the rotation, a separate model was fitted including crop type (wheat or legume), tillage system, and their interaction as fixed effects, with block as a random effect. In this context, the term “treatment” refers to the combination of crop type and tillage system.

Exploratory analysis indicated consistently lower weed biomass in wheat years (2011, 2013, 2015, 2017) than in legume years (2012, 2014, 2016), reflecting the crop rotation sequence. Subsequent models included crop type, year nested within crop type, treatment, and the crop type × treatment interaction. Significant interactions were analyzed within crop type.

The heteroscedastic model yielded the lowest BIC (data not shown) and was selected for final analyses. The model including crop type met assumptions of normality and homoscedasticity. Block variance was negligible relative to residual variance and was excluded from the final model.

4. Conclusions

This study shows that weed biomass in Mediterranean cereal–legume rotations is strongly influenced by the interaction between crop type and tillage system. Legume phases consistently exhibited higher weed biomass than wheat, confirming their lower competitive ability and their role as critical periods for weed proliferation within the rotation.

Minimum tillage resulted in the highest weed biomass, particularly in legume crops, likely due to shallow soil disturbance that favors weed emergence while lacking both deep seed burial (conventional tillage) and residue-mediated suppression (no-tillage). In contrast, no-tillage maintained relatively low and stable weed biomass, especially when combined with competitive wheat crops.

These findings highlight that tillage effects on weed biomass cannot be generalized across crops and must be evaluated within a crop-specific context. Effective weed management in Mediterranean dryland systems therefore requires integrated strategies that combine appropriate tillage practices with crop rotation design, emphasizing competitive cereal phases to enhance long-term weed suppression and system sustainability.

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Data Availability Statement: Data will be provided upon request to the corresponding author.

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Abbreviations

The following abbreviations are used in this manuscript:

CT	Conventional tillage
MT	Minimum tillage
NT	No-tillage
CA	Conservation agriculture
BIC	Bayesian Information Criterion

References

1. Zimdahl, R.L. *Weed–Crop Competition: A Review*, 2nd ed.; Blackwell Publishing: Ames, Iowa, 2004; pp. 220

2. Fernandez-Quintanilla, C.; Navarrete, L.; Gonzalez-Andujar, J.L.; Fernandez, A.; Sanchez, M.J. Seedling recruitment and age specific survivorship and reproduction in populations of Avena sterilis ssp. ludoviciana. *J. Appl. Ecol.* **1986**, *23*, 945-955. https://doi.org/10.2307/2403946
3. Chauhan, B.S.; Singh, R.G.; Mahajan, G. Ecology and management of weeds under conservation agriculture: A review. *Crop Prot.* **2012**, *38*, 57-65. https://doi.org/10.1016/j.cropro.2012.03.010
4. Gonzalez-Andujar, J.L. A matrix model for the dynamic population and vertical distribution of weed seedbanks. *Ecol. Model.* **1997**, *97*, 117-120. https://doi.org/10.1016/S0304-3800(96)00078-6
5. Colbach, N.; Roger-Estrade, J.; Chauvel, B.; Caneill, J. Modelling vertical and lateral seed bank movements during mouldboard ploughing. *Eur. J. Agron.* **2000**, *13*, 111-124. https://doi.org/10.1016/S1161-0301(00)00069-1
6. Hernandez Plaza, E.; Kozak, M.; Navarrete, L.; Gonzalez-Andujar, J.L. Tillage system did not affect weed diversity in a 23-year experiment in Mediterranean dryland. *Agric. Ecosyst. Environ.* **2011**, *140*, 102-105. https://doi.org/10.1016/j.agee.2010.11.016
7. Eager, E.; Haridas, C.; Pilson, D.; Rebarber, R.; Tenhumberg, B. Disturbance Frequency and Vertical Distribution of Seeds Affect Long-Term Population Dynamics: A Mechanistic Seed Bank Model. *Am. Nat.* **2013**, *182*, 180–190. https://doi.org/10.1086/670987
8. Pardo, G.; Cirujeda, A.; Perea, F.; Verdú, A.M.C.; Mas, M.; Urbano, J.M. Effects of Reduced and Conventional Tillage on Weed Communities: Results of a Long-Term Experiment in Southwestern Spain. *Adv. Weed Sci.* **2019**, *37*. https://doi.org/10.1590/S0100-83582019370100152
9. Légère, A.; Stevenson, F.C.; Benoit, D.L. The Selective Memory of Weed Seedbanks after 18 Years of Conservation Tillage. *Weed Sci.* **2011**, *59*, 98-106. https://doi.org/10.1614/WS-D-10-00092.1
10. Sans, F.X.; Berner, A.; Armengot, L.; Mäder, P. Tillage effects on weed communities in an organic winter wheat–sunflower–spelt cropping sequence. *Weed Res.* **2011**, *51*, 413-421. https://doi.org/10.1111/j.1365-3180.2011.00859.x
11. Hernandez Plaza, E.; Navarrete, L.; Gonzalez-Andujar, J.L. Intensity of soil disturbance shapes response trait diversity of weed communities: the long-term effects of different tillage systems. *Agric. Ecosyst. Environ.* **2015**, *207*, 101-108. https://doi.org/10.1016/j.agee.2015.03.031
12. Jurado-Exposito, M.; Lopez-Granados, F.; Gonzalez-Andujar, J.L.; Garcia-Torres, L. Characterizing population growth rate of Convolvulus arvensis in wheat-sunflower no-tillage systems. *Crop Sci.* **2005**, *45*, 2106-2112. https://doi.org/10.2135/cropsci2004.0502
13. Nichols, V.; Verhulst, N.; Cox, R.; Govaerts, B. Weed dynamics and conservation agriculture principles: A review. *Field Crops Res.* **2015**, *183*, 56-68. https://doi.org/10.1016/j.fcr.2015.07.012
14. Gonzalez-Sanchez, E.; Veroz-Gonzalez, O.; Blanco-Roldan, G.; Marquez-Garcia, F.; Carbonell-Bojollo, R. A renewed view of conservation agriculture and its evolution over the last decade in Spain. *Soil Tillage Res.* **2014**, *146*, 204-212. https://doi.org/10.1016/j.still.2014.10.016
15. Cordeau, S. Conservation agriculture and agroecological weed management. *Agronomy* **2022**, *12*, 867. https://doi.org/10.3390/agronomy12040867
16. Tuesca, D.; Nisensohn, L.; Bocanelli, S.; Torres, P.; Lewis, P. Weed seedbank and vegetation dynamics in summer crops under two contrasting tillage regimes. *Community Ecol.* **2004**, *5*, 247-255. https://doi.org/10.1556/ComEc.5.2004.2.12
17. Ministerio de Agricultura, Pesca y Alimentación. *Encuesta sobre Superficies y Rendimientos de Cultivos (ESYRCE): resultados 2015*. Madrid, Spain, 2016.
18. Alarcon, R.; Hernández Plaza, E.; Navarrete, L.; Sánchez, M.J.; Escudero, A.; Hernanz, J.L.; Sánchez-Giron, V.; Sánchez, A.M. Effects of no-tillage and non-inversion tillage on weed community diversity and crop yield over nine years in a Mediterranean cereal-legume cropland. *Soil Tillage Res* **2018**, *179*, 54-62. https://doi.org/10.1016/j.still.2018.01.014
19. Streit, B.; Rieger, S.B.; Stamp, P.; Richner, W. Weed populations in winter wheat as affected by crop sequence, intensity of tillage and time of herbicide application in a cool and humid climate. *Weed Res.* **2003**, *43*, 20-32. https://doi.org/10.1046/j.1365-3180.2003.00310.x

20. Santín-Montanyá, M.; Martín-Lammerding, D.; Walter. I.; Zambrana, E.; Tenorio, J. Effects of tillage, crop systems and fertilization on weed abundance and diversity in 4-year dry land winter wheat. *Eur J Agron* **2013**, *48*, 43-49. https://doi.org/10.1016/j.eja.2013.02.006
21. Demjanová, E.; Macák, M.; Đalović, I.; Majerník, F.; Týr, Š.; Smatana, J. Effects of tillage systems and crop rotation on weed density, weed species composition and weed biomass in maize. *Agron. Res.* **2009**, *7*, 785-792.
22. Dorado, J.; Barroso, J.; Peña, J.M.; Luna, I.M.; Fernández-Quintanilla, C. Weed control efficacy and economic profitability of three contrasting cropping systems in semi-arid rainfed zones. *Weed Sci.* **2025**, *73*, 1-13. https://doi.org/10.1017/wsc.2025.10052
23. Gonzalez-Andujar, J.L.; Saavedra, M. Spatial distribution of annual grass weed populations in winter cereals. *Crop Prot.* **2003**, *22*, 629-633. https://doi.org/10.1016/S0261-2194(02)00247-8
24. Izquierdo, J.; Blanco-Moreno, J.M.; Chamorro, L.; Gonzalez-Andujar, J.L.; Sans, F.X. Spatial distribution of weed diversity within a cereal field. *Agron. Sustain. Dev.* **2009**, *29*, 491-496. https://doi.org/10.1051/agro/2009009
25. Scordia, D.; Guarnaccia, P.; Calderone, F.; Maio, A.; La Malfa, T.; Scavo, A.; Gresta, F. Adoption of cereal–legume double cropping toward more sustainable organic systems in the Mediterranean area. *Agronomy* **2024**, *14*, 772. https://doi.org/10.3390/agronomy14040772
26. Dorado, J.; López-Fando, C. The effect of tillage system and use of a paraplow on weed flora in a semiarid soil from central Spain. *Weed Res.* **2006**, *46*, 424-431. https://doi.org/10.1111/j.1365-3180.2006.00526.x
27. Bybee-Finley, K.A.; Mirsky, S.B.; Ryan, M.R. Crop biomass not species richness drives weed suppression in warm-season annual grass–legume intercrops in the northeast. *Weed Sci.* **2017**, *65*, 669-680. https://doi.org/10.1017/wsc.2017.25
28. Barroso, J., Reardon, C.L.; Singh, S.; Machado, S.; Gourlie, J.A.; Namdar, G.F.; Oreja, F.H.; Pritchett, L.C.; Kriete, L.; Calderon, F.J.; Berry, P.A.; McGee, R.J.; Durfee, N.; Adams, C.B.; Hagerty, C.H. Biomass production, weed suppression, and soil water use of cover crops in dryland wheat production systems. *Agron. J.* **2025**, *117*, e70053. https://doi.org/10.1002/agj2.70053
29. Baraibar, B.; Mortensen, D.A.; Hunter, M.C.; Barbercheck, M.E.; Kaye, J.P.; Finney, D.M.; Curran, W.S.; Bunchek, J.; White, C.M. Growing degree days and cover crop type explain weed biomass in winter cover crops. *Agron. Sustain. Dev.* **2018**, 38. https://doi.org/10.1007/s13593-018-0543-1
30. Rougé, A.; Adeux, G.; Busset, H.; Hugard, R.; Martin, J.; Matejicek, A.; Moreau, D.; Guillemin, J.P.; Cordeau, S. Carry-over effects of cover crops on weeds and crop productivity in no-till systems. *Field Crops Res.* **2023**, *295*, 108899. https://doi.org/10.1016/j.fcr.2023.108899
31. Torra, J.; Gonzalez-Andujar, J.L; Recasens, J. Modelling the population dynamics of *Papaver rhoeas* under various weed management systems in a Mediterranean climate. Weed Res. **2008**, 48,136-146. https://doi.org/10.1111/j.1365-3180.2007.00610.x.
32. Sjursen, H.; Brandsæter, L.O.; Netland, J. Effects of repeated clover undersowing, green manure ley and weed harrowing on weeds and yields in organic cereals. *Acta Agric. Scand. B Soil Plant Sci*. **2012**, *62*, 138-150. https://doi.org/10.1080/09064710.2011.584550
33. Oreja, F.H.; Torcat-Fuentes, M.; Barrio, A.; Schiavinato, D.J.; Rosso, V.; de la Fuente, E. Weed Seedbank Changes Associated with Temporary Tillage After Long Periods of No-Till. *Agronomy* **2025**, *15*, 1410. https://doi.org/10.3390/agronomy15061410
34. Santín-Montanyá, M.I.; Casanova Pena, C.; Zambrana Quesada, E.; Sánchez Jiménez, F.J.; Tenorio Pasamón, J.L. Arable weed species associated with soil tillage systems under Mediterranean conditions. *Land Degrad. Dev.* **2018**, *29*, 865-874. https://doi.org/10.1002/ldr.2899
35. Knežević, M.; Đurkić, M.; Knežević, I.; Antonić, O.; Jelaska, S. Effects of tillage and reduced herbicide doses on weed biomass production in winter and spring cereals. *Plant Soil Environ.* **2003**, *49*, 414-421. https://doi.org/10.17221/4146-PSE
36. Mas, M.T.; Verdú, A.M. Tillage system effects on weed communities in a 4-year crop rotation under Mediterranean dryland conditions. *Soil Tillage Res.* **2003**, *74*, 15-24. https://doi.org/10.1016/S0167-1987(03)00079-5

doi:10.20944/preprints202601.1449.v1

37. Kurstjens, D.A. Precise tillage systems for enhanced non-chemical weed management. *Soil Tillage Res.* **2007**, *97*, 293-305.
38. Clements, D.R.; Weise, S.F.; Swanton, C.J. Integrated weed management and weed species diversity. *Phytoprotection* **1994**, *75*, 1-18. https://doi.org/10.7202/706048ar
39. Gonzalez-Andujar, J.L. Integrated Weed Management: a shift towards more sustainable and holistic practices. *Agronomy* **2023**, *13*, 2646. https://doi.org/10.3390/agronomy13102646
40. Sanchez-Giron, V.; Serrano, A.; Hernanz, J.L.; Navarrete, L. Economic assessment of three long-term tillage systems for rainfed cereal and legume production in semiarid central Spain. *Soil Tillage Res.* **2004**, *78*, 35-44. https://doi.org/10.1016/j.still.2004.01.001
41. Soil Survey Staff. *Soil taxonomy: A basic system of soil classification for making and interpreting soil surveys*, 2nd ed; U.S. Department of Agriculture Natural Resources Conservation Service: Washington, USA, 1999.
42. R Core Team. R: A Language and Environment for Statistical Computing; R Core Team: Vienna, Austria, 2025.