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Effects of Fertilizer Sources and Tillage Regimes on Weed Species Composition and Functional Diversity in the 4-Year of Durum Wheat–Potato Rotation

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ABSTRACT

Designing environmental-friendly approaches to mitigate weed pressure in agroecosystems is an urgent priority. A field experiment was conducted in 2014/2015 and 2015/2016 to evaluate fertilizer source and soil tillage on weed community composition in durum wheat–potato rotation. Experimental treatments included: (i) two fertilizer sources (mineral and organic fertilizers [MF and OF]) and (ii) three tillage regimes (plowing [CT], spading [RT1] and subsoiling [RT2]). A randomized complete block design with three replications was adopted. Weed characteristics were measured at the wheat tillering and potato flowering stage. In durum wheat, weed biomass ranged from 73.6 g m^{-2} (MF-CT) to 131.4 g m^{-2} (OF-RT2). In potato, weed biomass was the highest in OF-RT2 (156.3 g m^{-2} of DM), while weed density ranged from 29.0 n m^{-2} (MF-CT) to 46.5 n m^{-2} (OF-RT2). Overall, annual weeds were more abundant under OF compared to MF (75.3 vs. 64.5 n m^{-2}), regardless of tillage treatments. Within MF treatments, annual weed density was higher under RT2 than RT1 and CT (71.3 vs. 61.1 n m^{-2}). Relative frequency of perennial broadleaf species was highest in OF-RT2, with perennial weeds generally increasing under RT2 and RT1, regardless of the fertilization source. Across both crops, biomass production was higher under MF than OF. A negative relationship was observed between crop biomass and the density of broadleaf weeds ($R^2 = 0.3894$, $p < 0.001$) and perennial weeds ($R^2 = 0.2156$, $p < 0.01$). Further studies are required to investigate the long-term implications of these practices and refine weed management approaches across different cropping systems.

1 | Introduction

Ecological intensification practices in agroecosystems are essential in modern agriculture to meet the demands of a growing world population. This approach must be supported by sustainable weed management systems (Fawad et al. 2022). Inadequate weed management strategies can pose significant risks to crop production (Oerke 2006).

It is well-known that in crop rotation systems, weed density and biomass are typically lower compared to monocultures and depend on the previous crop (Woźniak and Soroka 2015). Crop rotation creates an unstable environment by diversifying agronomic practices, thereby disrupting the ecological conditions that favor the dominance of specific weed species. This diversification can reduce the spread and reproductive success of weeds by breaking their life cycles and limiting

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their adaptation to repeated selection pressures. In addition to limiting overall weed pressure, rotation practices can also shape the floristic composition of the weed flora, favoring or suppressing species depending on the sequence and management of crops. Indeed, crop rotation influences the development of specific weed communities (Oreja et al. 2023). Furthermore, Zarzecka et al. (2022) demonstrated that combining organic and mineral fertilizers (MFs) can limit weed establishment and growth, thereby enhancing the competitiveness of potato (*Solanum tuberosum* L.) crops. In contrast, the highest weed competitive ability was observed when MFs were applied alone. Additionally, to ensure high and stable yields, crop rotation systems enrich the soil with organic carbon (Woźniak 2020). When planning a crop rotation, selecting crops with optimal vegetative periods and competitive characteristics is crucial to gain an advantage over weeds (Scavo and Mauromicale 2020).

Another key aspect of preventing weed proliferation is choosing the appropriate fertilizer source. Nitrogen is the primary nutrient limited by crop–weed competition (Little et al. 2021). While MF can boost yields, it does not effectively maintain soil fertility over time (Pahalvi et al. 2021). High nitrogen inputs from MFs can lead to N_2O emissions and nitrate leaching (Han et al. 2024; Schmidt et al. 2019). A more environmentally favorable approach is to apply organic amendments (Salman et al. 2023), as organic fertilizers (OFs) improve the physical, chemical, and biological properties of soil, enhance fertility (Jamal et al. 2023), and replenish organic matter that undergoes natural MF (Butkevičienė et al. 2021). Hence, municipal organic waste increases soil moisture and stability, acts as a fertilizer, and contributes to the circular economy (Shahnazari et al. 2021).

Another important agronomic practice that influences weed pressure is the type of tillage used (MacLaren et al. 2021). Conservation tillage practices, such as minimum tillage and no-tillage (NT), allow for rapid soil preparation, improve soil aggregation, reduce energy consumption, and provide a more sustainable alternative to continuous tillage (Schlüter et al. 2018). It has been observed that these practices have a significant effect on reducing weed abundance (Cordeau et al. 2015). In conventional tillage (CT), plowing is foundational; whereas, subsoiling and spading are less invasive techniques that help to preserve soil structure (Guimaraes Júnnyor et al. 2022). Subsoiling, a noninversion tillage technique applied at depths of 35–40 cm, enhances soil aeration (Navarro-Rosales et al. 2023). Meanwhile, spading machines shred the soil, with a single pass typically sufficient to create a suitable seedbed. Given the importance of the fertilization source and soil tillage in the sustainability of agroecosystems, this study investigates the interactions between different combinations of these practices and their impact on the evolution of the weed species community and its functional traits and on the yields of the crops in rotation.

Potato (*S. tuberosum* L.) is characterized by a relatively low competitive ability against weeds, particularly during early developmental stages (Siddiqui and Jabran 2025). Its slow canopy closure, wide inter-row spacing, and shallow root system often allow for rapid weed establishment, which can significantly reduce tuber yield and quality if not adequately controlled (Colquhoun et al. 2009). In contrast, durum wheat (*Triticum durum* Desf.) exhibits a comparatively higher weed suppressive potential, primarily due to its faster canopy development and denser stand structure (Aharon et al. 2021). However, it is interesting to note that durum wheat is highly susceptible to competition from various weed species, including *Silene noctiflora* L. (night-flowering catchfly), *Anagallis arvensis* L. (scarlet pimpernel), *Viola arvensis* Murr. (field violet), Euphorbia spp., and Polygonum spp. Although these species do not significantly affect grain yield, others, such as *Raphanus raphanistrum* L. (wild radish) and *Galeopsis* spp. (hemp nettle), can cause severe crop yield losses (Esposito et al. 2023).

However, its competitiveness remains influenced by genotype, sowing density, and nutrient availability, and it can still suffer yield and quality reductions under high weed pressure, particularly from late-emerging or fast-growing broadleaf species (Mwendwa et al. 2020). The differential weed competitiveness between these two crops highlights the importance of tailored management strategies within rotational systems, especially under integrated or reduced-input scenarios. Driven by the hypothesis that specific combinations of fertilizer sources and tillage methods influence weed community dynamics and crop yields differently, the study evaluates weed community composition and species diversity after 4 years of a wheat–potato rotation. The experiment examined responses to two fertilizer sources (mineral vs. organic) and three soil tillage methods (conventional vs. subsoiler vs. spading machine). The objectives were to analyze the interaction effects of fertilizer source and soil tillage method on (i) aboveground weed biomass and density, (ii) weed species composition, (iii) functional weed structure in terms of morphotypes (monocots and dicots) and life cycle (annual, biennial and perennial), and (iv) crop yields.

2 | Materials and Methods

2.1 | Study Site and Soil Characteristics

A field trial was conducted at the experimental farm "Nello Lupori" of the University of Tuscia located in Viterbo, Italy (latitude 45°25′, longitude 12°04′ and altitude 310 m a.s.l.) during the 2014/2015 and 2015/2016 cropping seasons, respectively. The climate of the area is typically Mediterranean, characterized by warm/wet winters and hot/dry summers. The soil at the experimental site is of volcanic origin and is classified as *Typic Xerofluvent*. In the surface horizon (0–30 cm), particle size distribution was: 763 g kg^{-1} sand, 133 g kg^{-1} silt, and 104 g kg^{-1} clay. The soil contained 0.97% total organic C and 0.12% total N content with a pH (H_2O) of 6.9.

2.2 | Experimental Design, Field Setup, and Crop Management

A 2-year crop rotation (durum wheat [*T. durum* Desf.]—potato [*S. tuberosum* L.]), typical of the study area, was established during the 2010/2011 growing season to compare the combined effects of fertilizer sources and soil tillage practices. The experimental treatments included: (a) two fertilizer sources (MF as performed conventionally in the area, and organic fertilization

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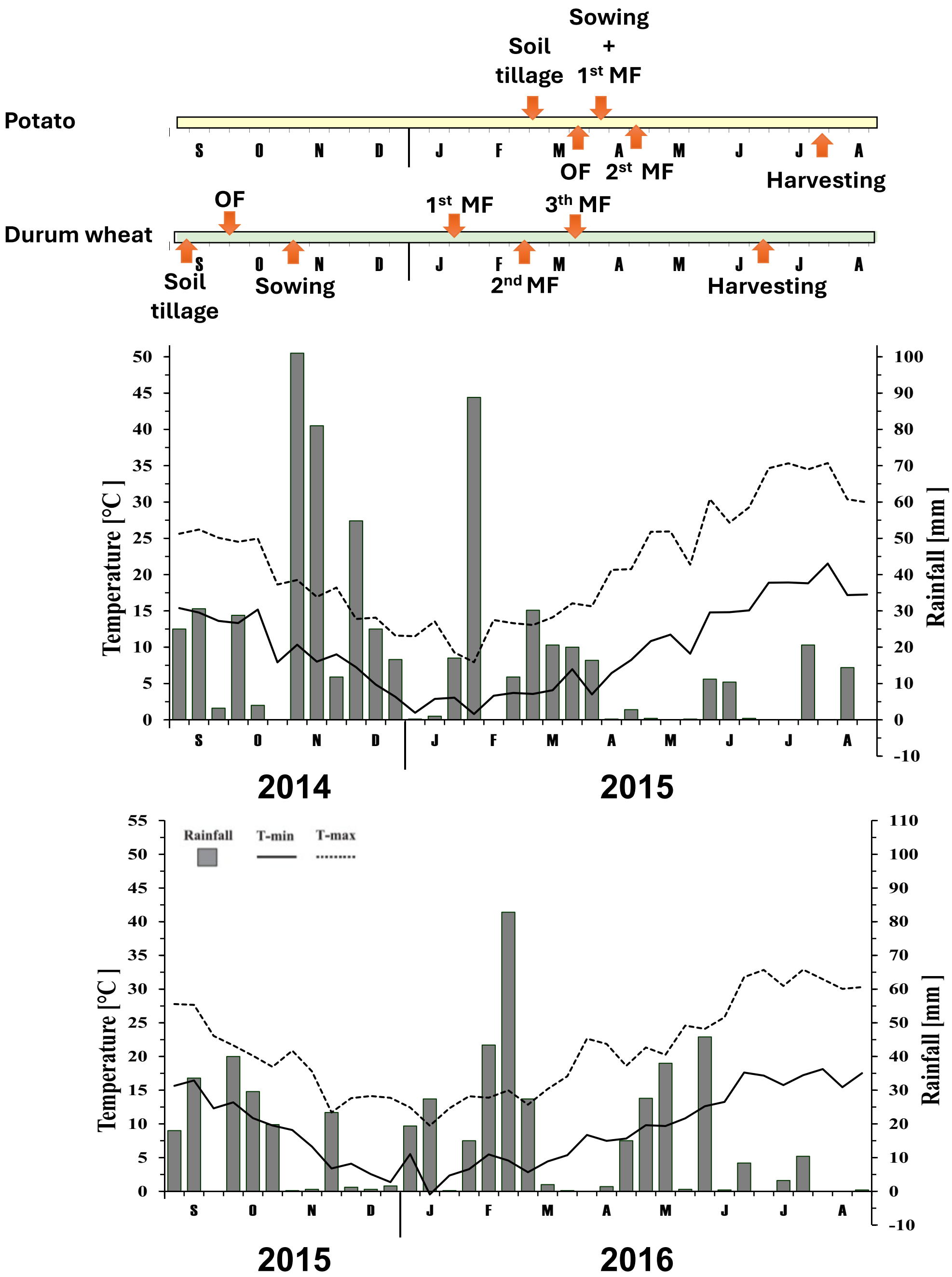

FIGURE 1 | Main agronomical practices adopted in durum wheat and potato crops and weather conditions, in terms of rainfall, minimum and maximum average air temperatures at 10-day intervals at the experimental site for the 2014/2015 and 2015/2016 growing seasons. OF = organic fertilizer; MF = mineral fertilizer.

using municipal organic waste [hereafter called OF]); and (b) three soil tillage methods (CT using a mouldboard plow; reduced tillage with a spading machine [hereafter called RT1]; and reduced tillage with a subsoiler [hereafter called RT2]). The treatments were replicated three times in a randomized complete block design. Since both crops in rotation were cultivated simultaneously each growing season, the experimental field included 36 plots (2 crops × 3 soil tillage × 2 fertilizer source × 3 blocks). Each plot measured 60 m^2 (6 × 10 m) and was separated by 3 m wide alleys to accommodate equipment operations. The data reported in this study correspond to the 2014/2015 and 2015/2016 growing seasons.

For durum wheat cultivation during 2014/2015 and 2015/2016, seedbed preparation was carried out in September. This process involved applying the selected soil tillage method to a depth of 30 cm, followed by two rounds of disk harrowing to a depth of 10 cm before sowing (Figure 1). The machines used for soil tillage were Angeloni APS M35 for CT, Dondi RHP-5 M for RT1, and the Tortella 005-100 for RT2, respectively. Under the MF treatment, fertilization followed a program commonly adopted by the local farmers. Specifically, 80 kg ha^{-1} of P_2O_5 as triple superphosphate was applied before the second disk harrowing during seedbed preparation. Additionally, a total of 120 kg ha^{-1} of N was applied in three stages: (i) at the beginning

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tillering stage in January (30 kg ha^{-1} of N as calcium nitrate); (ii) at the end of tillering stage in February (40 kg ha^{-1} of N), and (iii) at the beginning of stem elongation in March (50 kg ha^{-1} of N). For OF, 25 t ha^{-1} of municipal organic waste was applied, ensuring a theoretical MF that matched the 120 kg ha^{-1} of N requirement for durum wheat. This OF, compliant with the Italian Legislative Decree 75/2010, was applied before the second disk harrowing. In both growing seasons, durum wheat cv. Claudio was sown in November in all experimental plots using an experimental sowing machine. The seeding rate was 450 seeds m^{-2}, distributed in rows spaced 12.5 cm apart at a depth of 3–4 cm. Weed control was carried out chemically by applying a mixture of Mesosulfuron-Metile 3% + Iodosulfuron Metil-Sodium 3% + Mefenpir-Dietile 9% (ATLANTIS) for postemergence weeding against all graminaceous weeds and some important dicots at the tillering stage after weed sampling. Wheat harvesting was performed at full crop maturity stage in early July (Figure 1).

For the potato crop, in both experimental growing seasons, soil was tilled according to CT, RT1, and RT2 treatments in March, before tuber planting. The tillage procedures were consistent with those described earlier for durum wheat. After the primary soil tillage, all plots underwent two rounds of disk harrowing to a depth of 10 cm to break soil clods and prepare a uniform seedbed for potato planting (Figure 1). During potato cultivation, MFs were applied at the rates of 100 kg ha^{-1} of P_2O_5 as triple superphosphate, 50 kg ha^{-1} of K_2O as potassium sulfate, and a total of 170 kg ha^{-1} of N. Nitrogen fertilizer was applied in two stages: (i) 100 kg ha^{-1} of N as urea at potato sowing, and (ii) 70 kg ha^{-1} of N as ammonium nitrate before tuber formation. MF followed the practices commonly adopted by local farmers in the area. OF was conducted using the municipal organic waste at the rate of 18 t ha^{-1}. It was distributed on the soil surface after the main tillage and incorporated into the soil using disk harrowing. Tuber seed pieces of the potato cv. Monalisa were planted in April with rows spaced 70 cm apart and a planting density of 7.14 tubers m^{-2}. Weeds were controlled mechanically using a tractor-mounted cultivator, commonly employed for hilling potato plants, and applied at the flowering stage before weed sampling. Irrigation during both growing seasons was based on local weather conditions and ceased approximately 2 weeks before tuber harvesting in August (Figure 1).

2.3 | Data Collection

In each growing season for both durum wheat and potato crops, weed measurements were carried out at the tillering stage of durum wheat and flowering stage of potato, respectively. The measurements were performed simultaneously using a square of 1.0 m^2 (0.5 × 2.0 m) for durum wheat and 2.8 m^2 (1.4 × 2.0 m) for potato, respectively. The sampling area was placed randomly in the central area of each plot for each crop. Weeds collected from the sampling area in each treatment were pooled together to evaluate weed characteristics per unit area (1.0 m^2). Weeds were identified and counted by species to determine weed density per species and total. Additionally, all collected weeds were classified into eight functional groups (FGs), following the classification system proposed by Meiss et al. (2010) as detailed in Table 1. Weed community richness (S) was calculated as the

TABLE 1 | Functional groups of weeds based on the classification proposed by Meiss et al. (2010).

Functional group (FG)	Weed characteristic
FG 1	Annual broadleaf species, upright (species with erect morphology since seedling stage)
FG 2	Annual broadleaf species, climbing (species winding on neighboring plants)
FG 3	Annual broadleaf species, rosette (species with circular arrangement of the first leaves near to the soil surface)
FG 4	Annual broadleaf species, other (species comprising all other morphologies)
FG 5	Intermediate broadleaf species (species with biennial life cycles)
FG 6	Perennial broadleaf species
FG 7	Annual grass species
FG 8	Perennial grass species

number of weed species recorded per plot. Similarly, Shannon's diversity index was calculated using the formula proposed by Magurran (1988):

$$\text{Shannon's diversity index} = H' = -\Sigma\, \text{PA}_i \left(\ln\ \text{PA}_i\right)$$

where PA_i is the proportional abundance of species i ($\text{PA}_i = n_i/n_{\text{tot}}$). H' approaches 0 when the sample contains few species, while it reaches a maximum when all species (S) are evenly represented.

After determining weed density, all weeds were collected and oven-dried to a constant weight to measure total weed biomass. Grain yield and straw in durum wheat were measured at physiological maturity, while tuber yield and straw in potato were harvested when 90% of the plants exhibited aboveground senescence and skin-hardened tubers. Both crops were harvested manually, collecting plants from a 1.0 m^2 central area per plot. Each plot sample was weighed and dried to determine moisture content.

2.4 | Statistical Analysis

Data analysis was performed using JMP statistical software version 4.0. Before conducting the analysis of variance (ANOVA), Bartlett's test was applied to verify the homogeneity of variance. Weed density data were transformed into square root (x + 0.05), following the method described by Gomez and Gomez (1984). The reported data were back-transformed for interpretation. A two-way factorial design was used to analyze weed aboveground biomass, weed density, and biomass production (yield + straw) of both crops. Fertilizer source and soil tillage were considered

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treatments, while growing seasons were treated as repeated measures (Cody and Smith 1997). Treatment means were compared using Fisher's protected least significant differences (LSD) test at the significance level of $p < 0.05$.

Canonical discriminant analysis (CDA) was performed to examine the associations between fertilizer source and soil tillage groups with the occurrence of predefined FGs of weed species (Kenkel et al. 2002). The results of the CDA are presented in a canonical diagram, where the proximity of weed FGs and experimental treatments in the ordination space indicates associations (Kenkel et al. 2002). Linear regression analyses were conducted to explore the relationships between crop total biomass production in each durum wheat–potato rotation and the densities of annual, perennial, grass, and broadleaf weed species observed during the same period.

3 | Results

3.1 | Weather Conditions

The growing seasons of durum wheat lasted 224 and 212 days in 2014/2015 and 2015/2016, respectively (Figure 1). Total rainfall was higher in 2014/2015 compared to 2015/2016 (420.8 vs. 382.4 mm, respectively) over the entire period. However, the first growing season (2014/2015) experienced significantly more rainfall in November and December compared to the second growing season (189.2 vs. 27.4 mm, respectively). Conversely, in 2015/2016, higher rainfall was recorded during the tillering stage of durum wheat, as well as in April and May, leading up to durum wheat harvesting (140.2 vs. 42.0 mm, respectively). During the same period, average temperatures were similar between the growing seasons (on average 12.8°C). Temperatures gradually decreased after the cereal sowing, reaching their lowest values in January and February, before rising steadily, peaking in July during durum wheat harvesting. Minimum temperatures were rarely below 0°C, except in 2015/2016 when the air temperature fell below 0°C in January (Figure 1).

Potato cultivation lasted for 129 and 137 days in the 2015 and 2016 growing seasons, respectively (Figure 1). Weather data indicated that the 2016 growing season was rainier than the 2015 growing season, with cumulative rainfall of 150.8 mm compared to 62.6 mm. Notably, during the 2016 potato cultivation period, approximately 70% of the total precipitation occurred between potato sowing in April and the first days of June. Air temperatures were higher on average during the 2015 potato growing season than in 2016 (on average 19.7°C vs. 19.0°C, respectively). Minimum air temperatures were similar between the two seasons (on average 12.5°C), but maximum air temperatures were higher in 2015 than in 2016 (on average 26.8°C vs. 25.6°C, respectively, Figure 1).

3.2 | Weed Biomass and Density in Durum Wheat–Potato Rotation

Weed aboveground biomass and density across durum wheat and potato crops were significantly affected by the interaction fertilizer source × soil tillage ($p < 0.05$, Figure 2). The results indicate that weed characteristics in both crops were influenced by the same fertilization treatments. Specifically, OF resulted in the highest weed aboveground biomass and density, particularly, when combined with subsoil tillage (RT2, Figure 2).

3.2.1 | Weed Biomass and Density in Durum Wheat Crop

In durum wheat crop, weed aboveground biomass (Figure 2) ranged from 73.6 g m^{-2} of DM (MF-CT) to 131.4 g m^{-2} of DM (OF-RT2). Biomass was generally higher (+20%) in OF compared to MF, averaging 103.3 and 85.8 g m^{-2} of dry matter (hereafter called DM), respectively. A similar trend was observed across fertilizer treatments (Figure 2). Indeed, weed aboveground biomass was the highest under RT2 (131.4 and 97.4 g m^{-2} of DM in OF and MF), intermediate under RT1 (95.3 and 86.5 g m^{-2} of DM in OF and MF, respectively) and lowest under CT (83.2 and 73.6 g m^{-2} of DM in OF and MF, respectively). Under the OF-RT2, weed aboveground biomass value was significantly higher than under RT1 (+38%) and CT (+58%). Weed density followed a comparable pattern, with the highest density observed under OF-RT2 (65.5 n m^{-2}), followed by OF-RT1 (51.8 n m^{-2}), MF-RT2 (50.8 n m^{-2}), OF-CT (44 n m^{-2}), MF-RT1 (41.8 n m^{-2}), and MF-CT (39.3 n m^{-2}, Figure 2). Additionally, density was higher in RT2 than RT1 under both OF and MF (+26% and +22%, respectively).

3.2.2 | Weed Characteristics in Potato Crop

Weed aboveground biomass ranged from 101.7 g m^{-2} of DM (OF-CT) to 156.3 g m^{-2} of DM (OF-RT2). It was the highest under OF-RT2 (156.3 g m^{-2} of DM), intermediate under OF-RT1 and MF-RT2 (129.2 and 135.7 g m^{-2} of DM, respectively), and lowest under OF-CT, MF-RT1, and MF-CT (101.7, 113.4, and 104.5 g m^{-2} of DM). Similar to durum wheat, weed aboveground biomass was higher in OF (129.1 g m^{-2} of DM) compared to MF (117.9 g m^{-2} of DM, +10%). Across tillage practices, biomass was highest in RT2 (156.3 and 135.7 g m^{-2} of DM in OF and MF, respectively), intermediate in RT1 (129.2 and 113.4 g m^{-2} of DM in OF and MF, respectively) and lowest in CT (101.7 and 104.5 g m^{-2} of DM in OF and MF, respectively). Weed aboveground biomass under OF-RT2 was significantly higher than OF-RT1 (+21%) and OF-CT (+54%), and across all tillage practices under MF (+50%, +38%, and +15% for CT, RT1, and RT2, respectively). Weed density ranged from 29.0 n m^{-2} in MF-CT to 46.5 n m^{-2} in OF-RT2 and was higher under OF than MF treatments (on average 42.1 and 36.4 n m^{-2}, respectively, Figure 2). The weed density increased as tillage intensity shifted from CT to RT for both fertilizer sources. Weed density under RT2 was significantly higher than under CT (+21% and +41% for OF and MF, respectively), while MF treatments also showed a significant increase in weed density under RT1 compared to CT (+36%).

3.3 | Weed Species Composition and Their Characteristics

The density of each weed species collected during the tillering stage of durum wheat and potato crops over the experimental period is reported in Table 2 (durum wheat) and Table 3

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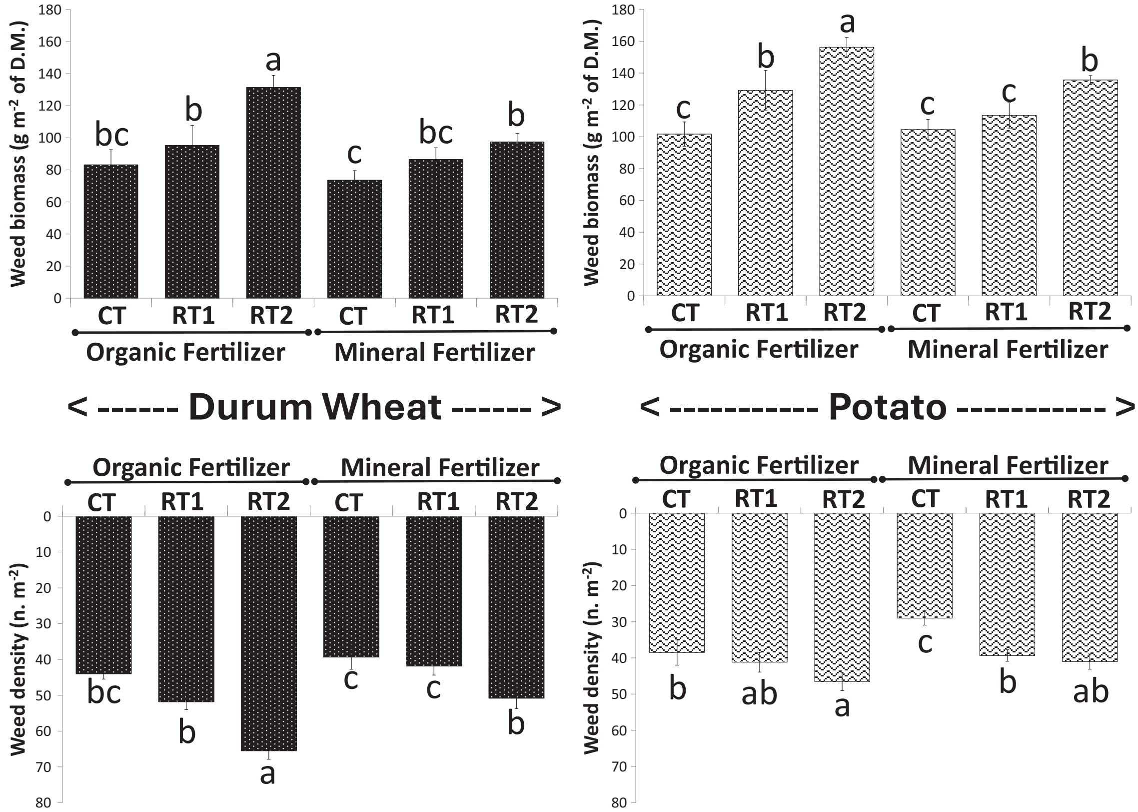

FIGURE 2 | The effect of fertilizer source × soil tillage interaction on the weed aboveground biomass and weed density in durum wheat and potato. Values belonging to the same crop with different letters are statistically different according to LSD (0.05). CT = conventional tillage; RT1 = reduced tillage based on spading machine; RT2 = reduced tillage based on subsoiler. Error bars represent ± standard error from mean (n = 6).

(potato). In durum wheat, a total of 27 weed species were identified, predominantly dicots compared to monocots (78% vs. 22%, Table 2). Interestingly, monocots were more abundant under MF than OF, with densities of 11.7 and 5.0 plants m^{-2}, respectively. On average, the weed flora consisted of 19 annual species, 2 biennials, and 6 perennials. The main weed species observed in durum wheat included *Galium aparine* L., *Stellaria media* (L.) Vill., *Fumaria officinalis* L., *Avena sterilis* (L.) P. Beauv., *Cirsium arvensis* (l.) Scop., and *Papaver rhoeas* L., collectively accounting for approximately 50% of the total weed abundance (Table 2). Regarding FGs (Table 1), FG 1, FG 6, and FG 7 were the most prominent, each represented by five weed species for durum wheat. FG 4 included four weed species, FG 3 included three weed species, and FG 2 and FG 5 each had two weed species. Only one weed species was classified under FG 8. The Shannon diversity index, calculated for weed species in durum wheat, was higher under OF compared to MF (on average 2.08 vs. 1.97). Within OF treatments, Shannon index values were consistent across soil tillage treatments. In contrast, under MF, the Shannon index was higher in CT and RT2 than RT1 (on average 1.99 vs. 1.85, respectively, Figure 3). Weed community richness followed a similar trend to the Shannon index, with higher values observed under OF than MF (16.3 vs. 15.0 n species m^{-2}, respectively). The highest values were observed under OF-RT1 and OF-RT2 compared to other treatments (on average 17.1 vs. 15.0 n species m^{-2}, respectively, Figure 3).

In potato, 19 weed species were identified, comprising 85% dicots and 15% monocots (Table 3). At the flowering stage, the weed composition primarily consisted of 15 annual species and 4 perennial species (Table 3). The dominant weed species collected included *Amaranthus retroflexus* L., *Chenopodium album* L., *Solanum nigrum* L., *Portulaca oleracea* L., *Convolvulus arvensis* L., *Echinochloa crus-galli* (L.) P.Beauv., and *Digitaria sanguinalis* (L.) Scop., which together accounted for approximately 61.0% of the total weed abundance (Table 3). Considering FGs in the potato crop, FG 1 was the most represented, comprising six weed species, followed by FG 6 (four species), FG 3 and FG 7 (three species each), and FG 4 (two species). Only one species was classified under FG 2, while no species were associated with FG 5 or FG 8. In the potato crop, the OF-RT2 treatment resulted in the highest values for both the Shannon index (2.03 vs. 1.77, respectively) and weed community richness (14.5 vs. 10.6 n species m^{-2}, respectively) compared with other treatments (Figure 3).

3.4 | Weed Association of FGs

CDA of FGs observed in durum wheat and potato crops is presented in Figure 4A,B, respectively. For the durum wheat crop, CDA revealed a tendency for weed communities to differentiate based on fertilization source and soil tillage treatments. The first two canonical variables explained 48.2% of the total between-group variance. FG 3, FG 7, and FG 8 were positioned within the same ordination space as MF. Specifically, FG 3 appeared to be associated with MF-CT, FG 7 with MF-RT1, and FG 8 with MF-RT2 (Figure 4A). In contrast, FG 1, FG 2, FG 4, FG 5, and FG 6 vectors were positioned within the ordination space corresponding to OF. Among these, FG 1, FG 4, and FG 5 were more closely

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TABLE 2 | The density of each weed species observed during the study period in durum wheat and its functional group number.

			Plants m^{-2}					
			Organic fertilizer			Mineral fertilizer		
Botanical name	FG		CT	RT1	RT2	CT	RT1	RT2
Bifora radians M. Bieb.	1	D	1.2	2.0	3.2	0.7	0.7	1.0
Centaurea cyanus L.	1	D	0.5	1.2	1.3	0.8	0.7	0.2
Fumaria officinalis L.	1	D	5.8	6.8	9.2	2.2	2.8	4.2
Matricaria chamomilla L.	1	D	2.0	2.8	3.0	1.0	1.2	2.3
Viola arvensis Murray	1	D	1.8	1.8	1.0	1.0	0.3	0.3
Galium aparine L.	2	D	5.3	6.3	7.0	6.5	6.5	6.3
Fallopia convolvulus (L.) Á. Löve	2	D	1.8	2.3	3.2	0.7	0.8	1.7
Anagallis arvensis L.	3	D	0.0	2.0	0.7	1.2	1.0	1.2
Stellaria media (L.) Vill.	3	D	5.7	4.8	4.8	8.2	6.5	6.5
Veronica hederifolia L.	3	D	2.8	1.3	0.7	1.8	0.3	0.0
Chrysantemum segetum (L.) Fourr.	4	D	0.8	0.5	1.3	0.2	0.2	0.7
Papaver rhoeas L.	4	D	2.0	2.8	3.7	0.5	1.3	1.8
Rapistrum rugosum (L.) All.	4	D	1.7	0.8	1.7	0.7	1.2	1.5
Sinapis arvensis L.	4	D	2.7	1.0	0.5	0.7	0.7	1.2
Capsella bursa-pastoris (L.) Medik.	5	D	1.2	1.7	0.7	0.8	0.5	0.5
Silybum marianum (L.) Gaertn.	5	D	3.2	2.2	2.0	1.2	0.0	0.5
Cirsium arvensis (L.) Scop.	6	D	0.7	3.2	2.8	1.0	3.0	1.7
Convolvulus arvense L.	6	D	0.2	0.7	3.5	0.2	1.2	3.2
Malva sylvestris L.	6	D	0.0	0.5	2.7	0.0	0.3	0.8
Sonchus arvensis L.	6	D	0.7	1.7	1.8	0.5	0.3	1.0
Taraxacum officinalis Weber	6	M	0.3	0.8	4.0	0.3	0.0	1.0
Apera spica-venti (L.) P.Beauv.	7	M	0.2	0.5	0.0	1.2	0.3	0.5
Avena sterilis L.	7	M	1.7	2.0	3.3	3.0	5.7	3.8
Bromus sterilis L.	7	M	0.0	0.3	0.0	0.7	1.2	0.5
Phalaris canariensis L.	7	M	1.0	0.5	1.2	2.5	2.3	2.0
Phalaris minor Retz.	7	M	0.5	0.5	0.0	1.7	1.5	2.0
Lolium perenne L.	8	M	0.3	0.7	2.3	0.3	1.3	4.5
SED			*0.8*	*0.9*	*0.9*	*0.7*	*0.7*	*0.9*

Note: See Table 1 for a description of the functional groups (FG).
Abbreviations: CT = conventional tillage; D = broadleaf species; FG = functional group; M = grass species; RT1 = reduced tillage based on spading machine; RT2 = reduced tillage based on subsoiler; SED = standard error of difference.

associated with OF-RT1, while FG 2 and FG 6 were primarily aligned with OF-RT2 (Figure 4A).

For the potato crop, the first two canonical variables of CDA accounted for 50.4% of the total between-group variance. The analysis indicated that all FGs were associated with OF, with no FGs linked to MF. Specifically, FG 1 and FG 3 were closely associated with OF under CT (OF-CT). Conversely, FG 2, FG 4, and FG 6 were associated with OF under both reduced tillage practices (OF-RT1 and OF-RT2).

The analysis of all weeds collected during durum wheat and potato cultivation across growing seasons revealed that annual weed density tended to be higher under OF compared to MF, regardless of soil tillage (on average 75.3 vs. 64.5 n m^{-2}, respectively). Under MF, annual weed density was greater in RT2 compared to RT1 and CT (on average 71.3 vs. 61.1 n m^{-2}, respectively, Figure 5). Concerning perennial weed species, the highest density was observed under the OF-RT2 treatment (31.8 n m^{-2}), followed by OF-RT1 and MF-RT2 (on average 20.3 n m^{-2}). Lower densities were recorded under MF-RT1 (13.3 n m^{-2}), while the

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TABLE 3 | The density of each weed species observed during the study period in potato and its functional group number.

Botanical name	FG		Plants m^{-2}					
			Organic fertilizer			Mineral fertilizer		
			CT	RT1	RT2	CT	RT1	RT2
Amaranthus retroflexus L.	1	D	6.7	3.5	3.7	5.7	4.7	4.5
Chenopodium album L.	1	D	5.7	3.5	2.3	4.3	4.7	4.8
Datura stramonium L.	1	D	3.8	3.0	1.3	1.0	0.3	0.5
Sinapis alba L.	1	D	5.3	2.3	1.0	1.0	0.5	0.7
Solanum nigrum L.	1	D	2.2	2.7	3.5	2.5	5.3	4.7
Xanthium spinosum L.	1	D	1.7	1.8	3.2	0.7	0.5	0.7
Fallopia convolvulus (L.) Á. Löve	2	D	0.8	0.2	2.5	0.2	0.2	0.7
Lamium amplexicaule (L.)	3	D	0.3	1.3	0.7	0.0	0.5	0.0
Polygonum aviculare (L.)	3	D	0.8	1.2	1.7	2.3	2.2	2.3
Portulaca oleracea L.	3	D	6.2	2.5	1.0	4.2	1.2	0.7
Galinsoga ciliata (Raf.) S.F. Blake	4	D	0.0	0.5	1.5	0.0	0.0	0.0
Heliotropium europaeum (L.)	4	D	0.3	0.5	0.7	0.0	0.0	0.0
Cirsium arvense (L.) Scop.	6	D	0.3	3.0	4.8	0.2	2.7	2.3
Convolvulus arvensis L.	6	D	0.7	7.7	7.0	0.0	3.2	3.0
Malva sylvestris (L.)	6	D	0.0	1.7	3.7	0.0	0.2	0.5
Sonchus arvensis L.	6	D	0.3	0.3	2.0	1.0	1.8	3.2
Digitaria sanguinalis (L.) Scop.	7	M	0.8	1.7	2.3	0.8	3.7	5.3
Echinochloa crus-galli (L.) P.Beauv.	7	M	0.8	3.0	2.8	1.5	5.0	5.2
Setaria viridis (L.) Beauv.	7	M	1.7	0.8	0.8	3.7	2.8	2.0
SED			*0.8*	*1.0*	*0.7*	*0.6*	*0.7*	*0.7*

Abbreviations: CT = conventional tillage; RT1 = reduced tillage based on spading machine; RT2 = reduced tillage based on subsoiler.

lowest values were associated with OF-CT and MF-CT (on average 3.8 n m^{-2}, Figure 5). The density of broadleaf weed species ranged from 98.2 n m^{-2} under OF-RT2 to 48.2 n m^{-2} under MF-CT. Broadleaf weeds were generally more prevalent under OF and RT2 treatments (on average 83.6 and 82.2 n m^{-2}, respectively). Conversely, grass weed density was higher under MF compared to OF (21.7 vs. 9.9 n m^{-2}, respectively). Within the MF treatment, perennial grass weed density was greater under RT2 and RT1 compared to CT (on average 24.8 vs. 15.3 n m^{-2}, respectively, Figure 5).

Across the durum wheat–potato rotation, the relative frequencies of the eight a priori defined FGs varied depending on the combination of fertilization source and soil tillage treatments (Figure 6). For upright annual broadleaf species (FG 1), relative frequencies were higher under OF than MF, with the highest value observed under OF-CT. Climbing annual species (FG 2) exhibited the highest relative frequencies under OF-RT2, with no significant differences between fertilization sources. Rosette annual species (FG 3) were most frequent under CT, particularly under MF-CT compared to OF-CT (Figure 6). Other annual weed species (FG 4) were more prevalent under OF, particularly in CT and RT2. Although perennial broadleaf species (FG 6) were most frequent under OF-RT2, their relative frequencies increased under RT2 and RT1 regardless of fertilization sources (Figure 6). The MF treatment increased the relative frequency of annual grass species (FG 7), particularly under RT1, while their frequency under OF was consistent through tillage treatments. Perennial grass species (FG 8) were most frequently found under MF-RT2 compared to the other treatments (Figure 6).

3.5 | Durum Wheat and Potato Biomass Production

The total aboveground biomass produced by durum wheat and potato during both growing seasons is presented in Figure 7. For durum wheat, MF resulted in significantly greater aboveground biomass compared to OF (on average 791.6 vs. 537.2 g m^{-2} of DM, respectively). Under MF, aboveground biomass production was consistent across soil tillage treatments. In contrast, under OF, aboveground biomass was the highest under CT, intermediate under RT1, and lowest under RT2, averaging 620.0, 513.8, and 477.8 g m^{-2} of DM, respectively. In the potato crop, aboveground biomass ranged from 848.6 g m^{-2} under OF-CT to

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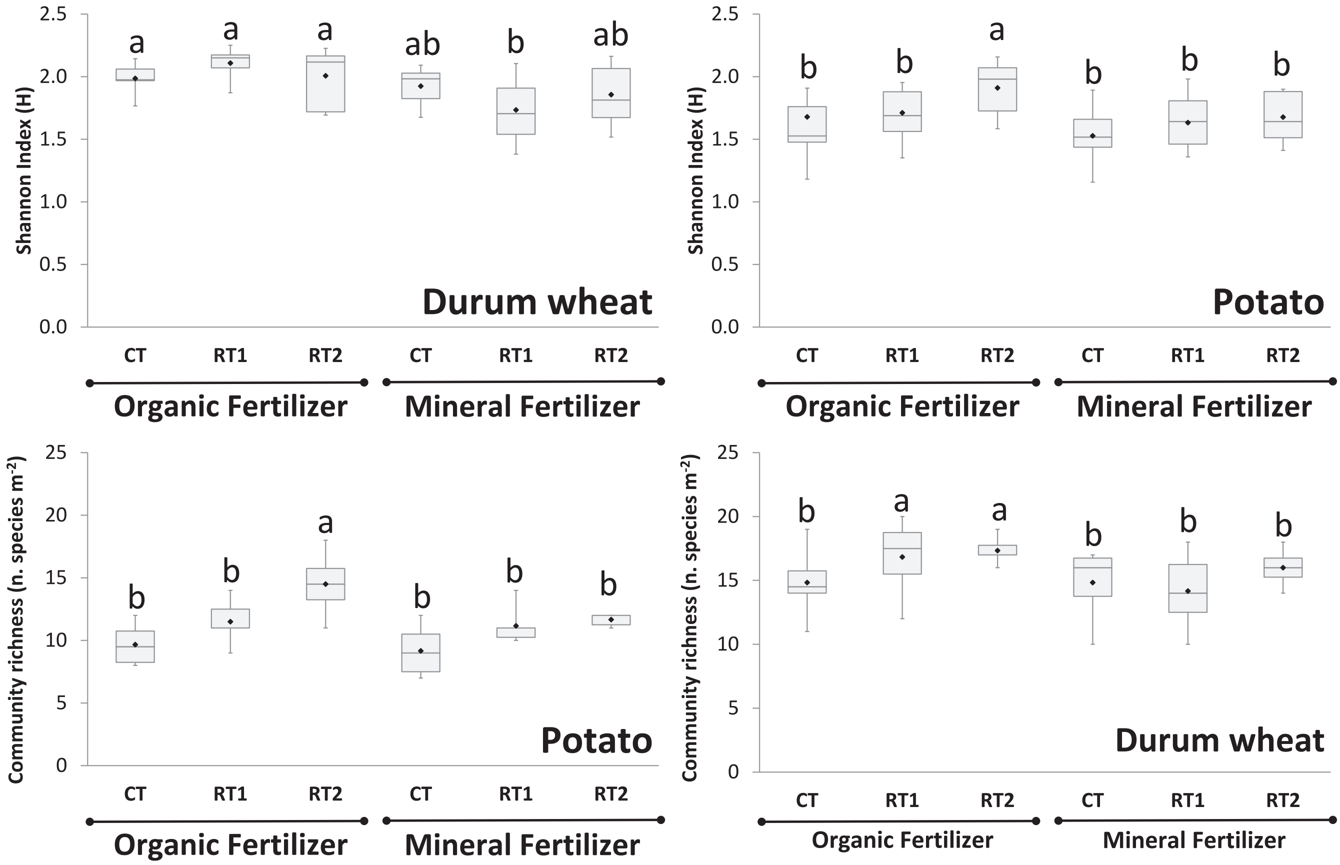

FIGURE 3 | Shannon index (H) and weed community richness measured in durum wheat and potato crops under fertilizer source × soil tillage interaction. Broad lines are medians, black dots are means, boxes show the interquartile range, and whiskers extend to the last data point within 1.5 times the inter-quartile range. Groups with different letters are significantly different. CT = conventional tillage; MF = mineral fertilizer; OF = organic fertilizer; RT1 = reduced tillage based on spading machine; RT2 = reduced tillage based on subsoiler.

555.3 g m^{-2} under OF-RT2. Despite these variations, potato aboveground biomass was slightly higher under MF than OF (757.8 vs. 727.5 g m^{-2} of DM, respectively, Figure 7). The relationships between the total aboveground biomass of both durum wheat and potato crops and various weed characteristics are shown in Figure 8. No significant relationships were observed between total crop aboveground biomass and the density of annual and grass weed species (Figure 8). However, a significant negative relationship was found between the cumulated aboveground biomass of both crops in rotation and the density of broadleaf weeds ($R^2 = 0.3894$, $p < 0.001$) as well as perennial weeds ($R^2 = 0.2156$, $p < 0.01$, Figure 8).

4 | Discussion

Under Mediterranean environmental conditions, agroecosystems are predominantly characterized by the prevalence of weed species with short biological cycles (therophytes), which remain dormant during adverse seasons of the year (Kanellou et al. 2023). These conditions favor the emergence of dominant and highly competitive weed species in the agroecosystems employing conventional practices such as intensive soil tillage and high MF inputs. These species compete aggressively for limited resources essential for optimal crop performance (Radicetti and Mancinelli 2021). Common weed species in Mediterranean agroecosystems include *C. arvensis*, *Daucus carota*, *Malva sylvestris*, *P. rhoeas*, *Sonchus oleraceus*), *A. retroflexus*, and *C. album*. These weeds can severely impact the yields of both winter and summer crops (Karkanis et al. 2018; Campiglia et al. 2018). The findings align with the existing literature, as the dominant weed species identified in both durum wheat and potato crops during a 2-year rotation are representative of Mediterranean agroecosystems. It is well-established that various agronomic practices, including soil tillage, fertilization methods, crop rotation, and the application of chemical herbicides, substantially influence weed species by altering the structure and composition of the weed community (Yvoz et al. 2021). Consistent with the findings of Nichols et al. (2015), agroecosystems based on high external inputs are associated with reduced weed diversity, leading to a simplification of the agroecosystem compared to lower-input systems. From an agroecological perspective, the observed decline in weed biodiversity in arable cropping systems raises concerns. As primary producers, weeds play a vital role in supporting the life cycles of other taxa within ecosystems. Their reduction can compromise agroecosystem functionality, potentially impacting ecosystem services and long-term productivity (Marshall et al. 2003). Currently, the adoption of regenerative agricultural practices, such as OF and RT, is strongly promoted under the common agricultural policy (CAP) in European countries (European Parliament 2009). These practices provide numerous agroecological benefits, including enhanced soil fertility, increased biodiversity, reduced soil erosion, and significant reductions in fossil fuel consumption and greenhouse gas emissions.

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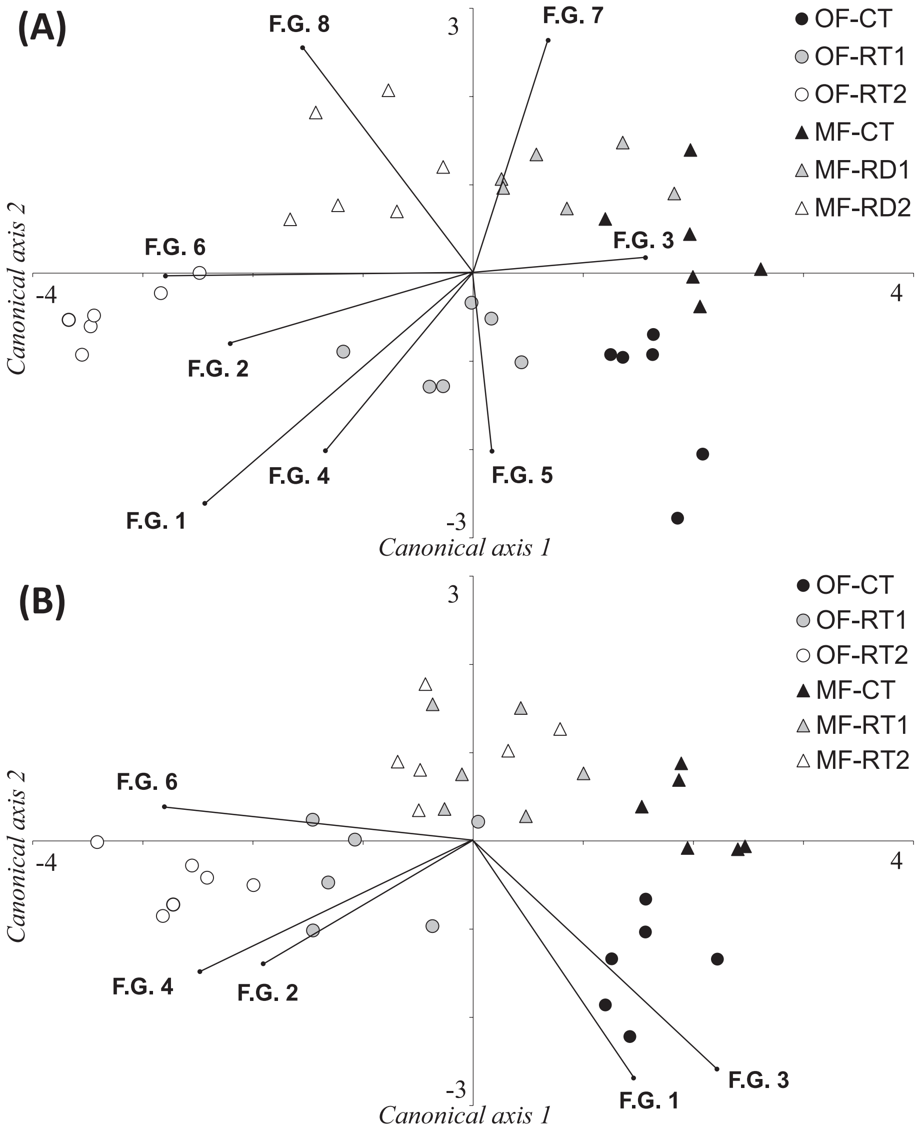

FIGURE 4 | Canonical discriminant analysis (CDA) based on the weed density in each functional groups (FGs) observed in durum wheat (A) and potato (B). Data were combined across the growing seasons. Points (triangles and/or circles) represent experimental treatments. The FG were represented as vectors whose length indicates the degree of association with direction in ordination space. See Table 1 for a description of the functional groups (FG). CT = conventional tillage; MF = mineral fertilizer; OF = organic fertilizer; RT1 = reduced tillage based on spading machine; RT2 = reduced tillage based on subsoiler.

It is important to clarify that the core objective of this study was to assess the ecological implications of noninversion tillage systems in combination with different fertilization strategies. Therefore, the discussion prioritizes the interpretation of weed biomass, density, and community shifts specifically under spading (RT1) and subsoiling (RT2) conditions, which have received limited attention in Mediterranean agroecosystems. The results of this study revealed a relationship between weed biomass and weed density, with both parameters showing an upward trend in the plots managed with alternative soil tillage methods, such as spading and subsoiling. Although RT is a cornerstone of regenerative agriculture, CT remains a key strategy for effective weed management, particularly, in organic farming systems where herbicides are not utilized. The reduction of soil inversion in conservation tillage systems often leads to a gradual increase in weed infestation. CT practices, such as plowing, significantly impact the vertical distribution of weed seeds, dispersing them more uniformly across the soil profile. In contrast, RT systems tend to concentrate weed seeds within the top 5 cm of the soil. This difference in seed distribution can influence weed emergence patterns. Gawęda et al. (2020) reported higher weed density and biomass under NT compared to CT systems. Our findings are consistent with this trend, as weed biomass and density were significantly higher under spading and subsoiling compared to conventional plowing. Furthermore, the results confirm that FGs of weeds are more influenced by RT, often favoring the selection of more competitive weed species (Campiglia et al. 2018). Soil tillage affects weed germination and establishment by creating stimuli, such as scarification, light exposure, and temperature fluctuation, that promote seed dormancy break and germination. These findings align with Armengot et al. (2015), who observed a more competitive weed flora under inversion tillage practices. While previous research has established general patterns of weed suppression

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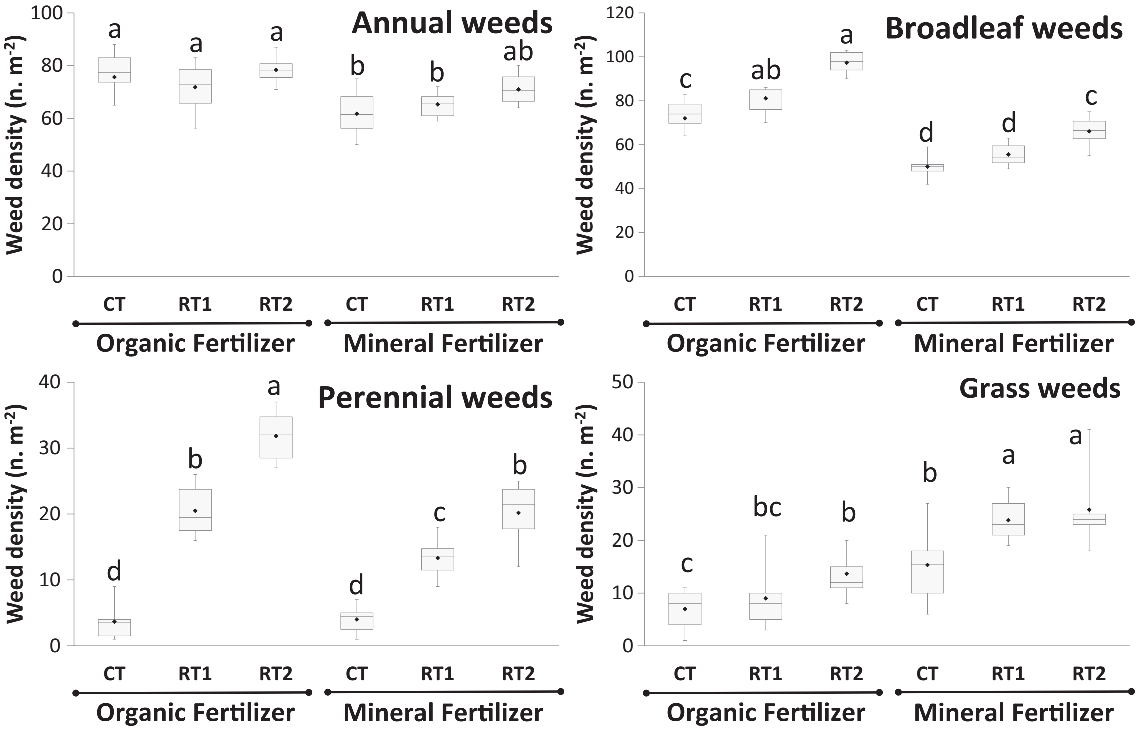

FIGURE 5 | Total weed density of annual and perennial, broadleaf and grass species measured in durum wheat–potato crop rotation under fertilizer source × soil tillage interaction. Broad lines are medians, black dots are means, boxes show the inter-quartile range and whiskers extend to the last data point within 1.5 times the inter-quartile range. Groups with different letters are significantly different. CT = Conventional tillage; RT1 = Reduced tillage based on spading machine; RT2 = Reduced tillage based on subsoiler.

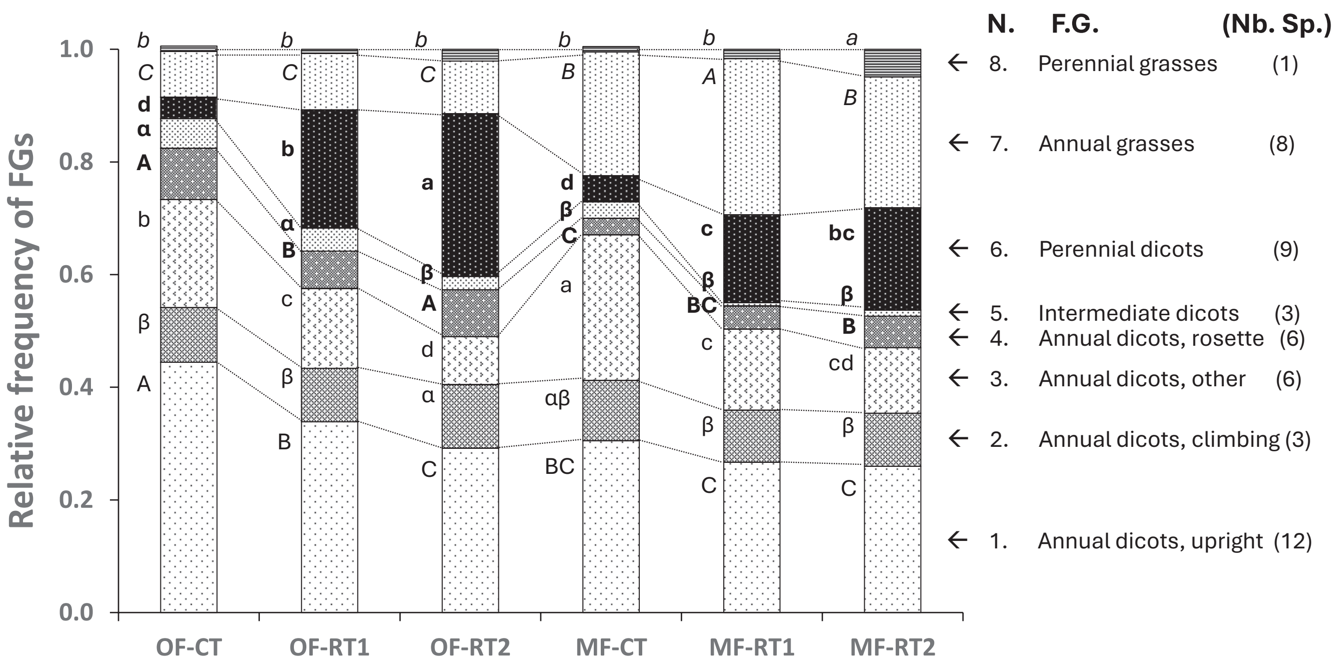

FIGURE 6 | Relative frequency of the eight functional groups related to the weed species across both durum wheat and potato crops in rotation representing the interaction of fertilizer source and soil tillage. The figure reports the main relative frequencies of each functional group (FG). Mean frequencies not reported with the same letter are significantly different between the treatments. CT = conventional tillage; MF = mineral fertilizer; Nb. Sp. = number of weed species within each FG. OF = organic fertilizer; RT1 = reduced tillage based on spading machine; RT2 = reduced tillage based on subsoiler.

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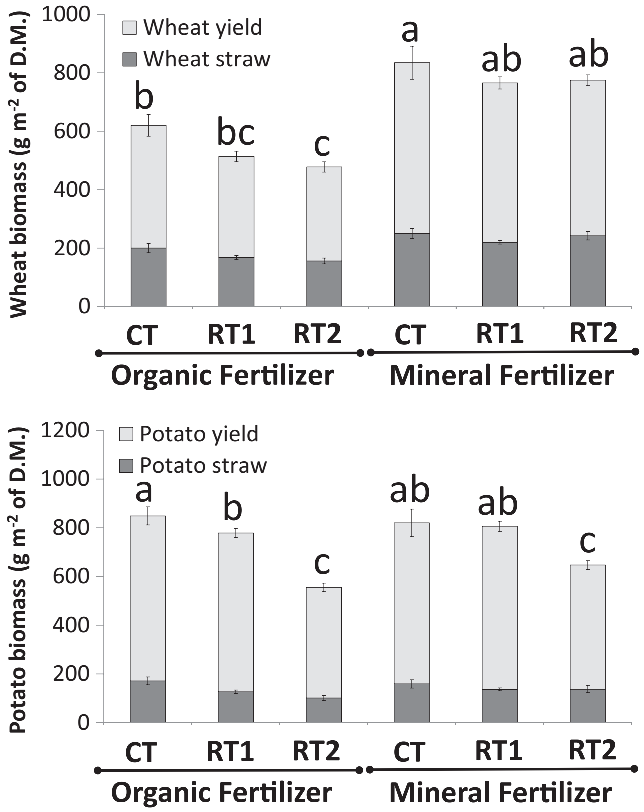

FIGURE 7 | The effect of fertilizer source × soil tillage interaction on the total aboveground biomass (yield + straw) of durum wheat and potato. Values belonging to the same crop with different letters are statistically different according to LSD (0.05). Error bars represent ± standard error from mean (n = 6). CT = conventional tillage; RT1 = reduced tillage based on spading machine; RT2 = reduced tillage based on subsoiler.

under plowing-based systems, the current findings highlight the distinct weed dynamics that emerge under noninversion tillage, particularly, when coupled with OF. Conversely, weed community richness was generally higher under spading and subsoiling systems than under CT (Figure 3). Increased weed abundance does not necessarily correlate with reduced crop yields (Armengot et al. 2015). Instead, diverse weed communities create varied ecological niches, potentially leading to less severe competition compared to systems dominated by a few aggressive weed species (Campiglia et al. 2009). Similarly, these findings are consistent with recent studies reporting higher weed abundance under RT (Hofmeijer et al. 2019; Woźniak 2018), and no tillage systems (Pratibha et al. 2021). The lack of soil inversion allows weed seeds to remain on the soil surface, where they contribute to the soil's food web, encourage predator activity, and enhance overall agroecosystem biodiversity. However, subsoiling tillage often results in higher weed density and biomass, particularly following OF (Campiglia et al. 2018). In this study, both durum wheat and potato crops exhibited greater weed community richness in spading and subsoiling systems, especially under OF compared to MF. Shifts in weed community composition are frequently reported with varying tillage intensities and fertilization programs. For example, perennial grasses and dicots are more common under RT due to lower soil disturbance (Weigel et al. 2024). Similarly, our results indicated an increase in perennial dicots (FG 6) under spading and subsoiling tillage (RT1 and RT2) compared to CT, particularly, in soils amended with OFs. This study confirms such dynamics, highlighting a strong interaction between tillage intensity and fertilization source, which contributes to the functional restructuring of weed communities, even if these findings demonstrate that under non-inversion tillage (spading and subsoiling), OF led to greater weed biodiversity but often lower yield responses in wheat compared to MF. Previous studies have also noted a higher abundance of perennials, such as *C. arvensis*, in RT systems. Shallow tillage implements, such as tines and discs, can facilitate the spread of perennial weeds with creeping roots or rhizomes, increasing their prevalence (Campiglia et al. 2018; Armengot et al. 2016). However, in this study integrating non-inversion tillage systems, such as spading and subsoiling, with OF can enhance weed species richness and ecological complexity, despite possible trade-offs in crop yield. Indeed, regarding crop yield, CT often produces higher yields than RT. For example, several studies reported higher wheat yields under CT (Woźniak and Gos 2014; Woźniak and Rachoń 2020; Jug et al. 2011), while Drakopoulos et al. (2016) observed a 13.4% reduction in potato tuber yield under RT compared to conventional practices. These yield differences are not solely attributable to tillage type but also reflect interactions between tillage and fertilization in increasing the total aboveground biomass (yield + straw) of durum wheat, as it provided nitrogen more efficiently to the crop.

Conversely, in potato, the difference in nitrogen utilization between MF and OFs is less pronounced. This is likely because wheat receives higher nitrogen inputs, which may be underutilized compared to potato, a crop that can utilize nitrogen more efficiently. Consequently, MF tends to perform better for autumn/winter crops. Supporting this, Mancinelli et al. (2023) observed that durum wheat performs better with mineral nitrogen fertilizers, which provide nitrogen in readily available forms critical for the crops during the cold season. A recent meta-analysis by Allam et al. (2022) showed that the effectiveness of OFs in enhancing both soil organic carbon (SOC) and crop yield varied with tillage intensity. The combination of organic and mineral fertilizers (MF) was most effective under CT, while performance decreased progressively under RT and NT systems. Specifically, SOC accumulation under MO treatments was significantly lower under NT compared to RT, likely due to reduced incorporation and mineralization of organic materials. Similarly, crop yield gains were attenuated under NT relative to RT, indicating a possible limitation in nutrient availability under minimal soil disturbance. However, the study also emphasized that the effectiveness of MF and OF depends on several agronomic factors, including climate conditions, soil properties, crop species, and water management. While CT often results in higher yields than RT, it tends to decrease biodiversity by reducing the abundance of perennial, grass, broadleaf, and annual weeds. In potato, CT further reduces weed community richness and biodiversity. OF, on the other hand, appears to favor weed proliferation, except for annual and perennial grasses, while MF enhances crop growth. Despite the high production values recorded under CT combined with plowing and MF, this system tends to reduce weed over time by selecting for a few competitive weed species, in agreement with previous studies (Travlos et al. 2018; Feledyn-Szewczyk et al. 2020). In contrast, Edesi

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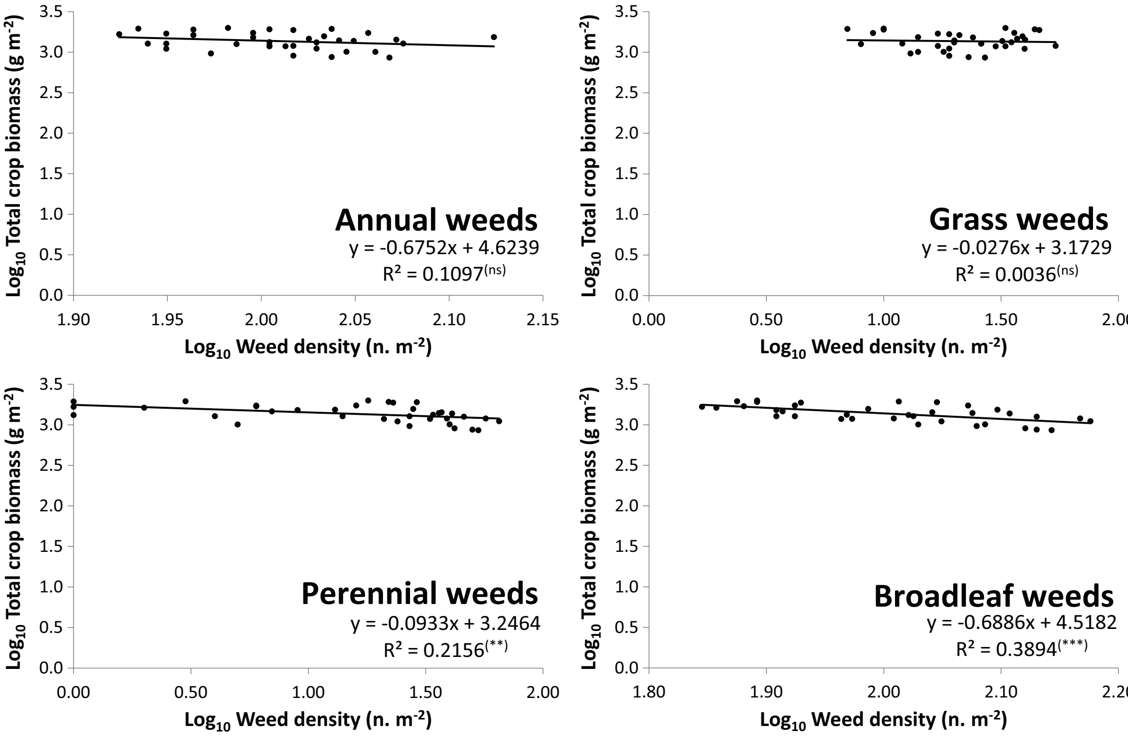

FIGURE 8 | Relationship between total crop biomass and annual weeds (A), perennial weeds (B), grass weeds (C), and broadleaf weeds (D).

et al. (2012) observed that organically managed systems tend to support a greater number of weed species compared to conventionally managed systems. According to Cordeau et al. (2021), nitrophilic dicots are often associated with high soil N concentrations derived from inorganic N fertilizer. Moreover, the higher association of FGs with OF in both crops suggests greater variability in weed species composition under organic systems. The relative frequency of weed FGs indicates that annual (FG 7) and perennial grass (FG 8) are strongly associated with MF. Grass weeds are well-known to be nitrophilic species (Prober and Lunt 2009). In contrast, weed biodiversity is generally higher under OF, as reported by (Petroselli et al. 2021). Numerous studies have demonstrated that in CT systems, which involve high soil disturbance, tend to favor annual broadleaf species, whereas NT or RT systems favor perennial weeds and species that can germinate on the soil surface, such as annual grasses (Bàrberi et al. 2001; Menalled et al. 2001; Taa et al. 2004). Similarly, Feledyn-Szewczyk et al. (2020) reported a higher proportion of perennial and invasive weed species under RT and NT systems. These findings suggest that nitrogen fertilization source significantly influences weed composition, with nitrophilic species benefiting from the readily available nitrogen provided by MFs. A similar trend was observed in both crops, with durum wheat showing a greater difference, possibly due to the higher nitrogen uptake by grass weeds compared to dicots during the winter season. In contrast, under OF, reduced soil nitrogen availability supports the establishment of more dicots in both crops. This is likely due to the nitrogen shortage limiting the proliferation of nitrophilous species. Annual weeds increased under OF in potato, whereas no significant differences were observed between fertilization sources in durum wheat. Overall, annual weed density was highest under OF, being 14% higher than under MF. Specifically, annual weed density under OF-CT and OF-RT1 were 23% and 10% higher than under MF-CT and MF-RT1, respectively. The integration of alternative management practices, such as subsoiling and OF, can help achieve ecological goals by enhancing weed species biodiversity and improving agroecosystem management and soil conditions.

5 | Conclusion

The results of this study confirm that the functionality of agroecosystems is intricately linked to complex interactions between agro-environmental conditions and agronomical practices. In this context, weeds are a vital component of the agroecosystems, and their biodiversity significantly contributes to agricultural system functionality and should not be overlooked. OF, particularly when combined with subsoil tillage, consistently results in a more diverse weed community. A negative relationship was observed between total crop biomass and the presence of broadleaf and perennial weed species, indicating that these weed groups may exert competitive pressure on crops, thereby reducing overall productivity. The integration of RT with OF presents a viable strategy for sustainable agriculture, benefiting the overall agroecosystem by promoting weed biodiversity. However, an increased frequency of perennial weeds under RT represents a critical challenge. Innovative approaches are required to effectively manage these weeds and mitigate their impact under RT systems. These findings underscore the intricate relationships between agricultural practices, weed communities, and crop productivity. They highlight the need for carefully planned and nuanced approaches to sustainable farming. Further research is essential to explore the long-term implications of these practices and to refine weed management strategies across diverse cropping systems.

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Author Contributions

Emanuele Radicetti and Roberto Mancinelli contributed to the study conception and design, material preparation, data collection, and data analysis. All authors contributed to data interpretation and discussions. The first draft of the manuscript was written by Mortadha Ben Hassine, Daniele Borgatti, Aftab Jamal, and Alireza Taab; the remaining authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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