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ORIGINAL ARTICLE

The effect of ground cover plants in apple orchards on soil-dwelling Collembola

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Abstract

Ground cover plants in orchards can effectively improve soil quality. One factor determining soil health is the presence of fauna, including mesofauna, which play a crucial role in soil ecosystems. However, the relationship between ground cover and Collembola assemblages in orchards remains underexplored. This study investigated how different ground cover plants sown in rows of apple trees influence the abundance and diversity of Collembola. Conducted at the Research Station of Wrocław University of Environmental and Life Sciences, Poland, the experiment utilized three cover species: *Tagetes patula*, *Festuca ovina*, and *Agrostis capillaris*, with fallow plots serving as control samples. Soil samples were collected over 2 years (2015–2016) to assess springtails richness and species composition. Results indicated that springtails were significantly more abundant in soils managed with ground cover plants than in conventionally managed fallow stands. Notably, the highest mean Collembola numbers were recorded in strips planted with *T. patula* and *F. ovina*. The springtail communities were primarily dominant in each of the treatments by two eudaphic species, *Mesaphorura macrochaeta* and *Hypogastrura assimilis*. These findings underscore the importance of cover crops in sustainable agriculture by reducing herbicide reliance, enhancing soil aeration, improving soil fertility through organic matter, and fostering biodiversity of soil biota.

Keywords: living mulches, species richness, springtails, sustainable horticulture

Introduction

The principles of integrated and organic farming systems include appropriate tillage to maintain well-structured soils, the use of techniques to prevent soil erosion (grazing, planting, mulching), and the reduction or even elimination of chemical inputs (Babita *et al.* 2020; Meena *et al.* 2020). One effective method to improve soil quality while simultaneously reducing the use of herbicides in agricultural crops is the utilization of cover crops (Mia *et al.* 2021; Devi *et al.* 2023). A cover crop is defined as any living ground cover that is planted either after the harvest of a main crop or during its growth, and is subsequently ploughed under before planting the next crop (Hartwig and Ammon 2002). Another form of soil cover management is living mulch, which consists of ground cover plants that are sown either before or simultaneously with a main crop and allowed to grow alongside it throughout the growing season (Qian *et al.* 2015). Fang *et al.* (2021) have demonstrated that living mulches can be beneficial in orchards, contributing to better fruit quality and yield. Living mulches are an example of a balanced soil management method that provides increased soil aggregate stability, improved soil water balance, adequate soil temperature and sufficient weed control (Cao *et al.* 2016; Dudás *et al.* 2016). Furthermore, there are indications that living mulches promote the abundance of soil biota. Consequently, the increased abundance of microflora, from the decomposition of plant residues, provides food for soil-dwelling arthropods (McDaniel *et al.* 2014; Gruss and Twardowski 2016; Birkhofer *et al.* 2019). As reported by Ji *et al.* (2022), the use of cover crops leads to an increase in the number of natural enemies of orchard pests. This suggests that ground covers have a beneficial effect on soil health at the biological level and hence on edaphic fauna biodiversity (Li *et al.* 2023).

Biodiversity has been shown to be a critical factor in promoting the stability of an agroecosystem and enhancing its services (Kazemi *et al.* 2018; Capelli *et al.* 2022; Mairata *et al.* 2023). A significant reservoir of global biodiversity exists below the soil surface, including microorganisms, nematodes, earthworms, arachnids, insects, and other arthropods (Nielsen *et al.* 2015; Anthony *et al.* 2023). Among the most abundant and diverse groups of soil fauna are Collembola (Potapov *et al.* 2020). These organisms are considered to provide essential agroecosystem services such as: decomposition of organic matter, nutrient cycling and suppression of plant pathogens or formation of soil microstructure (Nielsen *et al.* 2015; Hanisch *et al.* 2022; Bhattacharyya and Kumari 2023). The sensitivity of springtails to environmental perturbations predisposes them to be indicators of soil biological properties (Machado *et al.* 2019; Arenhardt *et al.* 2021; Chowdhury *et al.* 2023). The distribution of food resources in the soil, including plant roots and organic debris, is thought to trigger spatial aggregation of springtails (Rzeszowski *et al.* 2017; Zhang *et al.* 2023). Regarding the feeding behavior of Collembola, surface-dwelling species obtain nutrients mainly from litterfall, whereas deep-dwelling species often graze on plant roots. Several studies have shown that Collembola can feed on different groups of microorganisms (fungi, algae and bacteria) found in the vicinity of roots (Chauvat *et al.* 2014; Pommeresche and Løes 2014; Gruss *et al.* 2022). This could be the reason for the high abundance of Collembola reported in fields cultivated with certain types of living mulch, which increases the abundance of soil fungi (Bokova *et al.* 2023). This suggests that ground covers may have an effect on food chains (Dudás *et al.* 2016). Regarding the interactions between springtails and plant roots, the results reported by Fuji *et al.* (2014) demonstrated the dependence of Collembola on carbon from living roots and the contribution of springtails to nutrient uptake by plant roots. In general, this data can help soil management practices to improve the functioning of sustainable edaphic ecosystems in the future (Bokova *et al.* 2023).

This research hypothesized that the implementation of cover crops enhances soil health by promoting biodiversity, specifically beneficial soil-dwelling Collembola. To investigate this hypothesis, the effects of three cover plant species, French marigold (*Tagetes patula*), sheep fescue (*Festuca ovina*), and colonial bent grass (*Agrostis capillaris*) on soil Collembola abundance were studied and compared to herbicide-treated fallow plots used as a control.

Materials and Methods

Experimental site

The sampling sites were located at the Fruit Experimental Station in Samotwór, belonging to the Wroclaw University of Environmental and Life Sciences, in southwestern Poland (51°06'12"N, 16°49'52"E). The climate of the study area is transitional between maritime and continental. The study was carried out in the experimental sector of apple trees (*Malus domestica* Borkh.). Cv. 'Ligol' apple trees were planted in the spring of 2004 in rows with a spacing of 3.5 × 1.2 m, giving 2380 trees per hectare. The experimental factors included three types of cover crops: annual French marigold (*T. patula*), perennial sheep fescue (*F. ovina*) and perennial colonial bent grass (*A. capillaris*) and herbicide fallow as the control placed in rows of trees. The experiment was set up as a split plot with four replicates.

Soil conditions and agrotechnical practices

The trial was conducted on Luvisol soil with a granulometric structure of light silty loam, representing the third class of the Polish soil classification. The living mulches were sown in the spring of 2004 (*A. capillaris* cv. 'Frasek' – 34 kg of seeds · ha^{-1}, *T. patula* cv. 'Carmen' or 'Kolumbina' – 10 kg of seeds · ha^{-1}) and in spring of 2005 (*F. ovina* cv. 'Edolana' – 30 kg seeds · ha^{-1}). The sowing of *T. patula* in the following years was renewed with a dose of 15 kg of seeds per hectare.

In the year of sowing perennial species (*A. capillaris* and *F. ovina*), manual weed control was carried out within the living mulch sod. In the following years, weeds were removed with a hand hoe the only ones per growing season. In case of *F. ovina* herbicide mixture containing glyphosate (Klinik Duo 360 SL at a dose of 4 l · ha^{-1} or Glifogan 360 SL in 8 l · ha^{-1}) and 2-methyl-4-chlorophenoxyacetic acid (Agritox 500 SL at a dose of 1.5–2 l · ha^{-1}) was also used.

In the following years, mechanical weed control was carried out each time before sowing French marigold, and herbicides were also applied at the end of the French marigold growing season (November), with 2-methyl-4-chlorophenoxyacetic acid (Chwastox Extra 300 SL at a dose of 3.5 l · ha^{-1}) and glyphosate (Roundup at a dose of 360 SL (8 l · ha^{-1}).

The herbicide fallow was maintained yearly, with a herbicide mixture: glyphosate and MCPA (2-methyl--4-chlorophenoxyacetic acid) as the active ingredients at doses of $4\ l \cdot ha^{-1}$ and $2\ l \cdot ha^{-1}$, respectively. In the first year after orchard establishment, spraying was performed twice (June and November, glyphosate only). In subsequent years, the herbicide mixture was applied in May, July and in November, if necessary.

Soil samples and laboratory analysis

In 2015 and 2016 soil samples were collected three times during the growing seasons, in May, June and July. There were four plots with: French marigold (*T. patula*), sheep fescue (*F. ovina*), colonial bent grass (*A. capillaris*), and herbicide fallow. Each plot covered an area of 18 m^2 (1 meter wide and 18 meters long), in the tree rows. Five samples were collected at 2-meter intervals along the length of each plot. A metal core sampler (5 cm diameter, 10 cm depth) with a cutting edge was used for sampling. Soil arthropods were extracted using the Murphy modified Tullgren apparatus (Murphy 1956) and then preserved in 75% ethanol for 48 h. The soil fauna was observed under a stereomicroscope to detect springtails. Collembola were then mounted in Hoyer's medium and identified according to Hopkin (2007).

Statistical analysis

A General Linear Model (GLM) was used to analyze the relationship between explanatory variables (plant cover type and sampling time) and response variables (abundance of Collembola and diversity indices). To ensure that the model assumptions were met, we checked for normality, variance homogeneity, and residuals' independence. The normality of residuals was assessed using the Shapiro-Wilk test (the normal distribution was rejected), while the homogeneity of variance was checked using Levene's test. The explanatory variables were the type of plant cover (treatment) and sampling time (May, June and July), while the response variables were the abundance of Collembola or diversity indices values per sample. The analyses were performed separately for two seasons (2015 and 2016).

Additionally, the significance of differences between treatments was analyzed using the post-hoc Tukey test. The following diversity indices were calculated: Simpson diversity index, Shannon-Wiener index, Pielou evenness index based on the Collembola species or genera. The indices were calculated for each plot (the row data from five subsamples from each plot were summarized). The data were presented as means per treatment. Data analysis was carried out using SAS University Edition 9.4.

The Simpson diversity index was calculated according to the following formula:

$$D = 1 - \sum \frac{n(n-1)}{N(N-1)},$$

where:

n – number of individuals of a single species,

N – number of individuals in total population.

The Shannon-Wiener index was calculated using the following equation:

$$H' = -\sum_{i=1}^{R} p_i \ln p_{i,}$$

where:

R – number of species,

ln – natural log,

p_i – proportion of individuals in the sample belonging to the ith species.

For calculating the Pielou evenness index the following formula was used:

$$D_{pie} = 1 - \sum_{i=1}^{S} \left(\frac{n_i(n_i - 1)}{N(N-1)} \right),$$

where:

S – number of species,

n_i – the abundance of the nth species,

N – the total abundance of each species.

Results

The mean number of Collembola varied significantly between treatments (Tab. 1 and 3). In 2015, the highest mean abundance of Collembola was observed in the *T. patula* treatment, followed by *F. ovina*. The lowest numbers were recorded in the *A. capillaris* and fallow treatments, with the fallow treatment having significantly lower Collembola abundance than *A. capillaris* (Tab. 1). Similar trends were observed in 2016, with *T. patula* and *F. ovina* again showing higher Collembola abundance than *A. capillaris* and the fallow plots (Tab. 3).

In both years of the study, no significant differences were observed in the species diversity indices across treatments (Tab. 1 and 3). The mean number of species ranged from 12.33 to 15.00 in 2015 and from 11.33 and 16.67 in 2016 (Tab. 1 and 2). In 2016, there was a non-significant decrease in species diversity and the Simpson index in the fallow treatment compared to the cover crop treatments (Tab. 3). A similar trend was observed in 2015, where non-cover crop (fallow) also exhibited the lowest Simpson index value (Tab. 1).

There were significant fluctuations in the three sampling terms in Collembola abundance and diversity (Tab. 2 and 4). In 2015, while the mean number of

Table 1. Mean abundance, species richness, and diversity indices of Collembola in three ground cover treatments and fallow in the apple orchard (2015)

	Tagetes patula	*Festuca ovina*	*Agrostis capillaris*	Fallow	F	p	Df
Simpson index	0.32 ± 0.07 a*	0.34 ± 0.14 a	0.25 ± 0.09 a	0.22 ± 0.05 a	1.18	0.38	3
Shannon-Weaver index	1.58 ± 0.27	1.56 ± 0.42 a	1.74 ± 0.36 a	1.88 ± 0.19 a	0.66	0.60	3
Pielou index	0.58 ± 0.06 a	0.58 ± 0.13 a	0.70 ± 0.07 a	0.72 ± 0.05 a	2.24	0.16	3
Mean number	398.67 ± 146.32 a	321.67 ± 72.71 b	184.33 ± 115.01 c	212.67 ± 73.16 aab	45	< 0.001	3
Mean number of species	15.00 ± 2.65 a	15.33 ± 2.08 a	12.33 ± 3.06 a	13.33 ± 1.15 a	1.09	0.41	3

*the data are presented as means of treatment ± standard deviation. Different lowercase letters in columns indicate significant differences between variants ($p \leq 0.05$; GLM model, post hoc Tukey's test).
F, p – results of the general linear model (GLM)
Df – degrees of freedom

Table 2. Mean abundance, species richness, and diversity indices of Collembola in three sampling times (months) (2015)

	May	June	July	F	p	Df
Simpson index	0.36 ± 0.08 a*	0.21 ± 0.12 a	0.27 ± 0.10 a	4.04	0.056	2
Shannon-Weaver index	1.41 ± 0.19 b	1.97 ± 0.22 a	1.69 ± 0.25 b	7.20	0.013	2
Pielou index	0.57 ± 0.21	0.73 ± 0.38 a	0.64 ± 0.23 a	3.22	0.09	2
Mean number	212.75 ± 95.01 a	239.75 ± 31.40 a	385.5 ± 104.70 a	0.27	0.77	2
Mean number of species	15.00 ± 1.33 a	15.75 ± 2.31 a	14.25 ± 4.01 a	3.83	0.06	2

*the data are presented as means of treatment ± standard deviation. Different lowercase letters in columns indicate significant differences between variants ($p \leq 0.05$; GLM model, post hoc Tukey's test).
F, p – results of the general linear model (GLM)
Df – degrees of freedom

Table 3. Mean abundance, species richness, and diversity indices of Collembola in three ground cover treatments and fallow in the apple orchard (2016)

	Tagetes patula	*Festuca ovina*	*Agrostis capillaris*	Fallow	F	p	Df
Simpson index	0.30 ± 0.11 a*	0.28 ± 0.07 a	0.29 ± 0.06 a	0.24 ± 0.05 a	0.35	0.79	3
Shannon-Weaver index	1.66 ± 0.28 a	1.69 ± 0.23 a	1.59 ± 0.20 a	1.67 ± 0.20 a	0.12	0.95	3
Pielou index	0.60 ± 0.08 a	0.60 ± 0.08 a	0.58 ± 0.05 a	0.69 ± 0.07 a	0.96	0.46	3
Mean number	481.33 ± 69.17 a	474.67 ± 97.00 a	382.33 ± 43.32 ab	231.0 ± 17.09 c	45	< 0.001	3
Mean number of species	16.00 ± 2.65 a	16.67 ± 0.58 a	15.33 ± 2.08 a	11.33 ± 0.58 a	5.74	0.02	3

*the data are presented as means of treatment ± standard deviation. Different lowercase letters in columns indicate significant differences between variants ($p \leq 0.05$; GLM model, post hoc Tukey's test).
F, p – results of the general linear model (GLM)
Df – degrees of freedom

Table 4. Mean abundance, species richness, and diversity indices of Collembola in three sampling times (2016)

	May	June	July	F	p	Df
Simpson index	0.32 ± 0.06 a*	0.21 ± 0.10 b	0.30 ± 0.13 a	6.40	0.019	2
Shannon-Weaver index	1.62 ± 0.23 b	1.86 ± 0.19 a	1.47 ± 0.17 b	10.57	0.0043	2
Pielou index	0.59 ± 0.18 b	0.70 ± 0.24 a	0.57 ± 0.29 b	4.35	0.05	2
Mean number	410.25 ± 105.2 a	351.50 ± 40.6 a	415.25 ± 93.40 a	0.27	0.77	2
Mean number of species	16.25 ± 1.01 a	14.75 ± 0.95 a	13.50 ± 2.23 a	1.13	0.36	2

*the data are presented as means of treatment ± standard deviation. Different lowercase letters in columns indicate significant differences between variants ($p \leq 0.05$; GLM model, post hoc Tukey's test).
F, p – results of the general linear model (GLM)
Df – degrees of freedom

springtails remained consistent across the season (no significant differences), the Shannon-Weaver index showed significant variation, with the highest species richness occurring in June compared to May and July (Tab. 2). Similarly in 2016, the Simpson, Shannon--Weaver and Pielou indices showed significantly higher species diversity in June than in May and July. These results suggest that mid-summer conditions may enhance Collembola diversity, though overall abundance remained stable across the season.

Overall, *Mesaphorura macrochaeta* was the predominant species in the Collembola communities studied in 2015 and 2016, accounting for 28.3 to 51.3% of all individuals (Fig. 1 and 2), with the exception of *A. capillaris* treatment where *Hypogastrura assimilis* was the predominant species comprising 37% of all

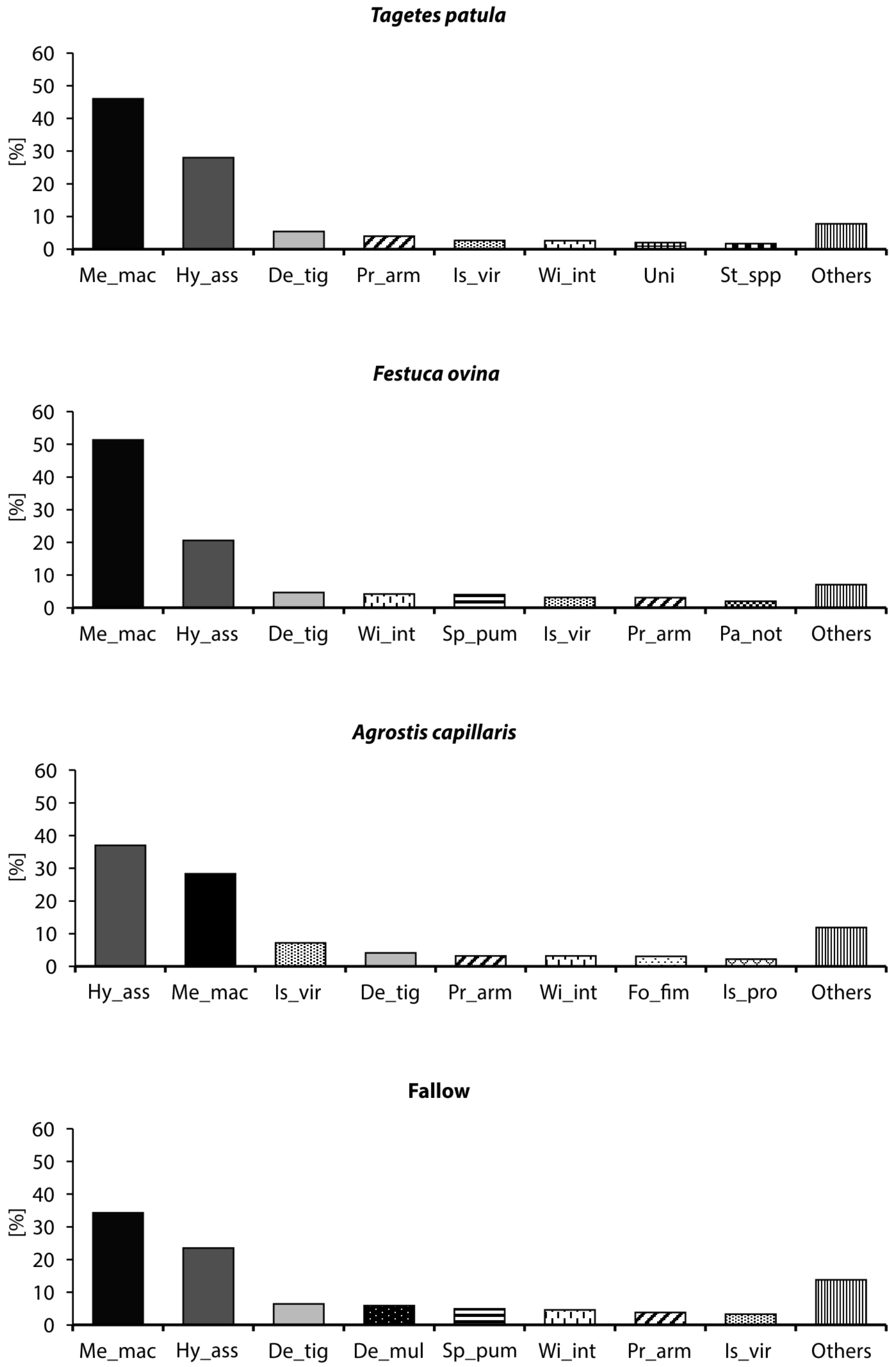

Fig. 1. Frequency of the most abundant species of springtails in three ground cover treatments and fallow in the apple orchard in 2015. Me_mac – *Mesaphorura macrochaeta* (Rusek), Hy_ass – *Hypogastrura assimilis* (Krausbauer), De_tig – *Desoria tigrina* (Nicolet), Pr_arm – *Protaphorura armata* (Tullberg), Is_vir – *Isotoma viridis* (Bourlet), Wi_int – *Willemia intermedia* (Mills), Uni – unidentified, St_spp – *Stenaphorura* spp. (Absolon), Sp_pum – *Sphaeridia pumilis* (Krausbauer), Pa_not – *Parisotoma notabilis* (Schäffer), Fo_fim – *Folsomia fimetaria* (Linnaeus), Is_pro – *Isotomodes productus* (Axelson), De_mul – *Desoria multisetis* (Carpenter & Phillips)

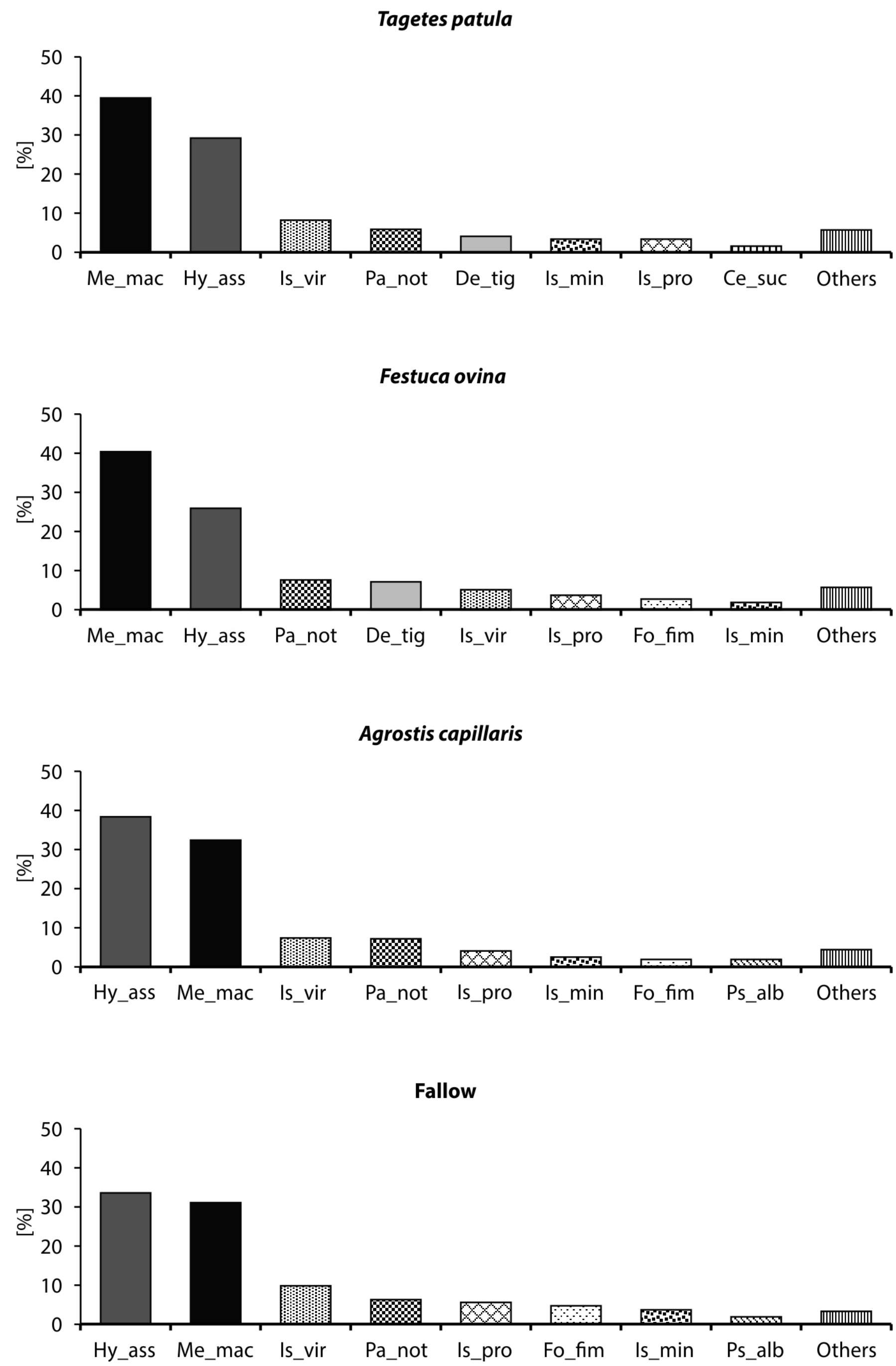

Fig. 2. Frequency of the most abundant species of springtails in three ground cover treatments and fallow in the apple orchard in 2016. Me_mac – *Mesaphorura macrochaeta* (Rusek), Hy_ass – *Hypogastrura assimilis* (Krausbauer), De_tig – *Desoria tigrina* (Nicolet), Is_vir – *Isotoma viridis* (Bourlet), Pa_not – *Parisotoma notabilis* (Schäffer), Ce_suc – *Ceratophysella succinea* (Gisin), Fo_fim – *Folsomia fimetaria* (Linnaeus), Is_pro – *Isotomodes productus* (Axelson), Is_min – *Isotomiella minor* (Schäffer), Ps_alb – *Pseudosinella alba* (Packard)

individuals in 2015 and 38.4% in 2016. In 2015, other species contributed less than 15% of the total Collembola population in each treatment, while in 2016, their contribution remained below 10%.

Further analysis of Collembola species composition showed that some of the species that did not occupy the orchard soil environment in one year of study (or appeared in small amounts) were present in another year. Two eudaphic species, *Protahporura armata* and *Willemia intermedia* inhabited the soil under all types of ground cover plants and fallow plots in 2015, whereas in 2016 none of these species were found in soil samples. The least numerous species, *Lepidocyrtus violaceus* and *Micranurida pigmaea,* were found in every treatment in 2015, but in 2016 these species were undetected (Tab. 5 and 6). In both years of the study the most common family was Isotomidae (38% of all examined species) with the most frequent species

Table 5. Collembola species described as Others in Fig. 1, extracted from soil samples in 2015

	Tagetes patula	*Festuca ovina*	*Agrostis capillaris*	Fallow
Sphaeridia pumilis (Krausbauer)	+		+	
Folsomia fimetaria (Linnaeus)	+	+		+
Isotomiella minor (Schäffer)	+	+	+	+
Parisotoma notabilis (Schäffer)	+		+	+
Desoria multisetis (Carpenter & Phillips)	+	+	+	
Unidentified		+	+	+
Stenaphorura spp. (Absolon)		+	+	+
Lepidiocyrtus violaceus (Geoffroy)	+	+	+	+
Isotomodes productus (Axelson)	+	+		+
Pseudosinella alba (Packard)	+	+	+	+
Micranurida pigmaea (Börner)	+	+	+	+
Folsomia spp.	+	+	+	
Folsomides parvulus (Stach)	+	+	+	+
Brachystomella parvula (Schäffer)	+	+	+	
Bourletiella hortensis (Fitch)		+		

(+) – means that the species was present in the treatment

Table 6. Collembola species described as Others in Fig. 2, extracted from soil samples in 2016

	Tagetes patula	*Festuca vina*	*Agrostis capillaris*	Fallow
Desoria tigrina (Tullberg)			+	+
Folsomia fimetaria (Linnaeus)	+			
Pseudosinella alba (Packard)	+	+		
Stenaphorura spp. (Absolon)	+	+	+	+
Sphaeridia pumilis (Krausbauer)	+	+	+	+
Folsomia spp.	+	+	+	+
Unidentified	+	+	+	+
Ceratophysella succinea (Gisin)		+	+	
Folsomides parvulus (Stach)	+	+	+	
Bourletiella hortensis (Fitch)	+	+	+	
Desoria multisetis (Carpenter & Phillips)	+	+	+	
Hypogastrura manubrialis (Tullberg)	+	+	+	+
Orchesella cincta (Linnaeus)		+	+	
Entomobyra marginata (Tullberg)	+		+	+
Orchesella villosa (von Linné)		+	+	
Mesaphorura florae (Simón, Ruiz, Martin & Luciáňez)	+		+	
Frisea truncata (Cassagnau)	+			

(+) – means that the species was present in the treatment

being: *Isotoma viridis, Desoria tigrina, Parisotoma notabilis, Isotomodes productus, Folsomia fimetaria* and *Isotomiella minor*. Analyzing the life forms of Collembola, it can be concluded that ground cover plants contributed to a domination of eudaphic species in the first year of study and then an increase in number of hemiedaphic species in the second year. The most noticeable change in the hemiedaphic Collembola number was observed for *T. patula,* totaling two species in 2015 (*H. assimilis* and *D. tigrina*) and six species in 2016 (*H. assimilis, P. notabilis, D. tigrina, I. minor, I. productus* and *Ceratophysella succinea*) (Fig. 1 and 2). Species with frequency below 3% were grouped as "others" and among them the most abundant were *Pseudosinella alba*, *F. fimetaria* and *I. minor* in 2015 in fallow plots (Tab. 5 and 6).

Discussion

Undisturbed or poorly used habitats in orchards can lead to an increase in the diversity of different organisms. The importance of undisturbed understory between olive trees has been demonstrated by Stavrianakis *et al.* (2024) for ground arthropods and flying insects. Plant cover and land-use management also can significantly influence Collembola density and diversity (Vanhée and Devigne 2018). For example, the control cultivated forest had a higher diversity of Collembola, possibly due to lower levels of disturbance than the arable and afforested areas (Harta *et al.* 2020). As noted by Cezar *et al.* (2015), the frequency and species composition of Collembola was higher in agroforestry systems undergoing regular pruning than in natural regeneration areas in the early stage of succession. Although the two types of ecosystems were similar in terms of low disturbance levels, the higher plant density in an agroforest was preferable due to favorable conditions for food supply and a prospect of protection against predatory species. Cognately, in the present study the ground cover plants provided appropriate conditions for springtails to live, whereas the fallow plots were depauperate in Collembola. In orchard agroecosystems there is limited data about the influence of ground cover on Collembola assemblages (Pfingstmann *et al.* 2019; de Pedro *et al.* 2020). This article represents the first attempt to show the influence of *T. patula* on the biodiversity of springtails.

Tagetes patula (Asteraceae), commonly known as French marigold, has been used as an ornamental plant, food coloring and insect repellent with potential biological activities. Also, it is a rich source of lutein (Gongalla 2020). The extract of *T. patula* shows significant potential for antifungal properties due to its high content of terthiophenes and insecticidal activity against hemipteran pests (Fabrick *et al.* 2020; Drumea *et al.* 2022). Furthermore, this asteraceous plant secretes a variety of lethal compounds against nematodes, which are killed as a result of physical contact with the root system or soil containing marigold root exudates (Karakas and Bolukbasi 2019). *Festuca ovina* and *A. capillaris* (Poaceae) are heavy metal uptake tolerant species (Woch *et al.* 2016; Dradrach *et al.* 2020). The main finding of this study is that *T. patula* as well as *F. ovina* may improve the density of springtail assemblages which may be due to the impact of Asteracae and *Festuca* plants on soil organisms and soil properties. The invasive species of the Asteraceae family in China alters soil nutrient levels, increasing nitrogen and decreasing phosphorus, with mycorrhizal symbiosis that potentially promotes their invasion (Chen *et al.* 2015). Perhaps the occurrence of a positive impact on the mycorrhizal fungal further promotes the Collembola, which is known from selective graze on arbuscular mycorrhizal (AM) fungi (Innocenti and Sabatini 2018). However, there is a lack of supporting data for this assumption. Furthermore, the positive effect of *F. ovina* on Collembola abundance could potentially be attributed to its positive impact on soil fertility. Another *Festuca* species (*F. arundinacea* Schreb.) was found to improve soil properties and fertility, mainly urease activity as an indicator of soil health (Peng *et al.* 2021).

As noted by Miyazawa *et al.* (2002), small changes in the soil environment due to biocide application may significantly affect springtail communities even before the changes in soil properties become detectable. In the present study seasonal changes had a clear impact on Collembola diversity, with diversity and evenness indices peaking in June in both 2015 and 2016, suggesting that favorable mid-summer conditions support a broader range of species. These patterns can reflect the influence of increased soil moisture and temperature (which was not measured in this study), which enhance Collembola activity and reproduction (Aupic-Samain *et al.* 2020). Considering the low abundance of Collembola in the fallow field, some studies suggest that certain herbicides may lead to reduced populations and biodiversity under field conditions (Altmanninger *et al.* 2023; Beaumelle *et al.* 2023). In this study, two herbicide active ingredients were used: MCPA and glyphosate. The effect of MCPA on Collembola is still unknown. However, glyphosate applications have been shown to reduce Collembola assemblages in both controlled environments (Torres-Moya and Dotor-Robayo 2020) and under field conditions (Pereira *et al.* 2018). Results from other laboratory studies showed that most glyphosate-based herbicides did not appear to affect Collembola at the recommended field dose (Niemeyer *et al.* 2018).

Generally, Collembola are known to be omnivorous, and they exhibit considerable diversity in their morphological and functional traits, with a wide range of food sources and some reports of predatory behavior towards nematodes (Manwaring *et al.* 2015; Beet *et al.* 2022). Research shows that these springtail species can be classified into three primary functional groups based on their habitat preferences in soil ecosystems: epigeic (living on the soil surface), hemiedaphic (associated with litter layers) and eudaphic (living within the soil) (Joimel *et al.* 2021). The dominant species in the *T. patula* and *F. ovina* strips was *M. macrochaeta*, the eudaphic Collembola species well adapted to soil life and with limited migratory abilities (Vanhée and Devigne 2018). In field experiments, eudaphic Collembola responded more obviously to soil degradation (Ma *et al.* 2023) or were more closely related to vegetation type (Potapov *et al*. 2016), indicating their potential role as better indicators of soil quality than hemiedaphic and epigeic Collembola. On the other hand, the dominant species in *A. capillaris* and fallow plots was the hemiedaphic *H. assimilis*. However, it has been shown that arbuscular mycorrhizal colonization of *A. capillaris* can be enhanced by *M. macrochaeta* (Cole *et al*. 2004), which was the second most abundant species in the vicinity of *A. capillaris* roots in both years of the subsequent study. In general, the analysis of Collembola species diversity showed a lack of relation between the species of a groundcover and the composition of springtails. Recent evidence shows that organically managed soils have highly abundant communities of *Mesaphorura* spp. (Pommeresche and Løes 2014; Jasiński *et al*. 2016). It has been shown that some Poaceae plants are able to suppress soil compaction, on which the abundance of eudaphic Collembola is highly dependent (Mottin *et al*. 2018; Betancur-Corredor *et al*. 2022; Lagendijk *et al*. 2022). This may explain the relatively high abundance of springtails in the *F. ovina* strips. Also, perennial grasses have been proven to be able to significantly enrich the soil with organic matter (Skersiene *et al.* 2024) that serves as the main source of food for soil organisms. On the other hand, given the toxic effect of marigold root exudates on nematodes, we can hypothesize that there is a certain amount of dead organic matter around marigold roots that attracts Collembola.

Conclusions

The use of living mulches has a significant positive impact on Collembola assemblages, contributing to enhanced soil biodiversity and ecosystem functioning. This study demonstrated that inter-row cover crops, particularly *T. patula* and *F. ovina*, significantly increased the abundance of Collembola compared to herbicide fallow treatments. The increased presence of these soil-dwelling organisms, which play a critical role in nutrient cycling and soil health, highlights the ecological benefits of cover crops in orchard management. By enriching the soil with organic matter and supporting beneficial soil fauna, cover crops promote a healthier, more resilient agroecosystem. These findings suggest that adopting *T. patula* and *F. ovina* as cover crops in apple orchards can not only reduce reliance on chemical inputs but also enhance soil biodiversity, making it a sustainable alternative to herbicide-based practices.

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