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> Effects of vineyard and olive orchards management practices on soil arthropods, with particular reference to Collembola

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## List of pubblications

- Guarino, R., Vrahnakis, M., Rodriguez Rojo, M.P., Giuga, L., and Pasta. S., 2020 Grasslands and shrublands of the Mediterranean region. In: Della Sala DA and Goldstein MI (eds.) The Encyclopedia of the World's Biomes, first edition, vol. 3: 638-655. Oxford: Academic Press— Elsevier.
- Giuga, L., Jordana, R., Baquero, E., & Lo Verde, G., 2022 New records of springtails (Collembola) from Sicily, Italy. REDIA, 105: 107-114. <u>https://doi.org/10.19263/REDIA-105.22.14</u>
- Giuga, L., Jordana, R. & Baquero, E., 2023 Three new species of Collembola from Sicily with first record of the genus *Superodontella* Stach, 1949 from Italy. ZOOTAXA, 5249 (5): 559-576. <u>https://doi.org/10.11646/zootaxa.5249.5.3</u>
- 4) **Giuga L**., Jordana R., Baquero E., Lo Verde G. 2023 Soil Collembola in Super-High Density and Traditional Olive Orchard Management System. (*Submitted to REDIA*)
- 5) **Giuga L**., Jordana R., Baquero E., Lo Verde G., *in prep*. Impact of soil management practices on soil arthropods in a Sicilian vineyard.

## **General introduction**

#### Soil biota

Soils are among the leading global reservoirs of biodiversity since more than 40% of living organisms in terrestrial ecosystems are associated during their life cycle directly with soils (Decaëns et al., 2006). Soils represent a complex habitat sustaining a massive diversity of organisms that are structured by and embedded within the physical matrix, together building the most diverse of all ecosystems (Bardgett & van der Putten, 2014; Curtis et al., 2002; Decaëns, 2010; Giller et al., 1997). Total biomass below ground equals or potentially exceeds that above ground, since nowhere in nature are species so densely packed as in soil communities.

It has been estimated that one gram of soil may contain millions of individuals and several thousands of species of bacteria. Billions of bacterial cells, tens of thousands of protist cells and kilometres of fungal hyphae typically inhabit a single gram of soil from anywhere on the planet, each represented by an enormous taxon diversity (Fierer, 2017; Geisen et al., 2018; Orgiazzi et al., 2016; Zhang et al., 2017).

Numerous groups are highly diverse, containing tens of thousands or more species (Geisen et al., 2019). These groups interact across different temporal and spatial scales and also adapt and coevolve (Geisen et al., 2019).

The soil's complex physical and chemical nature provides a range of habitats for many organisms of different sizes and ecology (Andre et al., 2002). They range from micro- to macro-levels and house microfauna (aquatic organisms like bacteria, protists, fungi, nematodes and tardigrades), mesofauna, macrofauna and megafauna (Zhang, 2013; Stork, 2018; Coleman & Whittman, 2005). Plants are part of this diversity and significantly impact the soil habitat and the rest of the biodiversity within the belowground matrix. Plants affect soil physics, chemistry and other soil biotas due to litter inputs and root growth (Berg and Smalla, 2009). Root exudates attract a variety of organisms that either feed directly on these secretions or graze on the microorganisms concentrated near the roots, making this busy environment called 'rhizosphere'. Other sources of carbon and nutrients deriving from roots and rhizodeposits/root exudates (Haichar et al., 2014) feed soil organisms involved in the soil food web.

The species numbers, composition and diversity in a particular ecosystem depend on many factors, including temperature, moisture, acidity, nutrient content and the nature of the organic substrates. Consequently, the patchy horizontal distribution of soil properties (soil temperature, moisture, pH, etc.) also drives the patchiness of the soil organisms across the landscape (Berg, 2012); soil communities also change in abundance and structure with soil depth (Berg and Bengtsson, 2007). But the structure of the soil communities strongly depends not only on the natural soil-forming factors but also on human activities (Briones, 2018).

Soil organisms exhibit various feeding preferences, life cycles and survival strategies while interacting within complex food webs (Briones, 2018). This diversity makes soil an extremely dynamic ecosystem. Seasonality, drought, and rainfall abundance significantly affect the soil properties and communities structure (Carini et al., 2018).

Not only are spatial patterns of soil biodiversity challenging, but their potential linkages to many soil processes and the overall ecosystem functioning remain under debate (Briones, 2018). Due to

experimental and observational limitations, the temporal factor in structuring soil communities is poorly understood (Fierer, 2017; Eisenhauer et al., 2018). Different communities of soil organisms differ in their lifespans and ecologies and in the various ways they interact with each other and their environment, making the soil a highly interactive system (Wolters, 2001).

Soil communities are diverse in size and number of species, yet they are poorly understood and need further assessment. Research has been limited by their immense diversity, small size and the technical challenges of identifying them.

The last three decades of soil ecology research have evidenced that the initial focus on distributions of specific faunal groups has turned significantly into understanding their acting roles, plant-soil interactions, and ecosystem functions and services (Briones, 2018).

#### Soil fauna - linkages to soil processes and to ecosystem functions and services

The capacity of soil to function and to support life is provided by soil organisms interacting in complex food–webs. Soil organisms play key roles in the terrestrial ecosystem (including agroecosystem) functions and services (Bardgett and van der Putten, 2014; Bender & van der Heijden, 2015; Bender et al., 2016) such as the physical and chemical transformation of litter, the significant contribution to soil formation, the nutrient cycling, the stable organic matter formation (humification) and decomposition (Brussaard et al., 1997; Seastedt & Crossley, 1984). The latter is an example of complex ecosystem processes that includes several soil functions and is supported by the whole soil food web. Soil organisms are directly or indirectly involved in decomposing dead organic matter to recover their energy, carbon and nutrient needs (de Ruiter et al., 1994).

Through their biological activity, soil organisms transform complex chemical forms into simple molecules or compounds that plants can absorb, providing feedback to plant productivity and the maintenance of soil fertility (Battigelli & Marshall, 1993).

Soil communities influence soil structure by changing conditions of drainage and aeration and contribute to soil formation (Brussaard *et al.*, 1997; Seastedt & Crossley, 1984); they also control populations of other organisms. Although their biological activities operate at small scales, their activities aggregate at larger scales, resulting in primary functional outputs (Kibblewhite et al., 2008) such as carbon transformation, nutrient cycling, formation of soil structure, and biocontrol (Gao et al., 2019; Abate et al., 2017; Azevedo et al., 2018; Yeates et al., 2009).

These biodiversity-based soil functions define soil health, sustain soil ecosystem processes, influence above-ground diversity (Bardgett & Wardle, 2010) and contribute to climate regulation, and at the same time, provide and regulate ecosystem services essential for human well-being (Hedlund et al., 2004; IPBES, 2019; Lavelle et al., 2006; Jeffery et al., 2010; Bardgett & van der Putten, 2014; Orgiazzi et al., 2016).

Soil organisms and their functional diversity perspective in the soil food web context may help understand soil biodiversity's role. Therefore, soil organisms interacting in the soil food web are assigned to functional groups based on size, shape, feed source, taxon and trophic level characteristics.

Each of these functional groups may contribute to one or more soil functions. Still, much of the soil biodiversity is just functionally "redundant" (i.e. a variety of soil organisms having the same

functional ability), especially for broad processes like decomposition (Liiri et al., 2002; Schimel, 1995).

Human activities, such as the intensification of land use, play a critical role in soil functioning leading to the modification of soil abiotic properties and tremendous changes in the structure, composition, and diversity of the soil communities.

Although driven by multiple factors, land use change due to agricultural expansion is the primary driver of biodiversity loss (Cardoso et al., 2020; IPBES, 2019). Many farming practices, such as tillage (Roger-Estrade et al., 2010; Fiera et al., 2020; Gonçalves et al., 2020), the use of fertilisers and pesticides and the homogenisation of landscapes leading to the loss of semi-natural habitats tend to reduce biodiversity (Brühl & Zaller, 2019; Habel et al., 2019; Tilman et al., 2017; IPES-Food, 2016; Zabel et al., 2019). Biodiversity, especially soil-dwelling arthropods, of agroecosystems where intensification occurs is impoverished (Biaggini et al., 2007; Cotes et al., 2010; Ruano et al., 2004; Santos et al., 2007).

Soil health (Kibblewhite et al., 2008) and sustainability depend on the maintenance of the biodiversity-based soil functions (carbon transformation, nutrient cycling, formation of soil structure, and biodiversity regulation); it is recognised that these functions are under threat (Gardi et al., 2013).

It is important to understand how soil food webs respond to helpful management and restoration regimes under the perspective of global climate change to sustain soil functions at specific levels. For that purpose, it is crucial to focus research on a better understanding of the links between biodiversity and soil functions and ecosystem services (de Vries et al., 2013) and among abiotic properties, soil organisms and climate (Bhusal et al., 2015; Orgiazzi and Panagos, 2018), to develop efficient monitoring tools and maps, increasing the bioindication potential at scales that are appropriate for management decisions (Stone et al., 2016; van Leeuwen et al., 2017; van den Hoogen et al., 2019).

#### Collembola

Collembola are small (0.12-17 mm), wingless, entognathous hexapods commonly known as 'springtails'. The body of Collembola basically comprises three tagmata, a head capsule, a thorax with three segments, and an abdomen with five segments and a terminal periproct. Thoracic and abdominal segments may be indistinct. The head bears two antennae, two optional postantennal organs, two optional composed eyes and the mouthparts. The antennae principally consist of four articulations each of which may be subdivided or annulated. Each composed eye consists of 0-8 ommatidia. Each thoracic segment bears ventrally a pair of walking limbs. The first abdominal segment bears a ventral tube, the third abdominal segment ventrally optionally bears the retinaculum and the fourth abdominal segment ventrally optionally bears the furca (Bellinger et al., 1996–2022).

The current systematics of Arthropoda supports Insecta as an independent class of Hexapoda subphylum, with Collembola, Protura and Diplura as the other non-insect classes (Grimaldi & Engel, 2005; Misof et al., 2014), characterised by entograthy, a condition in which the mouthparts are hidden inside the head capsule.

Collembola are classified into four orders: Entomobryomorpha, Poduromorpha, Symphypleona and Neelipleona. Main characters used for identification are: chaetae number, disposition and shape; scale presence; shape of antennae, head, thorax, abdomen, collophore, legs, furca, etc.; antennae segments number; shape of sensory organ of the third antennal segment; number of ocelli; presence and shape of postantennal organ; anal spines presence and shape; trichobothria presence, number, position, disposition; abdominal segments coalescence.

Collembola are among the most widespread terrestrial arthropods and are incredibly abundant in soil and leaf litter in almost any environment (Hopkin, 1997). Being the most abundant hexapods in the world, they occur at densities between 10 000 and 100 000 individuals per square metre in most terrestrial ecosystems.

Despite their wide global distribution, including Antarctica (Hopkin, 1997), the collembolan fauna of many geographic regions is poorly known. The number of species described up to now is 9485 (Bellinger et al., 1996–2022), while the number of still undescribed species is estimated to be about 50 000 (Hopkin, 1998) or even 65 000 (Porco et al., 2013). Moreover, recent molecular studies led to hypothesising that species richness within the class Collembola is underestimated by morphological approaches (Emerson et al., 2011; Cicconardi et al., 2013).

They occupy all trophic levels in belowground detritus food webs (Moore et al., 1988) and constitute an essential component of soil mesofauna in almost all terrestrial ecosystems (Rusek, 1998). Collembola are, together with Acari, the dominant arthropods in soils (Haarløv, 1955; Petersen and Luxton, 1982), usually accounting for 95 % of the microarthropods in soils (Seastedt, 1984). Collembola comprises a high number of species, occupying highly diverse habitats over a broad biogeographic area (Hopkin, 1997) and play an important role in soil functioning and ecosystem services.

Most Collembola feed on fungal hyphae, spores, bacteria, and decaying plant material and are responsible for up to 30% of total soil invertebrate respiration. However, some species are predators, feeding on nematodes or other Collembola and their eggs. In turn, springtails are prey for predatory mites and other Arachnida, Coleoptera, and even Vertebrata, such as reptiles and frogs. They can host parasitic Protozoa or nematodes, bacteria and fungal pathogens (Rusek, 1998). Consequently, Collembola are a relevant part of the soil food web and contribute to ecosystem functioning, as they directly and indirectly, regulate the soil microbial activity and nutrient cycling (Kaneda and Kaneko, 2011; Pieper and Weigmann, 2008).

Collembola enhance nitrogen mineralisation directly through their excreta and indirectly by interacting with microorganisms, thus increasing plant nutrient availability and growth (Filser, 2002; Kaneda and Kaneko, 2011). Collembola influence microbial and fungal species composition and biomass and thus indirectly impact mineralisation rates and mobilisation of nutrients from fungal biomass (Hopkin 1997; Coulibaly et al., 2019), i.e. helping in the release of nutrients locked in fungal biomass. They also participate in the dispersal of active fungal spores and bacteria cells modifying the composition of the rhizosphere microbiome (Crowther et al., 2012; Soong et al., 2016) and can inoculate microbes on materials to decompose, then affecting carbon turnover and soil carbon composition at the molecular level (Chamberlain et al., 2006).

Springtails do not actively burrow like other organisms (Rusek, 1998) but play a role in soil structure alteration through litter comminution, casting and other disintegration mechanisms. They

also indirectly contribute to soil structure dynamics and aggregate formation through mucilage secretion and their interaction with arbuscular mycorrhizal fungi (Siddiky et al., 2012).

Some species have been found to feed preferably on pathogenic rather than on antagonistic or arbuscular mycorrhizal fungal propagules; however, their biocontrol effect might depend on their density and field conditions (Coleman et al., 2018; Innocenti and Sabatini, 2018). Collembola might also indirectly reduce aphid reproduction depending on the plant host (Scheu et al., 1999; Schütz et al., 2008).

Springtails contribute to agroecosystem functioning; their distribution and abundance in arable fields influence the nutrient cycle, plant productivity, and the spatial patterns and abundance of their predators. Collembolan communities have been related to various habitat factors, such as soil fertility (Hågvar, 1982), soil chemistry (Hågvar and Abrahamsen, 1984), soil pH (Vilkamaa and Huhta, 1986; Van Straalen and Verhoef, 1997; Ponge, 2000; Loranger *et al.*, 2001). Crop management practices can also change species assemblages and diversity (Nakamura, 1988; Dekkers *et al.*, 1994; Filser *et al.*, 1995; Loranger *et al.*, 1999; Alvarez *et al.*, 2001; Gardi *et al.*, 2002). They are among the most sensitive soil fauna groups to pesticides (Joimel et al., 2022).

Given their trophic position in agroecosystems, maintaining Collembola communities is economically and ecologically important.

#### Soil fauna in agroecosystem - State of the art and goals

From the above, enhanced biodiversity in agricultural ecosystems would contribute to sustainability and productivity (Dainese, 2019; FAO, 2019; Jarvis et al., 2007).

If correctly assembled, a good level of agroecosystem biodiversity provides several ecosystem services (bioturbation, support of soil fertility, pollination, biocontrol, recycling of organic matter) which directly affect production (Altieri, 1999; Dangles & Casas, 2019; Schowalter et al., 2018). The part of the agro-biodiversity that provides desired services is called 'functional' (Bàrberi, 2013; Moonen and Bàrberi, 2008). Soil arthropod communities can provide adequate services in agroecosystems in terms of biocontrol (Daane and Johnson, 2010), nutrient cycling and decomposition (Mocali et al., 2020), litter fragmentation (Hagvar, 2016), grazing on microflora, and improvement of soil structure (Reichle, 1977). It is, therefore, foreseeable that their loss will have severe economic as well as ecological consequences (van der Sluijs, 2020).

While biodiversity in the soil exceeds that of other terrestrial ecosystems by orders of magnitude and soil organisms are pivotal for ecosystem functioning, soil communities (either from a taxonomic or functional perspective) are still poorly understood (Daily, 1997; Geisen et al., 2019; Pimentel et al., 1997; Swift and Anderson, 1994), and soil is, as yet, one of the most poorly investigated habitats of our planet (Giller, 1996; Hall, 1996). For example, the impact of microarthropods on soil aggregation is almost unknown (Maaß et al., 2015).

There is still no single method to fully characterise biodiversity, even within a single group of soil biota (Geisen et al., 2019). There are few causal studies linking biodiversity across different groups of soil organisms with soil ecosystem functioning (de Vries et al., 2013; Wagg et al., 2014; Delgado-Baquerizo et al., 2017; Morriën et al., 2017; Wang et al., 2019), and most of food web models are limited to certain components of the food web (Brose and Scheu, 2014; Heidemann et

al., 2014). Research has been limited by the immense diversity of soil organisms, their small size and the technical challenge of identifying them. Perhaps this problem derives mainly from the decline in human resources devoted to taxonomy, generally due to diminished institutional support for systematic research, particularly from agricultural and natural resource agencies. This decline has been particularly severe for soil taxa (Brussaard et al., 1997; Freckman, 1994).

Most of the knowledge on species characteristics has been accumulated by the few taxonomists of soil biota, valuable information for other disciplines to understand the mechanisms and effects of observed biodiversity patterns. Integrative methodological approaches, ideally in collaborative interactions across (soil) disciplines, are crucial to improving our understanding of soil biodiversity (Geisen et al., 2019; Kühl et al., 2020).

We still lack a generalised framework of when and where, and which aspects of soil biodiversity matter for ecosystem functioning (Mathieu et al., 2022).

The exponential growth of environmental sustainability certification programs, especially in the wine industry, in recent years can undoubtedly be related to greater consumer awareness of the environmental sustainability of agricultural production. Increasing the functional biodiversity in the vineyard is a new goal of the wine-growing industry. For this purpose, some cultural practices have been proposed to increase the genetic diversity of the crop, as well as the taxonomic and community diversity. Some of these sustainable farming practices may include, but are not limited to, the reduction of the use of pesticides, the use of natural predators of pests, or the inclusion of ecological infrastructures (e.g. woodlands, ground covers, etc.) adjacent to vineyards (OIV, 2018).

Various agricultural practices have been developed to increase sustainability and conservation of biodiversity and its functions within agroecosystems to stop the loss of biodiversity and their related services.

Most studies in fruit agroecosystems have focused up to date on above-ground factors, and the biological aspects and threats to the soil matrix that supports wine production are largely neglected (Diti *et al.*, 2020; Renaud *et al.*, 2004). Furthermore, few studies focus on the response of fauna or flora communities in perennial crops under different management systems (Bruggisser et al., 2010); few are carried out in the Mediterranean region (Ponce et al., 2011). Only a few studies have evaluated the effects of farming practices in vineyards or olive orchards on soil biological communities. Research focusing on functional subgroups of soil arthropods is scarce.

Conservation agriculture is increasingly adopted to improve soil fertility and reduce the depressive effects of intensive farming on soil functions and associated services. However, conservation agriculture systems may vary significantly regarding time since conversion and the quality and biomass production of cover crops. In addition, the effects of these variables on soil fauna are still poorly known.

The restoration of semi-natural habitats in agricultural areas by cover crops and spontaneous vegetation cover has been widely shown to improve soil quality (Capó-Bauçà et al., 2019; Ruiz-Colmenero et al., 2013) and soil's capacity to deliver ecosystem services (Garcia et al., 2019; Jian et al., 2020; Paiola et al., 2020; Winter et al., 2018) like pest regulation (Blaise et al., 2021; Hofmann et al., 2017) and the refuge function for insect biodiversity (Eckert et al., 2019a; Sáenz-Romo et al., 2019). In vineyards, integrating annual and perennial plant species with other than the cultivated species is therefore expected to provide multiple benefits for perennial crops (Franin et al., 2019).

al., 2016; Garcia et al., 2019; Gonçalves et al., 2020; Hendgen et al., 2018; Nicholls et al., 2008; Schreck et al., 2012; Shapira et al., 2018).

The high variability in response to management (Buchholz et al., 2017; Eckert et al., 2019b; Fiera et al., 2020; Gagnarli et al., 2015; Geldenhuys et al., 2021; Ghiglieno et al., 2019; Judt et al., 2019; Landi et al., 2022; Linder & Juvara-Bals, 2006; Renaud et al., 2004; Sánchez-Moreno et al., 2015; Sáenz-Romo et al., 2019; Simoni et al., 2018; Vignozzi et al., 2019; Wood et al., 2017) and when looking at arthropod guilds separately (Blaise et al., 2022) demonstrate the need to improve the understanding of soil management effects on arthropods and to investigate more finely the relationships among the different functional groups.

The objective of my PhD project entitled "Effects of vineyard and olive orchards management practices on soil arthropods community, with particular reference to Collembola" was to evaluate the effects of management practices in Mediterranean vineyards and olive orchards on arthropod conservation. Using taxonomic and eco-morphological approaches, I studied the response of arthropods to different modes of soil management. I focused on soil arthropods and, in particular, on Collembola occurring in vineyards and olive orchards located in Sicily, Italy.

My PhD project aims to a) better understand the effects of different management practices (cover crops, plant density, etc.) in Mediterranean vineyards and olive orchards on Collembola; b) increase the knowledge on distribution and ecology of Collembola occurring in agricultural lands and typical Mediterranean habitats of Sicily.

Achieving greater knowledge about soil arthropods is necessary to allow significant assessments of the state of soil quality and constitutes an aid tool in ecosystem management; the study of the Collembola community is an important part of this goal.

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## Chapter 1 - New records of springtails (Collembola) from Sicily, Italy

#### Abstract

Along with some studies on soil fauna in various Sicilian sites, both agricultural lands and typical Mediterranean habitats, particular attention was paid to the Collembolan. Specific identification allowed to assess the occurrence in Sicily of 39 species not previously recorded, belonging to 10 families: Hypogastruridae (six species), Brachystomellidae (one species), Neanuridae (six species), Onychiuridae (three species), Tullbergiidae (seven species), Isotomidae (nine species), Entomobryidae (four species), Cyphoderidae (one species), Dicyrtomidae (one species), and Katiannidae (one species). Of these 39 species, 21 are new also for Italy. Furthermore, this is the first record of the genus Doutnatcia Rusek, 1974 for the fauna of Italy. The discovery of such a number of new species for Sicily (i.e. 35 % of those known) and Italy by sampling a limited number of habitats, shows that the knowledge relating to this group of arthropods in Sicily is still very scarce.

Keywords: Soil Fauna, Soil Arthropods, Mediterranean, Agroecosystems

#### Introduction

Collembola are extremely abundant in soil and leaf litter in almost any environment (HOPKIN, 1997), as they occupy all trophic levels belowground, detritus, and food-webs (MOORE *et al.*, 1988), and constitute an important component of soil mesofauna in almost all terrestrial ecosystems (RUSEK, 1998). Together with the Acari, usually account for 95 % of the microarthropods in soils (SEASTEDT, 1984). Collembola comprises a high number of species that occupy highly diverse habitats over a broad biogeographic area (HOPKIN, 1997), and play an important role in soil functioning and ecosystem services.

Despite their wide global distribution, including Antarctica (HOPKIN, 1997), the collembolan fauna of many geographic regions is poorly known. The global number of species described up to now is 9393 (BELLINGER *et al.*, 1996–2022) while the number of still undescribed species is estimated to be about 50000 (HOPKIN, 1998) or even 65000 (PORCO *et al.*, 2013). Moreover, recent molecular studies led to hypothesize that species richness within the class Collembola is clearly underestimated by morphological approaches (EMERSON *et al.*, 2011, CICCONARDI *et al.*, 2013).

More than 2000 species are known from Europe (ULRICH & FIERA, 2010; DEHARVENG, 2011), while 437 species of springtails are known from Italy: 419 are reported in the Checklist of the Italian fauna (DALLAI *et al.*, 1995), 18 more in later articles (FANCIULLI & DALLAI, 1995; FANCIULLI, 1999; FANCIULLI *et al.*, 2005; FANCIULLI *et al.*, 2006; FANCIULLI & DALLAI, 2008; DALLAI & FANCIULLI, 2009; FANCIULLI *et al.*, 2010; JORDANA *et al.*, 2011; MATEOS & PETERSEN, 2012; GIUGA *et al.*, 2013; ARBEA, 2014; FANCIULLI *et al.*, 2017; FANCIULLI *et al.*, 2018; VALLE *et al.*, 2021).

The species known for Sicily are 111: 104 are reported in the Checklist of the Italian fauna (DALLAI *et al.*, 1995); further 7 species, of which 6 new to science, are reported in more recent papers (FANCIULLI & DALLAI, 1995; FANCIULLI *et al.*, 2006; JORDANA *et al.*, 2011; GIUGA & JORDANA., 2013). The poverty of these numbers is evident when considering that a great variety of habitats and geographical areas of Sicily remain unexplored from the point of view of soil arthropods in general and of collembolan fauna in particular.

Achieving greater knowledge about soil arthropods is necessary to allow significant assessments of the state of soil quality and constitutes an aid tool in ecosystem management; the study of the Collembola community is an important part of this goal.

In this paper, we present a species list of springtail new to Sicily collected during some studies on soil fauna in agroecosystems (vineyards and olive groves) and Mediterranean habitats typical of Sicily.

## Materials and methods

Soil samples were collected at various locations (Fig. I) from agricultural lands and some typical Mediterranean habitats of Sicily, listed in Table 1. Some results presented here are part of a previous study (Giuga, 2011).

n	Locality (Province)	Latitude	Longitude	Altitude (m a.s.l.)	Land use
1	Capo Zafferano (Palermo)	38°06'40"N	13°32'17"E	30-40	Annual dry grasslands
2	Rocca Busambra (Palermo)	37°50'40"N	13°26'20"E	1130-1140	Annual dry grasslands
3	Santa Cristina Gela (Palermo)	37°58'58"N	13°20'06"E	670-680	Annual dry grasslands
4	Mt. Pellegrino (Palermo)	38°11'11''N	13°20'59''E	80	Coniferous forest
5	Mt. Pelato, Mt. Nebrodi (Messina)	37°53'40"N	14°33'51"E	1560	Beech forest
6	Caronia, Mt. Nebrodi (Messina)	38°00'45"N	14°32'28"E	400-600	Cork-oak forest
7	Santo Pietro (Catania)	37°05'59"N	14°27'52"E	230-240	Cork-oak forest
8	Santo Pietro (Catania)	37°05'34"N	14°28'35"E	250-270	Mediterranean maquis
9	I. Lampione, Pelagian Islands (Agrigento)	35°33'16"N	12°19'59"E	30	Bare soil
10	Camporeale (Palermo)	37°54'22"N	13°04'22"E	350-500	Vineyard
11	Sambuca di Sicilia (Agrigento)	37°38'40"N	13° 02'18"E	205	Vineyard
12	Ballata (Trapani)	37°58'27"N	12°40'49"E	240-250	Olive orchard

Table 1. Geographical features (Datum WGS84) and land use of the investigated sites.



Figure I. Sampling locations; numbers on the map follow the *n* of Table 1.

Sampling and extraction of microarthropods were carried out in 2010 and 2019-2021 following standard methodologies applied in soil biology (PHILLIPSON, 1971; PARISI 2001; PARISI *et al.*, 2005). Soil samples (including litter when present) were transferred to the laboratory avoiding thermal shock and evaporation. Arthropods were extracted using a modified Berlese-Tullgren funnel (WALLWORK, 1976; PARISI, 2001; ANDRÉ *et al.*, 2002; PARISI *et al.*, 2005) and stored in 70 % ethyl alcohol.

For each sample, springtails were sorted, counted, and identified to the morphospecies level under a binocular stereomicroscope (Zeiss Wild M5A, 7–45x magnification). For each morphospecies, at least one specimen was mounted on a slide in Hoyer's medium for observation and identification with a light microscope. When necessary, samples were rinsed in Nesbitt's fluid and then washed for one hour in 70 % ethyl alcohol prior to slide mounting. The slides then were observed with an Olympus BX51-TF microscope with multiple viewing systems and phase contrast, and a Zeiss «Axio Imager.A1» with differential interference contrast (DIC).

Specific identification was carried out using the main literature in the springtails taxonomy (GISIN, 1960; JORDANA *et al.*, 1997; BRETFELD, 1999; POTAPOV, 2001; THIBAUD *et al.*, 2004; DUNGER & SCHLITTT, 2011; JORDANA, 2012) and consulting the original descriptions, when needed. The identified species are deposited in the Museum of Zoology of the University of Navarra (MZNA). For nomenclature and geographic distribution we followed specialized literature and GBIF (2021). The Italian geographic distribution is obtained from the Checklist of the Italian Fauna (DALLAI *et al.*, 1995), which reports a division into four areas: Northern Italy, Southern Italy, Sardinia, and Sicily.

#### Results

Overall, about 4700 springtails were collected, of which more than 500 were mounted for identification. In this paper, only the species that are new citations for the Sicilian or Italian fauna are presented.

Among them, 21 species, belonging to seven families (Hypogastruridae, Neanuridae, Onychiuridae, Tullbergiidae, Isotomidae, Entomobryidae, Katiannidae), are new for Italy and further 18 species are new for Sicily. Furthermore, the genus *Doutnatcia* Rusek, 1974 is recorded for the first time in Italy.

Hypogastruridae

Ceratophysella Börner, 1932

#### Ceratophysella denticulata (Bagnall, 1941)

EXAMINED MATERIAL. Sambuca di Sicilia (Agrigento), 5 December 2019, 1 ex., vineyard, L. Giuga & G. Lo Verde legit.

DISTRIBUTION. Cosmopolitan (FJELLBERG, 1998). Already recorded in northern and southern Italy. Notes. First record for Sicily.

#### Ceratophysella engadinensis (Gisin, 1949)

EXAMINED MATERIAL. Santa Cristina Gela (Palermo), 13 March 2010, 2 ex., annual dry grasslands. Santo Pietro (Catania), 26 February 2010, 4 ex., in Mediterranean maquis, L. Giuga & P. Alicata legit.

DISTRIBUTION. Probably Palaeartic and Thailand (JANTARIT *et al.*, 2016); its presence in Thailand could be doubtful.

NOTES. First record for Italy.

## Ceratophysella gibbosa (Bagnall, 1940)

EXAMINED MATERIAL. Sambuca di Sicilia (Agrigento), 5 December 2019, 1 ex., vineyard, L. Giuga & G. Lo Verde legit. Camporeale (Palermo), 1 April 2021, 1 ex., in vineyard, L. Giuga legit. DISTRIBUTION. Cosmopolitan (FJELBERG, 1992). NOTES. First record for Italy.

## *Ceratophysella succinea* (Gisin, 1949)

EXAMINED MATERIAL. Santo Pietro (Catania), 26 February 2010, 93 ex., Mediterranean maquis, L. Giuga & P. Alicata legit. Camporeale (Palermo), 1 April 2021, 129 ex., in vineyard, L. Giuga legit. Sambuca di Sicilia (Agrigento), 12 November 2021, 1 ex., vineyard, L. Giuga & G. Lo Verde legit. Sambuca di Sicilia (Agrigento), 1 June 2021, 12 ex., vineyard, L. Giuga & G. Lo Verde legit. DISTRIBUTION. Holarctic (FJELBERG, 1998). Known from southern Italy. NOTES. First record for Sicily.

## Xenylla Tullberg, 1869

## Xenylla brevicauda Tullberg, 1869

EXAMINED MATERIAL. Lampione Island (Agrigento), 1 June 2021, 1 ex., seabird nest, T. La Mantia legit.

DISTRIBUTION. Palearctic (FJELBERG, 1998). In Italy present in northern regions and Romagna as f. *atypica*.

NOTES. First record for Sicily.

## Xenylla xavieri Da Gama, 1959

EXAMINED MATERIAL. Lampione Island (Agrigento), 1 June 2021, 1 ex., seabird nest, T. La Mantia legit. Mt Pellegrino (PA) 12 June 2021, 1 ex., coniferous forest, G. Lo Verde & H. Tsolakis legit. DISTRIBUTION. W Palaearctic and Macaronesian (JORDANA *et al.* 1997) NOTES. First record for Italy.

#### Brachystomellidae

## Brachystomella Ågren, 1903

## Brachystomella parvula (Schäffer, 1896)

EXAMINED MATERIAL. Caronia (Messina), 5 March 2010, 9 ex., cork-oak forest, L. Giuga & P. Alicata legit. Santa Cristina Gela (Palermo), 13 March 2010, 1 ex., annual dry grassland, L. Giuga & R. Guarino legit.

DISTRIBUTION. Cosmopolitan (FJELLBERG, 1998), but its presence as native in the tropics and Southern Hemisphere is uncertain (MARI-MUTT & BELLINGER, 1990; GREENSLADE, 1994; THIBAUD, 2013; GBIF, 2021). In Italy reported for peninsular regions. NOTES. First record for Sicily.

#### Neanuridae

#### Deutonura Cassagnau, 1979

#### Deutonura conjuncta (Stach, 1926)

EXAMINED MATERIAL. Santo Pietro 26.ii.2010, 1 ex., cork-oak forest, L. Giuga & P. Alicata leg.

DISTRIBUTION. France, Germany, Hungary, Austria, Poland, Czechia (GBIF, 2021). In Italy present only in Bosco del Cansiglio (Venetian pre-alps). NOTES. First record for Sicily.

## *Deutonura ibicensis* (Ellis, 1974)

EXAMINED MATERIAL. Santo Pietro (Catania), 26 February 2010, 1 ex., Mediterranean maquis, L. Giuga & P. Alicata legit. Santo Pietro (Catania), 26 February 2010, 4 ex., cork-oak forest, L. Giuga & P. Alicata legit. DISTRIBUTION. Ibiza (ELLIS, 1974). NOTES. First record for Italy.

## Friesea Dalla Torre, 1895

## Friesea claviseta Axelson, 1900

EXAMINED MATERIAL. Capo Zafferano (Palermo), 13 March 2010, 4 ex., annual dry grassland, L. Giuga legit. DISTRIBUTION. Holarctic (JORDANA *et al.*, 1997). In Italy reported for peninsular regions.

NOTES. First record for Sicily.

#### Friesea ladeiroi Da Gama, 1959

EXAMINED MATERIAL. Santo Pietro (Catania), 26 February 2010, 6 ex., Mediterranean maquis, L. Giuga & P. Alicata legit. DISTRIBUTION. Portugal and Azores (JORDANA *et al.*, 1997). NOTES. First record for Italy.

## Friesea steineri Simón, 1973

EXAMINED MATERIAL. Caronia (Messina), 5 March 2010, 5 ex., cork-oak forest, L. Giuga & P. Alicata legit. DISTRIBUTION. Iberian (JORDANA *et al.*, 1997).

NOTES. First record for Italy.

Pseudachorutes Tullberg, 1871

## Pseudachorutes palmiensis Börner, 1903

EXAMINED MATERIAL. Mt. Pelato - Nebrodi Mountains (Messina), 23 June 2021, 2 ex., beech forest, T. La Mantia legit.

DISTRIBUTION. Palaearctic (JORDANA et al., 1997), Belize (GBIF, 2021).

NOTES. First record for Sicily. In the Checklist of the Italian Fauna (DALLAI *et al.*, 1995) recorded for Southern Italy as *Pseudachorudina palmiensis*.

## Onychiuridae

Deuteraphorura Absolon, 1901

#### Deuteraphorura cebennaria (Gisin, 1956)

EXAMINED MATERIAL. Caronia (Messina), 5 March 2010, 2 ex., cork-oak forest, L. Giuga & P. Alicata legit.

DISTRIBUTION. Europe (ARBEA *et al.*, 2011), introduced in southern Hemisphere (GREENSLADE *et al.*, 2012). In Italy recorded in Abruzzo region.

NOTES. First record for Sicily.

## Protaphorura Absolon, 1901

## Protaphorura campata (Gisin, 1952)

EXAMINED MATERIAL. Lampione Island (Agrigento), 1 June 2021, 9 ex., T. La Mantia legit. Sambuca di Sicilia (Agrigento), 1 June 2021, 18 ex., vineyard, L. Giuga & G. Lo Verde legit. *Ibidem* 12 November 2021, 1 ex., L. Giuga & G. Lo Verde legit. Camporeale (Palermo), 1 April 2021, 51 ex., vineyard, L. Giuga legit.

DISTRIBUTION. Palaearctic (FJELLBERG, 1998).

NOTES. First record for Italy.

## Protaphorura florae Simón-Benito & Luciáñez, 1994

EXAMINED MATERIAL. Sambuca di Sicilia (Agrigento), 5 May 2019, 5 ex., vineyard, L. Giuga & G. Lo Verde legit. DISTRIBUTION. Sierra de Gredos (SIMÓN-BENITO & LUCIÁÑEZ, 1994), Mexico (GBIF, 2021). NOTES. First record for Italy.

## Tullbergiidae

## Doutnatcia Rusek, 1974

## Doutnatcia xerophila Rusek, 1974

EXAMINED MATERIAL. Santo Pietro (Catania), 26 February 2010, 34 ex., cork-oak forest, L. Giuga & P. Alicata legit.

DISTRIBUTION. Europe (DUNGER & SCHILTT, 2011).

NOTES. First record of this genus for Italy. Cited also from Italy by DUNGER & SCHILTT (2011) but without bibliography or location information.

## Mesaphorura Börner, 1901

#### Mesaphorura critica Ellis, 1976

EXAMINED MATERIAL. Santo Pietro (Catania), 26 February 2010, 1 ex., Mediterranean maquis, L. Giuga & P. Alicata legit. Caronia (Messina), 5 March 2010, 1 ex., cork-oak forest, L. Giuga & P. Alicata legit. Lampione Island (AG), 1 June 2021, 3 ex., seabird nest, T. La Mantia legit.

DISTRIBUTION. Palaeartic (DUNGER & SCHILTT, 2011). In Italy recorded in the southeastern Abruzzo region.

NOTES. First record for Sicily.

## *Mesaphorura italica* (Rusek, 1971)

EXAMINED MATERIAL. Santo Pietro (Catania), 26 February 2010, 1 ex., Mediterranean maquis, L. Giuga & P. Alicata legit. Caronia (Messina), 5 March 2010, 3 ex., cork-oak forest, L. Giuga & P. Alicata legit.

DISTRIBUTION. Palaeartic (DUNGER & SCHILTT, 2011). In Italy recorded in peninsular regions and Sardinia.

NOTES. First record for Sicily.

## Mesaphorura orousseti Najt, Thibaud & Weiner, 1990

EXAMINED MATERIAL. Santo Pietro (Catania), 26 February 2010, 18 ex., cork-oak forest, L. Giuga & P. Alicata legit. Caronia (Messina), 5 March 2010, 5 ex., cork-oak forest, L. Giuga & P. Alicata legit.

DISTRIBUTION. Guyana, Iberica (DUNGER & SCHILTT, 2011). NOTES. First record for Italy.

## *Mesaphorura sylvatica* (Rusek, 1971)

EXAMINED MATERIAL. Sambuca di Sicilia (Agrigento), 1 June 2021, 2 ex., vineyard, L. Giuga & G. Lo Verde legit. DISTRIBUTION. Palaeartic (DUNGER & SCHILTT, 2011). In Italy reported from northern regions and Sardinia. NOTES. First record for Sicily.

## Metaphorura Stach, 1954

#### Metaphorura denisi Simón Benito, 1985

EXAMINED MATERIAL. Rocca Busambra (PA), 13 March 2010, 4 ex., annual dry grassland, L. Giuga & R. Guarino legit. Santa Cristina Gela (Palermo), 13 March 2010, 1 ex., annual dry grassland, L. Giuga & R. Guarino legit. DISTRIBUTION. Mediterranean (DUNGER & SCHILTT, 2011).

NOTES. First record for Italy.

#### Metaphorura riozoi Castaño-Meneses, Palacios-Vargas & Traser, 2000

EXAMINED MATERIAL. Ballata (TP), 14 October 2019, 1 ex., olive orchard, R. Rizzo legit. DISTRIBUTION. Hungary (CASTAÑO-MENESES *et al.*, 2000). NOTES. First record for Italy.

#### Isotomidae

#### Proisotomodes Bagnal, 1949

#### Proisotomodes debilis (Cassagnau, 1959)

EXAMINED MATERIAL. Capo Zafferano (Palermo), 13 March 2010, 8 ex., annual dry grassland, L. Giuga legit.

DISTRIBUTION. Recorded from France, Spain, Portugal, and Crete (POTAPOV, 2001). NOTES. First record for Italy.

#### Folsomia Willem, 1902

#### Folsomia manolachei Bagnall, 1939

EXAMINED MATERIAL. Caronia (Messina), 5 March 2010, 130 ex., cork-oak forest, L. Giuga & P. Alicata legit. Rocca Busambra (Palermo), 13 March 2010, 5 ex., annual dry grassland, L. Giuga & R. Guarino legit.

DISTRIBUTION. Probably all over the Palaearctic (POTAPOV, 2001). In Italy reported for northern regions (as *Folsomia nana* Gisin, 1957).

NOTES. First record for Sicily.

## Folsomia quadrioculata (Tullberg, 1871)

EXAMINED MATERIAL. Mt. Pelato - Nebrodi Mountains (Messina), 23 June 2021, 50 ex., beech forest, T. La Mantia legit.

DISTRIBUTION. Holartic (POTAPOV, 2001). Known from peninsular Italy. NOTES. First record for Sicily.

## Folsomides Stach, 1922

## Folsomides unicus Fjellberg, 1993

EXAMINED MATERIAL. Lampione Island (Palermo), 1 June 2021, 2 ex., seabird nest, T. La Mantia legit.

DISTRIBUTION. Macaronesian (FJELLBERG, 1993). NOTES. First record for Italy.

## Isotoma Bourlet, 1839

## Isotoma anglicana Lubbock, 1862

EXAMINED MATERIAL. Rocca Busambra (Palermo), 13 March 2010, 1 ex., annual dry grassland, L. Giuga & R. Guarino legit. DISTRIBUTION. Arctic & Sub-arctic, W Palaearctic (POTAPOV, 2001). NOTES. First record for Italy.

## Isotomiella Bagnall, 1939

#### Isotomiella paraminor Gisin, 1942

EXAMINED MATERIAL. Camporeale (Palermo), 1 April 2021, 4 ex., vineyard, L. Giuga legit. DISTRIBUTION. Mountainous areas of Central Europe (POTAPOV, 2001), E Russia (GBIF, 2021). Cited also from N Italy by Potapov (2001). NOTES. First record for Sicily.

Micranurophorus Bernard, 1977

## *Micranurophorus musci* Bernard 1977

EXAMINED MATERIAL. Sambuca di Sicilia (Agrigento), 1 June 2021, 2 ex., vineyard, L. Giuga & G. Lo Verde legit. DISTRIBUTION. Holartic (POTAPOV, 2001). NOTES. First record for Italy.

Scutisotoma Bagnall, 1949

*Scutisotoma variabilis* (Gisin, 1949)

Syn: Proisotoma (Subisotoma) variabilis Gisin, 1949

Subisotoma variabilis (Gisin, 1949) sensu Potapov, 2001.

EXAMINED MATERIAL. Capo Zafferano (Palermo), 13 March 2010, 61 ex., annual dry grassland, L. Giuga legit. Santa Cristina Gela (Palermo), 13 March 2010, 73 ex., annual dry grassland, L. Giuga & R. Guarino legit. Santo Pietro (Catania), 26 February 2010, 30 ex., Mediterranean maquis, L. Giuga & P. Alicata legit.

DISTRIBUTION. Central Europe (POTAPOV, 2001).

NOTES. First record for Italy.

## Tetracanthella Schött, 1891

## Tetracanthella serrana Steiner, 1955

EXAMINED MATERIAL. Rocca Busambra (PA), 13 March 2010, 2 ex., annual dry grassland, L. Giuga & R. Guarino legit.

DISTRIBUTION. Iberian (POTAPOV, 2001). NOTES. First record for Italy.

## Entomobryidae

Entomobrya Rondani, 1861

## Entomobrya lindbergi Stach, 1960

EXAMINED MATERIAL. Sambuca di Sicilia (Agrigento), 1 June 2021, 18 ex., vineyard, L. Giuga & G. Lo Verde legit. DISTRIBUTION. Afghanistan, Iran, Egypt, UAE and Yemen (JORDANA, 2012). NOTES. First record for Italy.

## Entomobrya vergarensis Baquero, Arbea & Jordana, 2010

EXAMINED MATERIAL. Santo Pietro (Catania), 26 February 2010, 1 ex., Mediterranean maquis, L. Giuga & P. Alicata legit. DISTRIBUTION. El Egido, Malaga (BAQUERO *et al.*, 2010). NOTES. First record for Italy.

## Lepidocyrtus Bourlet, 1839

## Lepidocyrtus violaceus (Geoffroy, 1762)

EXAMINED MATERIAL. Capo Zafferano (Palermo), 13 March 2010, 19 ex., annual dry grassland, L. Giuga legit. Caronia (Messina), 5 March 2010, 30 ex., cork-oak forest, L. Giuga & P. Alicata legit. Santo Pietro (Catania), 26 February 2010, 36 ex., Mediterranean maquis, L. Giuga & P. Alicata legit. Santo Pietro (Catania), 26 February 2010, 9 ex., cork-oak forest, L. Giuga & P. Alicata legit. DISTRIBUTION. European species (MATEOS *et al.*, 2021). In Italy known only from Reatini Mountains (Central Apennines).

NOTES. First record for Sicily.

Orchesella Templeton, 1836

## Orchesella cincta (Linnæus, 1758)

EXAMINED MATERIAL. Santo Pietro (Catania), 26 February 2010, 3 ex., Mediterranean maquis, L. Giuga & P. Alicata legit.

DISTRIBUTION. Holarctic (GBIF, 2021). In Italy reported for peninsular regions and Sardinia. NOTES. First record for Sicily.

## Cyphoderidae

## Cyphoderus Nicolet, 1842

## Cyphoderus albinus Nicolet, 1842

EXAMINED MATERIAL. Sambuca di Sicilia (Agrigento), 5 December 2019, 2 ex., vineyard, L. Giuga & G. Lo Verde legit.

DISTRIBUTION. Palaearctic (FJELLBERG, 2007). In Italy reported for peninsular regions. NOTES. First record for Sicily.

#### Dicyrtomidae

#### Dicyrtomina Börner, 1903

#### *Dicyrtomina ornata* (Nicolet, 1842)

EXAMINED MATERIAL. Santo Pietro (Catania), 26 February 2010, 2 ex., cork-oak forest, L. Giuga & P. Alicata legit.

DISTRIBUTION. Western Palaearctic region (BRETFELD, 1999). Already recorded in Northern and Southern Italy and Sardinia.

NOTES. First record for Sicily.

#### Katiannidae

#### Stenognatellus Stach, 1956

#### Stenognatellus cassagnaui Yosii, 1966

EXAMINED MATERIAL. Santa Cristina Gela (Palermo), 13 March 2010, 1 ex., annual dry grassland, L. Giuga & R. Guarino legit. DISTRIBUTION. Himalayan, Grecia (BRETFELD, 1999).

NOTES. First record for Italy.

#### Discussion

To date 111 species are recorded for Sicily. In this work, the study of eight habitats (including agricultural soils) at twelve localities led to the identification of one genus and 21 species new to Italy and 39 species, belonging to 10 families, new for Sicily. The discovery of such a number of new species for Sicily (i.e. 35% of those known) and Italy by sampling a limited number of habitats, two of which were agricultural, indicates that the degree of exploration for this group of arthropods is very poor in this region.

The availability of information for this group in general, except for a few areas, is scarce. Soil biology studies should be encouraged which, in addition to providing for the use of synthetic indexes, involve identification to species level in order to understand their ecology and distribution. This list of new records from Sicily and included notes on the habitats of each species is a contribution to the knowledge of ecology and distribution of these species.

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# Chapter 2 - Three new species of Collembola from Sicily with first record of the genus *Superodontella* Stach, 1949 from Italy

#### Abstract

Three new species of soil Collembola from both agricultural lands (vineyards and olive orchards) and a natural habitat (beech forest) located in Sicily (Italy) are described: *Superodontella eleonorae* **sp. nov.**, the first record of this genus for Italy; *Lepidocyrtus rapitalai* **sp. nov.** and *Pseudosinella francae* **sp. nov.**.

Keywords: Entomobryidae, Lepidocyrtus, Pseudosinella, Odontellidae, new species, soil fauna, Taxonomy

#### Introduction

As part of a study on the soil fauna carried out in several Sicilian sites (Giuga *et al.*, 2022) in which new species were added to the Sicilian and Italian fauna, some species were found that are new to the science: *Superodontella eleonorae* **sp. nov.**, *Lepidocyrtus rapitalai* **sp. nov.**, *Pseudosinella francae* **sp. nov.**. This is the first record of the genus *Superodontella* Stach, 1949 for Italy.

#### Material and methods

Soil samples (including litter when present) were collected at three localities: a mature beech forest (*Fagus sylvatica*) at Mt. Pelato, on the northern slope of Nebrodi Mountains; a vineyard at Camporeale, Western Sicily; an olive orchard at Erice, Northwestern Sicily.

Arthropods were extracted using a modified Berlese-Tullgren funnel (Wallwork, 1976; André *et al.*, 2002) and stored in 70% ethyl alcohol. The specimen was mounted on a slide in Hoyer's medium for observation and identification at species level.

In the laboratory the specimens were studied with an Olympus BX51-TF microscope with multiple viewing systems, phase contrast, and drawing device; and a Zeiss «Axio Imager.A1» with differential interference contrast (DIC).

In the descriptions of *Superodontella* we used the morphological nomenclature as follows: antennal sensilla (S-chaetae) are numbered after D'Haese (2003); the nomenclature of tergite chaetotaxy follows the classical a, m, p pattern, after Yosii (1956) and Arbea & Jordana (1997); for the perilabial chaetotaxy, after Gama (1969) and Yosii (1971); for the labial chaetotaxy, after Massoud (1967); for the structure of maxilla, after Deharveng (1981) and Bedos & Deharveng (1990); for the chaetae classification of anal valves, after Hüther (1962). The macrochaetotaxy for *Pseudosinella* and *Lepidocyrtus* follows Gisin & Gama (1969), Szeptycki (1979), Mateos (2008), Soto-Adames (2010), Zhang & Deharveng (2015), and Zhang *et al.* (2019). The characters defined by Christiansen *et al.* (1990) for *Pseudosinella*, and those used in a Delta key by Christiansen in Jordana *et al.* (2018), were used for identification and descriptions. In the descriptions of *Lepidocyrtus* and *Pseudosinella*, the reduced formula of Gisin (1965, 1967a, b) has been considered, which for Abd II is based on the presence and appearance of the chaeta a<sub>2</sub> (a, mic; A, Mc),  $a_{2p}$  (p, presence; '-', absence), m<sub>3</sub> (b, mic; B, Mc), m<sub>3e</sub> and p<sub>4</sub> (q, mic; Q, Mc).

Abbreviations: Abd—abdomen or abdominal segment I-VI, al—anterolateral s-chaeta, am anteromedial s-chaeta, Ant—antennal or antenna/ae, a.s.l.—above sea level, Mc—macrochaeta/ae, mes—mesochaeta, mic—microchaeta, ms—microsensillum/a, PAO—postantennal organ, psp pseudopore, Th—thorax, or thoracic segments, Tita— Tibiotarsus/a.

Material deposited. MZNA-Museo de Zoología, University of Navarra, Pamplona (Spain).

#### **Species description**

#### Superodontella eleonorae Giuga & Jordana sp. nov.

Figs 1–3, Tables 1–3

**Type locality.** Northeastern Sicily, Messina Province, Caronia, Mt. Pelato; 37.894444 N, 14.564167 E, 1560 m a.s.l.

**Type material.** Holotype: Male on slide (#NEBRODI\_FAGGETA\_06-II), Mt. Pelato, Caronia, Messina Province, Sicily, beech forest on soil, 23.VI.2021, leg. T. La Mantia. Paratypes: four specimens on slide (#06-I male, #06-III male, # 06-IV male, # 06-V male), same data as holotype. Holotype and four paratypes deposited in MZNA.

**Other material.** Five juveniles on slide (#06-VI, #06-VII, #06-VIII, #06-IX, #21-II), same data and locality as type material and deposited at MZNA.

**Diagnosis.** Habitus typical for the genus *Superodontella* Stach 1949. 5+5 eyes present. Ant IV with eight dorsal and two ventral subcylindrical curved sensilla (S-chaetae). Head with two c chaetae ( $c_2$ ,  $c_3$ ) and p row with three or four chaetae. Buccal cone rather short. Labium (per half) with two small papillate chaetae and six ordinary chaetae: F as Mc, G as mes, D, E, c and e as mic. Perilabial area with 2+2 subequal a-chaetae ( $a_2$ ,  $a_3$ ). Furca well developed with five identical chaetae on each dens. Each anal valve with three hr-chaeate, of which two laterals are bifid. Tita I, II, and III with 17, 17 and 17 chaetae, respectively. Empodial appendage absent. Anal spines absent.

**Description.** Body length up to 1.4 mm, including head (mean 1.19 mm, n = 5 adults); holotype length 1.2 mm. Dimensions in Table 1. Colour in alcohol grey, ocular area blue-black. Body integument strongly granulated. The granulated area on head as in Figure 1A and Figure 3D, Abd tergum V granulation as in Figure 3E. Ant I, II and III with 6, 10 and 12 ordinary chaetae, respectively. Sensory organ of Ant III with two small rounded internal sensory rods (S<sub>2</sub> and S<sub>3</sub>), two long bent external sensilla (S<sub>1</sub> and S<sub>4</sub>), and with ventral S<sub>5</sub> microsensillum (Figs 1D, 1E); Ant IV dorso-distally with small ovoid organite (or); microsensillum (ms) absent; eight dorsal subcylindrical bent sensilla S<sub>0</sub>, S<sub>1</sub>, S<sub>2</sub>, S<sub>4</sub>, S<sub>6</sub>, S<sub>7</sub>, S<sub>8</sub> and S<sub>9</sub> rather short, subequal; two ventral sensilla straight and rather long subequal; S<sub>3</sub> long and slender and tree more long sensilla-like macrochaetae; ten long, slender and pointed chaetae, two more (including dorsal chaeta i) short chaetae, and 6–8 ventral trumped-chaetae. Apical exertile vesicle absent. No eversible sac between Ant III and IV (Fig. 1D). Ocelli 5 + 5. PAO as long as ocellus B, with four or five lobes (Figs 1B, 3A–B). Buccal cone rather short. Labium (per half) with two small apical papillate chaetae and six ordinary chaetae: F as Mc, G as mes, D, E c and e as mic (Figs 1C, 3C). Perilabial area with 2 + 2 subequal chaetae a (a<sub>2–3</sub>), 3 + 3 chaetae m (m<sub>1–3</sub>) and 2 + 2 chaetae p (p<sub>1–2</sub>) (Figs 1C, 3C).

Specimen	Body	Antennae	Head	Mucro	Dens
Holotype	1200	130	180	30	52
06-I male	1400	150	230	25	70
06-III male	1100	-	-	36	58
06-IV male	1150	130	180	30	-
06-V male	1100	140	-	34	46
06-VI juvenile	1100	100	110	36	46
06-VII juvenile	1000	95	130	28	50
06-VIII juvenile	850	60	150	25	34
06-IX juvenile	1000	-	-	32	38
21-II juvenile	760	75	124	28	36
mean adults	1190	137,5	196,7	31,0	56,5
mean juveniles	942	82,5	128,5	29,8	40,8

**TABLE 1**. Dimensions in  $\mu$ m of holotype and paratypes of *S*. *eleonorae* **sp. nov.** 

Dorsal chaetotaxy as in Fig. 2A and Table 2. Ordinary chaetae subequal, smooth and pointed on all terga. Formula of sensory chaetae s per half: 022/11111. 'ms' present on Th II and III. Head without chaeta  $a_0$  and with chaetae  $c_2$  and  $c_3$ . Row p with three to four chaetae. Th I with 4+4 chaetae; Th II with 10-11+10-11 chaetae ( $p_1$  absent in one specimen); Th III with 12+12 chaetae; Abd I–III with 6+6 chaetae between  $p_{5s}$ , Abd IV with 7-8+7-8 chaetae ( $a_4$  present or absent) between  $p_{5s}$  chaetae; Abd V with 4+4 chaetae between  $p_{3s}$  chaetae. Without anal spines.

Ventral chaetotaxy. Th I–III without chaetae. Ventral tube with 3 + 3 chaetae. Abd I without chaetae, Abd II with 3 + 3 chaetae, Abd III with 5 + 5 chaetae. Furca well developed with five identical chaetae on each dens (Fig. 2B). Manubrium (posterior side) with 8 + 8 chaetae. Mucro typical for the genus, ratio mucro/dens as 0,6. Tenaculum with 3 + 3 teeth. Each anal valve with three hr-chaetae of which the two external ones are bifid (Figs 2D–E); the last character observed only in adult specimens.

Tibiotarsi I, II and III with 17, 17 and 17 pointed chaetae (10 on distal whorl and seven on proximal one). Femora I, II and III with 9–10 chaetae, trochanters I, II and III with 4–5 chaetae, coxae I, II and III with three, six and six chaetae, respectively, subcoxae I, II and III with 1–2 chaetae. Legs without spine-like chaetae. Claws with a basal inner tooth and a pair of lateral teeth (Fig. 2C). Empodial appendage absent.

Tergites chaetotaxy	a	m	р		Chaetae		Sensory chaetae
Th I	-	4	-	-	m1,2,3,4	-	-
Th II	3	4	4	a1,4,6	m1,4,5,6+ms	p1,2,4,6	p4, m6
Th III	5	3	4	a1,3,4,5,6	m1,5,6+ms	p1,2,4,6	p4, m6
Abd I-III	4	-	5	a1,4,5,7	-	p1,2,4,5,6	p5
Abd IV	4	3	5	a1,4,5,7	m1,5,7	p1,2,4,5,6	p5
Abd V	4	-	4	a1,3,5,6	-	p1,2,3,4	p3
Abd VI	2	2	2	a1,2	m1,2	p1,2	-

**TABLE 2**. Dorsal chaetotaxy of S. eleonorae sp. nov.

**Ecology and distribution.** All specimens were obtained in the same locality, in beech forest (*Fagus sylvatica* L.) soil at Mt. Pelato, Nebrodi Mountains. According to the available data of presence, *S. eleonorae* **sp. nov.** occurs with a low frequency.

Etymology. The species is lovely dedicated to the marine biologist Dr Eleonora Curcuraci.

**Discussion.** The new species is related to *S. aspinata* Deharveng & Izarra, 1979 from France, *S. conglobata* Arbea & Jordana, 1991 from Spain, and *S. euro* Weiner & Stomp, 2003 from Luxembourg. These four species have five subequal chaetae on dens and lack anal spines. All them differ in the presence or absence of bifid hr-chaetae on anal valves, presence or absence of capitated chaetae on Abd VI, number and position of chaetae on labium, number of tibiotarsal chaetae and the sensillar chaetotaxy of Ant IV.

The new species is also related to *S. arvensis* (Paclt, 1961) from Slovakia and *S. scabra* (Stach, 1946) from Austria which are insufficiently described; *S. arvensis* differs in the number of sensilla on Ant IV (10 in the new species, five in *S. arvensis*), *S. scabra* differs in the number of sensilla on Ant IV (10 in the new species, eight in *S. scabra*), number and shape of tenant hairs. Main differences between *S. eleonorae* **sp. nov.** and these species are summarized in Table 3.
Characters/Species	S. arvensis	S. aspinata	S. conglobata	S. euro	S. scabra	S. eleonorae sp. nov.
Number of sensilla on Ant IV	5*	9	7*	7*	8*	9
Empodial appendage	+*	-	-	-	-	-
Bifid hr-chaetae on anal valves	_*	_*	_*	_*	_*	+
Number of chaetae on Tita I, II, III	?	15-17, 15-17, 15-16*	15, 15, 15*	16, 16, 15*	?	17, 17, 17
Number of lobes on PAO	4	4	4	4	4	4-5
Capitated chaetae on Tita	?	+*	-	+*	+*	-
Capitated chaetae on Abd tr VI	?	+*	+*	-	-	-
Labial chaetotaxy (*)	?	3+3*	7+7*	6+6	?	6+6
А	?	?	mes*	mes*	?	-
В	?	?	mes*	mes*	?	-
D	?	?	_*	_*	?	mic
Е	?	?	mes*	_*	?	mic
F	?	?	Mc	Mc	?	Mc
G	?	?	mes	mes	?	mes
b	?	?	mes*	mes*	?	-
с	?	?	_*	_*	?	mic
e	?	?	mes*	_*	?	mic
f	?	?	-	mes*	?	-
Differences	3	5	12	12	3	

**TABLE 3**. Comparison of *S. eleonorae* **sp. nov.** with species of Superodontella with five subequal chaetae on dens without Asp. **\*** = differences; + = present; - = absent; ? = unknown. Abbreviations: see Material and methods.

#### Lepidocyrtus rapitalai Giuga & Jordana sp. nov.

Figs 4–6; Table 4

Type locality. Western Sicily, Palermo, Camporeale; 37.906002 N, 13.072977 E; 510–520 m a.s.l.

**Type material.** Holotype: Female on slide (RAPITALÀ\_FSA\_#1L\_05-II), Camporeale, Palermo Province, Sicily, vineyards on soil, 01.iv.2021, leg. L. Giuga. Paratypes: three specimens (#1L\_05-I female, #1L\_05-III male, #3i\_08 male) on slide, same data as holotype. Holotype and three paratypes deposited at MZNA.

**Diagnosis.** Ant I–III and legs scaled. Ant III sense organ with two curved and expanded sensilla. Head Mc Pa<sub>5</sub> present; A<sub>0</sub>, A<sub>2</sub> and A<sub>3</sub> as Mc; posterior labial row with M<sub>1</sub>, M<sub>2</sub>, R, E, L<sub>1</sub> and L<sub>2</sub> ciliated Mc; labrum with a<sub>1</sub> apically branched chaetae. Th II-III without Mc; Abd II with m<sub>3</sub> as ciliated Mc; Abd IV with four median Mc (Sm, B<sub>4-6</sub>), three non-fan-shaped ciliated mic above anterior bothriotrichum; claw with three internal teeth: two basal and one unpaired; empodium acuminate and serrate; manubrial plate with two internal and 2–3 external chaetae and two pseudopores.

**Description.** Body length up to 1.45 mm, including head (mean 1.11 mm, n = four adults), excluding antennae (holotype: 0.95 mm). Colour pale except blue dark on ocular patches, interocular area, coxae, end of Ant II–IV. Scales on Ant I–III ventral and dorsal head and body, coxae I–III, femora-tibiotarsus I–III, dorsally and ventrally on manubrium and only dorsally on dens; manubrium and dens length 200 µm and 228 µm respectively (n = 4); non-annulated part of dens 2.5 times the length of mucro.

Head. Antennal head ratio 1.51 (n=4). Ant IV without apical bulb; subapical organite and accessory sensillum as in Fig. 4E; Ant III sense organ with two curved and expanded sensilla (Fig.

4F), and three guard sensilla; Ant II with one distal similar to Ant III expanded sensillum. Head Mc Pa<sub>5</sub> present, A<sub>0</sub>, A<sub>2</sub> and A<sub>3</sub> as ciliated Mc smaller than An (although of a different size from the large Mc present in other species, or in other parts of its body; on the other hand the insertion is clearly greater than those of the mic); also with pair of smaller supplementary Mc A<sub>2a</sub> between A<sub>0</sub> and A<sub>2</sub>; present only the chaeta p intraocular; head dorsal chaetotaxy (Fig. 4A) with 7–8 Ant (An) ciliated Mc; four prelabral ciliated chaetae; labrum with three rows, 'a' row with a<sub>1</sub> apically branched chaetae (more than two branches), a<sub>2</sub> smooth chaetae, 'm' and 'p' with five smooth chaetae (Fig. 4B). Four labral papillae, with two or three projections (Fig. 4D). Maxillary palp bifurcated with three sublobal appendages. Labial papilla (l.p.) E with finger-shaped process not reaching at base of apical appendage. Labial row with M<sub>1</sub>\*, M<sub>2</sub>, R\*, E, L<sub>1</sub>, and L<sub>2</sub> ciliated Mc (M<sub>1</sub> and R 85 % of M<sub>2</sub>) (Fig. 4G). Postlabial chaetotaxy with 3 + 1 ciliated central Mc along the groove (Fig. 4C). 11 + 11 spinelike chaetae on posterior dorsal head (Fig. 4A).

Thorax (Fig. 5A). Th II and Th III without Mc; Th II with 'ms' and 'al' in antero-lateral position, at level of 'a' row; Th III with  $a_1-a_3$ ,  $a_5-a_6$ ,  $m_2-m_4$ ,  $m_6$ ,  $p_1-p_6$  present; lateral tergite with three mes and the lateral sensillum (al).

Abdomen. Abd I with 12 chaetae:  $a_1-a_3$ ,  $a_5-a_6$ ;  $m_2-m_6$ ;  $p_5-p_6$  (with the 'ms' near  $a_6$ ) (Fig. 5A). Ventral tube as in Figure 6D. Abd II (Fig. 5A)  $a_2(a)$ ,  $a_3$ ,  $a_6$  and  $a_7$  as smooth mic,  $a_5$  as bothriotrichum, and lm and ll above  $a_5$ ;  $a_{2p}(p)$  present as smooth mic;  $m_2$  as bothriotrichum,  $m_3(B)$  and  $m_5$  as Mc,  $m_{3e}(q_1)$ ,  $m_4$ , and  $m_6$  as smooth mic, and  $m_i$  above  $m_2$ ;  $a_{2p}$ ,  $p_4(q_2)$ ,  $p_5-p_7$  as smooth mic, 'as' above  $m_3$ . Abd III (Fig. 5B) with two Mc (pm<sub>6</sub> and p<sub>6</sub>) and with 18 microchaetae; three bothriotricha  $a_5$ ,  $m_2$ , and  $m_5$ . Bothriotricha  $a_5$  surrounded by three anterior chaetae (li, lm, and ll) and three posterior ones ( $am_6$ , em, and im). Abd IV without s chaetae over bothriotrichum T<sub>2</sub>. In central area of the tergum four Mc present: Sm, B<sub>4</sub>, B<sub>5</sub>, and B<sub>6</sub>. The remaining chaetotaxy as in Fig. 5B. Abd V with S-chaetae as, acc.p4 and acc.p5 (Fig. 5B).

Legs. Scales on legs (including all coxae). Trochanteral organ 'V' shaped with about seven spine-like chaetae (n = 4) three on each rami and one on the centre. Claw with three teeth on inner edge: basal pair at 50 %, an unpaired median at 65 %; two lateral teeth intermediate to base and paired, and one more basal dorsal tooth. Empodium acuminate, 0.61 times the length of claw, with pe lamella serrated, other lamellae smooth (ae, ai, pi). Tita III distally with one inner smooth chaeta longer than empodium; tenent hair spatulated, smooth, as long as claw (22  $\mu$ m in length) (Fig. 6C).

Furcula. Manubrium with scales dorsally and ventrally. Dens with scales only dorsally (Fig. 6A); manubrium and dens length  $200 \,\mu\text{m}$  and  $228 \,\mu\text{m}$  respectively (n=4); manubrial plate (dorsally) with two internal chaetae, with 2–3 external chaetae, and 2 psp (Fig. 6B). Non-ringed area of dens 2,5 times the length of mucro (Fig. 6A).

Pseudopores. Following the nomenclature of Mateos *et al.* (2021) the pseudopores observed are: antenna: Ant I, 1; Ant II, 1; Ant III, 1. Head dorsal (cephalic int Ant I), 1. Body dorsal: Th II, 1; Th III, 1; Abd I, 1; Abd II, 1; Abd III, 1; Abd IV, 1. Body ventral: Th I, 1; Th II, 1; Th III, 1; Abd I - post. VT base, 1; Abd II, (4). Legs: coxa I, 2; coxa II, 2; coxa III, 2. Furca dorsal: manubrial base, 1; manubrial plate, 2.

Macrochaetotaxy. Reduced formula (from Gisin 1965, 1967a, b):  $R_0R_1R_2001/00/0101+3/0$ , paBq1q2, M1\*M2R\*EL1L2 (\*85 % of M2).

**Ecology and distribution.** All specimens were obtained in the same locality, in vineyard soil. According to the available data of presence, *L. rapitalai* **sp. nov.** occurs with a low frequency.

**Etymology.** The specific epithet refers to the presence of this species in the Rapitalà district and to the homonymous winery that is located on it

**Discussion.** *L. rapitalai* **sp. nov.** belongs to the species of *lignorum*-group; all of them have trunk macrochaetotaxy formula 00/0101+3 (Mateos 2011); the species of this group with A<sub>0</sub>, A<sub>2</sub>, A<sub>3</sub> and Pa<sub>5</sub> as Mc are: *L. barbulus* Mateos, 2011, *L. chorus* Mateos & Lukić, 2019, *L. fuscocephalus* Mateos, 2022 in Mateos & Álvarez-Presas 2022, *L. instratus* Handschin, 1924, *L. intermedius* Mateos, Escuer & Alvarez-Presas, 2018 in Mateos *et al.*, 2018, *L. juliae* Mateos, 2011, *L. labyrinthi* Baquero & Jordana, 2021 in Baquero *et al.*, 2021, *L. lignorum* (Fabricius, 1793), *L. milagrosae* 

Mateos, 2022 in Mateos & Álvarez-Presas, 2022, *L. paralignorum* Baquero & Jordana, 2021 in Baquero *et al.*, 2021, *L. peisonis* Traser & Christian, 1992, *L. pulchellus* Denis, 1926, *L. ruber* Schött, 1902, *L. semicoloratus* Mateos, 2022 in Mateos & Álvarez-Presas, 2022, *L. tellecheae* Arbea & Jordana, 1990, *L. traseri* Winkler, 2016, *L. uzeli* Rusek,1985, *L. vexillosus* Loksa & Bogojević, 1967, *L. violaceus* (Geoffroy, 1762), and *L. rapitalai* **sp. nov**. With respect to all of them the new species is separated by having labial R chaeta as Mc, labrum with a<sub>1</sub> apically branched chaetae, claw with three teeth on inner edge, and by other characters shown in Table 4. The species *L. intermedius*, *L. vexillosus* and *L. ruber* have not been included in the Table 4 because, although they can be considered as belonging to the lignorum-group (Mateos 2020), they differ from the rest of species by lacking A<sub>3</sub> (like Mc), the first two, and Pa<sub>5</sub> (as Mc), the third.

In relation to the new denomination of the Mc Sm on Abd IV (Zhang *et al.* 2019), it must be considered that this chaeta has been called  $C_1$  in the descriptions of *Lepidocyrtus*, *Pseudosinella* and other genera since the work of Szeptycki (1979). This must be taken into account when referring to previously described species since in them it is not known whether the chaeta is  $C_1$  or Sm.

**TABLE 4.** Comparison of *L. rapitalai* **sp. nov.** with species of lignorum-group that share A0, A2, A3 and Pa5 as Mc. \* = differences; + = present; ? = unknown; acu = acuminate; b = bifurcate; br = branched; c = ciliated; exp = expanded; Mc = macrochaeta; mes = mesochaeta; mic = microchaeta; mul = multispinate; p = pointed; rl = rod-like; s = smooth; sl = chaeta-like; tru = truncate.

Characters/Species	L. barbulus	L. chorus	L. fuscocephalus	L. instratus	L. juliae	L. labyrinthi	L. lignorum	L. milagrosae	L. paralignorum	L. peisonis	L. pulchellus	L. semicoloratus	L. tellecheae	L. traseri	L. uzeli	L. violaceus	L. rapitalai sp. nov.
Ant segments with scales	I–III	I-II*	I–III	I-II*	I–II*	I–II*	I–II*	I–II*	I–II*	I-III(1)	I-III	I–II*	I–III	I–II*	I–II*	1-II*	I–III
Apical organ of Ant III	rl*	rl*	rl*	?	rl*	exp	?	rl*	exp	rl*	rl*	rl*	exp	rl*	exp	?	exp
Labral papillae	mul	mul	sl*	?	mul	sl*	mul	mul	mul	s*	mul	sl*	sl*	sl*	sl*	mul	mul
Labral a <sub>1</sub>	p*	b*	p*	?	b*	b*	b/br	br	b*	p*	b*	br	p*	b/br	p*	b*	br
Labral a2	р	b*	р	?	b*	c*	b/br*	b*	b*	p*	b*	b*	р	р	р	?	р
R ventral labial chaeta	c mic or mes*	c Mc															
$a = a_2$ on Abd II	s mic	c mic*	s mic	? mic	s mic												
Claw teeth number	4*	4*	4*	3	4*	4*	4*	4*	4*	4*	4*	4*	4*	4*	2*	4*	3
Empodium shape	acu	tru*	tru*	acu	acu	acu	tru*	acu	acu								
Inner chaetae number on manubrial plate	3*	3*	3-4*	?	2	3-4*	3*	2-3	3*	2-3	3-4*	3*	3*	2	?	3*	2
Outer chaetae number on manubrial plate	17*	6*	16*	?	8*	5-8*	7*	8*	5-12*	4-7*	8-11*	9*	15*	9*	?	9*	2-3
Differences	6	9	7	2	7	8	6	6	7	8	8	8	6	6	6	6	

#### Pseudosinella francae Giuga & Jordana sp. nov.

Figs 7-9; Table 5

**Type locality.** Northwestern Sicily, Trapani, Erice, Ballata; 37.974367 N, 12.680431 E, 240–250 m a.s.l.

**Type material.** Holotype: Female on slide (#TRAPANI\_ULIVETO\_SIC1-01), Ballata, Erice, Trapani Province, Sicily, olive orchard on soil, 14.x.2019, leg. R. Rizzo. Paratypes: one specimen on slide (#SIC3-04 without visible sexual plate), same data as holotype. Holotype and one paratype deposited at MZNA.

**Other material.** Same data and locality as type material; two specimens preserved in ethyl alcohol and deposited at MZNA.

**Diagnosis.** Body with some blue pigment on antennae and first leg segments. Head with 3+3 eyes (B, C, H); A<sub>0</sub>, A<sub>2</sub>, A<sub>3</sub>, S<sub>3</sub> and Pa<sub>5</sub> as Mc; basomedial labial fields chaetae smooth; posterior labial row with M<sub>2</sub>, E, L<sub>1</sub> and L<sub>2</sub> Mc, R absent; Th II with one Mc, Th III without Mc; Abd II with chaetae a<sub>2</sub>(a) and p<sub>2</sub>(p) absent, a<sub>3</sub> near of 'as' sensillum and forward m<sub>3</sub> Mc; Abd IV with one median Mc (B<sub>5</sub>), B<sub>6</sub> as mic or mes, two (instead of three) smooth mic above anterior bothriotrichum, and without 's' mic; claw with three internal teeth: two basal and one unpaired; empodium acuminate and external lamella smooth; manubrial plate with one internal and one external chaetae.

**Description.** Body length up to 0.60 mm, head included, excluding antennae (holotype: 0.60 mm). Blue colour is evenly dispersed throughout the body, dorsal and ventral head, antennae, and legs. Scales absent on antennae and legs, present on the ventral and dorsal head, thorax and abdomen dorsally, and furcula only ventrally.

Head. Ant head ratio 1.3 (n=2). Ant IV with simple apical bulb, apical organite and accessory sensillum; Ant III sense organ with two rod-shaped sensilla (individually encased in a pit) and three spiny guard sensilla; on Ant II one distal similar to Ant III sensillum. 3+3 eyes in a longitudinal line (B, C, H). Head dorsal chaetotaxy with 5–6 antennal (An) ciliated Mc; A<sub>0</sub>, A<sub>2</sub>, A<sub>3</sub>, S<sub>3</sub>, and Pa<sub>5</sub> as Mc (Fig. 7A); 4/554 labral chaetae: prelabral ciliated, labral row 'p' and 'm' ciliated; labral row 'a' smooth, a<sub>2</sub> bi-furcated and a<sub>1</sub> multi-furcated (Fig. 7B). Labral papillae absent. Maxillary palp bifurcate with three smooth sublobal chaetae. Labial papilla (l.p.) E with finger-shaped process reaching the base of apical appendage. Labial row with M<sub>2</sub>, E, L<sub>1</sub> and L<sub>2</sub> as ciliated Mc, R absent. Postlabial chaetotaxy with 3+1-3+1 ciliated central Mc along the groove and with four smooth mic (Fig. 7C).

Thorax (Fig. 8). Th II with one Mc ( $p_3$ ), al and ms in anterolateral position; Th III without Mc, with  $a_1-a_4$ ,  $m_2$ ,  $m_4$  and  $m_6$ ,  $p_1-p_4$  and  $p_6$ , and three Mc above the sensillum (al).

Abdomen (Figs 8, 9A). Abd I with  $a_1-a_3$ ,  $a_5-a_6$ ,  $m_2-m_6$ ,  $p_5$  and  $p_6$ , ms near  $a_6$ . Abd II, mi and ml chaetae present over bothriotrichum ( $m_2$ );  $a_{2p}(p)$  and  $a_2(a)$  absent;  $m_3(B)$  as Mc; sensillum as and  $a_3$  mic above  $m_3$  Mc;  $m_{3e}$  and  $p_4$  ( $q_1$  and  $q_2$ ) as smooth mic; lm, ll,  $a_6$  and  $m_4$  present as smooth mic over bothriotrichum ( $a_5$ );  $m_5$  as Mc; Abd III, mi, ml,  $a_2$ , as smooth pointed mic over bothriotrichum ( $m_2$ ); 'as' near  $m_3$  apparently smooth;  $a_3$ ,  $m_4$  and  $p_3$  as smooth and pointed mic;  $a_3$  present or absent; li, lm, and  $a_6$ , as smooth pointed mic surrounding bothriotrichum ( $a_5$ ); em, am<sub>6</sub> and  $a_7$  as smooth pointed mic bellow  $a_5$  bothriotrichum;  $m_6$  an  $p_6$  as Mc,  $p_3$ ,  $p_5$  and  $p_7$  mic. Abd IV with one median Mc ( $B_5$ ),  $B_6$  as mic or mes, and four lateral mes ( $D_3$ ,  $D_{e3}$ ,  $E_3$ ,  $F_1$ ); two smooth mic (m and  $D_1$ ) above  $T_2$  bothriotrichum (Fig. 9A); all mic smooth and pointed.

Legs. Legs without scales. Trochanteral organ with 6–7 stiff chaetae (Fig. 9B). Claw with three teeth on inner edge: basal pair at 40 % and 50 % with respect to the internal claw edge length, unpaired at 70 %; two lateral teeth at 40 %, dorsal tooth not seen. Empodium acuminate, all with smooth external lamella (pe), other lamellae smooth (ae, ai, pi); claw/empodium ratio: 0.57 (n = 2). Tita III distally with one smooth inner chaeta (6  $\mu$ m in length), tenent hair capitate, smooth, and same lengh of claw (10  $\mu$ m) (Fig. 9C).

Furcula. Manubrium and dens with scales only ventrally and with the same length; manubrial plate (dorsally) with one internal and one external Mc, and 1 psp. Mucro, with subapical tooth similar to apical one, and with a basal spine.

Macrochaetotaxy. Reduced formula (from Gisin 1965, 1967a, b):  $R_1R_2011/10/0100+1/0$ , --  $Bq_1q_2$ , - $M_2$ -EL<sub>1</sub>L<sub>2</sub>.

**Ecology and distribution.** All specimens were obtained in the same locality, in olive orchard soil. According to the available data of presence, *P. francae* **sp. nov.** occurs with a low frequency.

Etymology. This species is lovely dedicated to Franca Muccio the mum of the first author.

**Discussion.** The species that share the traditional reduced formula of Gisin: 011/10 on the head and Th II and III, presence of 3 + 3 corneolae, and without supplementary chaeta 's' on Abd IV are: *P. apuanica* Dallai, 1970, *P. fallax* (Börner, 1903) *sensu* Dallai 1976, *P. trioculata* Gama, 1988, *P. zaragozana* Arbea, 2006, and *P. francae* **sp. nov.** The differences between these species are shown in Table 5. The new species shows ten differences with respect to *P. apuanica*, twelve with *P. fallax*, and fourteen with *P. zaragozana*. The more similar species is *P. trioculata* but its description is short and lacks a lot of information; nevertheless, it shows six differences with respect to new species: R present on labium as smooth mic; 'a' present on Abd II as smooth mic; two Mc on medial dorsal Abd IV; three chaetae above T<sub>2</sub> on Abd IV; claw wing tooth absent; bigger body length. Furthermore *P. trioculata* is from Canary Islands. What was said in the previous species (*L. rapitalai* **sp. nov.**) regarding the identity of the chaeta Sm is also valid for this species.

**Table 5.** Comparison of *P. francae* **sp. nov.** with species group that share the traditional macrochaetae formula of Gisin (1965, 1967a, b) 001/10 on the head and Th II and III, with 3 + 3 corneolae, and without supplementary chaeta 's' on Abd IV. \* = differences; + = present; - = absent; ? = unknown.

Characters/Species	P. apuanica	P.fallax	P. trioculata	P. zaragozana	P. francae sp. nov.
Ant apical bulb	?	?	?	_*	+
Apical organ of Ant III	?	Rod like	Rod like	Expanded*	Rod like
Position/Number of eyes per side	A, B, C*	A, B, C*	3	A, B, D*	В, С, Н
M1 ventral labial chaeta	Ciliated Mc*	Ciliated Mc*	-	Ciliated Mc*	-
R ventral labial chaeta	Smooth mic*	Smooth mic*	Smooth mic*	Smooth mic*	-
E ventral labial chaeta	Smooth or Ciliated Mc	Smooth Mc*	Ciliated Mc	Ciliated Mc	Ciliated Mc
L <sub>1</sub> ventral labial chaeta	Ciliated Mc	Smooth Mc*	Ciliated Mc	Ciliated Mc	Ciliated Mc
p2(p) on Abd II	+*	+*	+ or -	-	-
a2(a) on Abd II	Ciliated Mc*	Ciliated Mc*	Smooth mic*	Smooth mic*	-
m3e(q1) on Abd II	Smooth mic	Smooth mic	Smooth mic	Ciliated Mc*	Smooth mic
Anterior lateral ( $P = Sm \text{ or } C_1$ ) dorsal Mc	+*	+*	-	+*	-
Medial (M = B) dorsal Mc number on Abd IV	2*	2*	2*	2*	1+mes
Chaetae number above bothriotricum T2 on Abd IV	3*	3*	3*	3*	2
Claw wing tooth	_*	_*	_*	+	+
Empodium wing tooth	Smooth	Smooth	Smooth	Serrate*	Smooth
Inner chaetae number on manubrial plate	?	?	?	2*	1
Outer chaetae number on manubrial plate	?	?	?	2*	1
Maximum length	1.14*	1*	0.9*	1.1*	0.6
Differences	10	12	6	14	

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**FIGURE 1.** Superodontella eleonorae **sp. nov.**: A, dorsal chaetotaxy of head; B, postantennal organ and ocelli; C, labial and perilabial chaetotaxy; D, right Ant I–IV, dorsally; E, sensory organ of Ant III and right Ant IV, dorsally. Scale bar: A and  $D = 50 \mu m$ ; B and  $C = 25 \mu m$ .



**FIGURE 2.** Superodontella eleonorae **sp. nov.**: A, habitus and dorsal chaetotaxy; B, furca; C, distal part of leg III; D, Abd terga V and VI; E, anal valves chaetotaxy. Scale bar:  $A = 100 \mu m$ ;  $B - D = 50 \mu m$ ;  $E = 25 \mu m$ .



**FIGURE 3.** *Superodontella eleonorae* **sp. nov.**, DIC micro-photographs: A, PAO with four lobes; B, PAO with five lobes; C, buccal cone and perilabial area; D, granulated area of dorsal head (front on the right); E, Abd terga V granulation. Scale bar: 10 µm.



**FIGURE 4.** *Lepidocyrtus rapitalai* **sp. nov.**: A, dorsal head chaetotaxy (hollow circles are proportional to reality); B, prelabral chaetae (pr) and labral chaetae (rows 'p', 'm' and 'a'); C, ventral head chaetotaxy; D, labral papillae; E, organite (or) and accessory sensillum (as) on Ant IV; F, sensory organ of Ant III; G, postlabial area. Scale bar: A, C, E and  $G = 25 \mu m$ ; B, D and  $F = 10 \mu m$ .



**FIGURE 5.** *Lepidocyrtus rapitalai* **sp. nov.**: A, Th II to Abd II dorsal chaetotaxy; B, Abd III to Abd V dorsal chaetotaxy, with detail of the chaetae Sm and  $E_1$  at the same scale (hollow circles are proportional to reality); C, detail of Sm,  $E_1$ ,  $B_{4-6}$  Abd IV chaetae. Scale bar for all drawings: 50 µm.



**FIGURE 6.** *Lepidocyrtus rapitalai* **sp. nov.**: A, tip of furcula showing the non-ringed area of dens, mucro and mucronal spine; B, manubrial plate chaetae and pseudopores; C, apical part of tibiotarsus, claw and empodium of leg III; D, ventral tube. Scale bar: A and  $C = 10 \mu m$ ; B and  $D = 25 \mu m$ .



**FIGURE 7.** *Pseudosinella francae* **sp. nov.**: A, dorsal head chaetotaxy (hollow circles are proportional to reality); B, prelabral chaetae (pr) and labral chaetae (rows 'p', 'm' and 'a'); C, ventral head chaetotaxy and detail of the postlabial area. Scale bar: A and  $C = 25 \mu m$ ;  $B = 10 \mu m$ .



FIGURE 8. *Pseudosinella francae* sp. nov.: Th II to Abd III dorsal chaetotaxy, and detail of  $p_3$  chaeta of Th II. Scale bar: 30  $\mu$ m.



**FIGURE 9.** *Pseudosinella francae* **sp. nov.**: A, Abd IV dorsal chaetotaxy (hollow circles are proportional to reality); B, trochanteral organ; C, apical part of tibiotarsus, claw and empodium of leg III. Scale bar:  $A = 20 \mu m$ ;  $B-C = 10 \mu m$ .

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# Chapter 3 – First data on soil collembola in super-high density and traditional olive orchard management systems

#### Abstract

The Collembola community plays an essential role in soil ecosystem services. This preliminary study aimed to assess the effect of super-high density olive orchards systems on the Collembola community and biological soil quality using the QBS-ar index in Sicily. Two super-high density managed olive orchards (SHD-A and SHD-B) were compared to one adjacent traditional orchard (TRAD) system. The same fertilization management and phytosanitary measures were applied. Soil management in the two SHD orchards consisted of green manure, while conventional tillage was adopted in TRAD. The soil Collembola community was evaluated using the species abundances and eco-morphological life forms. Multivariate analysis showed that the different management systems significantly influenced the Collembola community assemblage. A negative impact of super-high density management system on Collembola abundance and QBS-ar was also highlighted.

Moreover, the management system seems to affect the soil Collembola composition in terms of eco-morphological life forms. Euclaphobionts were more abundant in the two SHD than TRAD. However, conservative and sustainable soil management maintains or improves the soil Collembola community's functionality and biological soil quality.

Keywords: Soil Fauna, Springtails, Olea europaea, olive orchard management systems, Sicily.

## Introduction

Soil arthropods significantly contribute to soil formation, soil organic matter transformation, nutrient cycling and are involved in a wide range of interactions with micro-organisms and other invertebrates (Lavelle *et al.*, 2006). Enhanced agroecosystem biodiversity, when correctly assembled, provides several services, supporting soil fertility, nutrient cycling and improvement of soil structure (Reichle, 1977), crop protection and productivity (Altieri, 1999). The part of agrobiodiversity delivering such desired services is regarded as 'functional' (Bàrberi, 2013; Moonen and Bàrberi, 2008). Despite it, the role and function of soil fauna in ecosystem services in productive orchards have often been overlooked (FAO, 2017), while it should be seriously considered in land management strategies oriented not only to fruit production but also to soil fertility restoration (Battigelli & Marshall, 1993).

Olive groves (*Olea europaea* L.) are widespread in the Mediterranean basin due to their adaptability to soil and environmental constraints (Loumou and Giourga, 2003). Italy represents one of the leading olive oil producers in the world, with a cultivated area of about 1.1 million hectares distributed in the country's Central and South (Pupo D'Andrea, 2021). To reduce production costs and, at the same time, increase yields per hectare, super-high density olive orchards are gaining popularity in Italy (Landi *et al.*, 2022).

Intensification of disturbance by agricultural management usually has adverse effects on biodiversity (Benton *et al.*, 2002; Biaggini *et al.*, 2007; Tilman *et al.*, 2001), and soil arthropod fauna is significantly affected (Cotes *et al.*, 2010; Ruano *et al.*, 2004; Santos *et al.*, 2007). Nevertheless, a limited number of studies have focused up to date on the response of fauna or flora communities in perennial crops under different management systems (Bruggisser *et al.*, 2010; Gagnarli *et al.*, 2015). Most of these were carried out in middle or high latitudes but rarely in the Mediterranean region (Ponce et al., 2011). Even further, only a few studies (e.g. Cotes *et al.*, 2009; Gkisakis *et al.*, 2016; Gonçalves and Pereira, 2012; Jerez-Valle *et al.*, 2014; Landi *et al.*, 2022; Sofo *et al.* 2020; Vignozzi *et al.*, 2019) have evaluated the effects of farming practices applied in olive production systems biodiversity, while soil biological communities have been poorly investigated.

This study aims to evaluate the effects of a super-high density olive orchard system on the soil Collembola community, compared to the traditional olive orchard system in a farm in Sicily, Italy. This study supports the hypothesis that the density and the investigated cultivars could affect the soil Collembola structure. In detail, the effect of the management system was assessed on: (i) the abundance and diversity of soil Collembola communities, (ii) soil Collembola composition in terms of eco-morphological life forms (Rusek, 2005), and (iii) soil biological quality using QBS-ar index (Parisi, 2001; Parisi *et al.*, 2005).

## Material and methods

## STUDY AREA

The study areas were in Trapani province (Sicily, South Italy) (Fig. I). Soil texture is classified as Clay Loam (USDA); the area is characterized by a semi-arid climate, with an annual rainfall of 680 mm and a mean annual temperature of 17.6 °C (Landi *et al.*, 2022). To compare the soil collembola community, three organic olive orchards, two managed by a super-high density system (SHD-A and SHD-B) and one traditional (TRAD), were selected. The area's characteristics are summarized in Tab. 1. Phytosanitary practices and organic fertilization (manure 1000 kg/ha) were the same in all orchards. Soil management in the two SHD orchards consisted of green manure with *Vicia faba minor* L., while conventional tillage was adopted in TRAD. Complementary irrigation and mechanical harvest were applied in both SHD orchards.

Management system	Code	Coordinates	Altitude a.s.l (m)	Cultivar	Surface (ha)	Density (trees/ha)	Tree age	Soil management
Super-High Density	SHD-A	37°57'49.55"N 12°43'35.43"E	195	Arbequina	6	2600	10-12	green manure, organic fertilization
	SHD-B	37°58'27.74"N 12°40'49.60"E	245	Arbequina	0.6	2600	10-12	green manure, organic fertilization
Traditional	TRAD	37°58'19.63"N 12°40'58.17"E	220	Biancolilla, Cerasuola, Nocellara del Belice	1.5	125	about 40	conventional tillage, organic fertilization

Table 1 – Characteristics of the olive orchards in which the study was conducted.



Fig. I – Study area.

## ARTHROPOD SAMPLING

Three soil samples were collected per orchard along a linear transect in the middle of an inter-row, in October 2019, before mechanical harvesting in the two SHD orchards. Soil samples, with a volume of 1 l each, were taken at a 0–15 cm depth after removing surface residues. Each sample was placed in a plastic bag, labelled, and transferred to the laboratory –being protected from thermal shock and avoiding evaporation– where the fauna was extracted using a modified Berlese-Tullgren funnel (Wallwork, 1976; Parisi, 2001; André *et al.*, 2002; Parisi *et al.*, 2005) and stored in 70 % alcohol.

Collembola were counted and identified at morpho-species level for each sample under a binocular stereomicroscope (Zeiss Wild M5A stereomicroscope, 7–45× magnification). At least one specimen for each morpho-species was slide mounted in Hoyer's medium for optical microscope observation and identification. Mounted specimens were examined using an Olympus BX51-TF microscope with a multi-viewing system and phase contrast, and a Zeiss «Axio Imager.A1» with differential interference contrast (DIC). The specimens analyzed are deposited in the Museum of Zoology of the University of Navarra (MZNA).

Collembola were identified at species level using the proper literature in the springtails taxonomy (Gisin, 1960; Dunger, 1994; Jordana *et al.*, 1997; Bretfeld, 1999; Potapow, 2001; Thibaud *et al.*, 2004; Dunger and Bettina, 2011; Jordana, 2012). When attribution at the species level was impossible (e.g. immature stage), specimens were assigned to higher taxa and included in the respective groups' total abundance.

Moreover, the identified taxa were classified into eco-morphological life form groups and subgroups following Rusek (2005).

Finally, all collected microarthropods besides Collembola were examined for each sample under a binocular stereomicroscope. An Eco-morphological Index (EMI) was assigned considering their morphological features and adaptation to the edaphic environment to calculate the QBS-ar index (Parisi, 2001; Parisi *et al.*, 2005).

## DATA ANALYSIS

For each orchard, the following indices were calculated to provide information on springtail soil richness and diversity: taxa richness (S), Shannon diversity index (H'), and Simpson evenness (e'). Collembola community composition variability in the three orchards was assessed by a multivariate analysis (according to Rodgers and Kitchinj, 1998) of the species abundance based on the Bray-Curtis similarity index after performing a quadratic transformation of the data. One-way Analysis of Similarity (ANOSIM) was conducted based on the different management systems (SHD and TRAD orchard management systems) as the factor and transformed species abundance data as the response variable. A similarity of per cent contribution (SIMPER) analysis was performed to identify the species that mainly drove the dissimilarities in the Collembola community composition between the different orchards. The CLUSTER analysis on the respective resemblance matrix visually identified similarities in soil communities between plots.

All analyses were performed using the PRIMER 6 v.6.1 software package (PRIMER-E Ltd., Plymouth, UK). Diversity indices values were compared by applying the Kruskal–Wallis tests to detect differences among sites using the Minitab<sup>®</sup> software (Minitab Inc., State College, Pennsylvania, USA).

## **Results and discussion**

Overall, 662 springtail individuals were collected, of which 62 specimens were mounted for identification. Nine families were identified with 23 species (Tab. 2).

Family	Species	SHD-A	SHD-B	TRAD
	Acherontiella bougisi Cassagnau & Delamare,			18
Hypogastruridae	1955			
Brachystomellidae	Brachystomella parvula (Schäffer, 1896)		2	3
Neanuridae	Deutonura ibicensis (Ellis, 1974)			1
Onychiuridae	Protaphorura armata (Tullberg, 1869)	5	4	12
	Protaphorura spp.*		5	
	Metaphorura riozoi Castaño-Meneses,			
Tullbergiidae	Palacios-Vargas et Traser, 2000		1	
Isotomidae	Folsomia penicula Bagnall, 1939			28
	Folsomides parvulus Stach, 1922	26	51	90
	Hemisotoma pontica (Stach, 1947)	7	21	
	Hemisotoma thermophila (Axelson, 1900)	18	60	41
	Isotomiella minor (Schäffer, 1896)	6		
	Isotomurus sp. 1	1		
	Pseudanurophorus quadrioculatus von Törne,			
	1955			45
	N.D.*	82		1
Entomobryidae	<i>Entomobrya</i> sp. 1	3		2
	Lepidocyrtus lignorum (Fabricius, 1775)	1		
	Pseudosinella fallax (Börner, 1903)	23	8	17
	Pseudosinella sp.1		4	
	Seira sp. 1			1
	N.D.*			10
Orchesessildae	Heteromurus major (Moniez, 1889)	2	2	25
	Heteromurus nitidus (Templeton, 1836)	16	1	

*Table 2* – Families and species (or higher taxa) list identified in the three orchards. (\*indicates taxa not included in the statistical analysis).

	Heteromurus spp.*			12
	Orchesella sp. 1	1		
Paronellidae	Paronellidae sp. 1	1		
	Troglopedetes sp. 1			4
	Total number of specimens	191	161	310

Abundance was lowest within SHD-A and SHD-B, showing that the SHD olive orchard system negatively affected the density of Collembola. The richness Taxa (S) did not show a significant difference between orchard systems; the diversity indices H' and e' reached the lowest value in SHD-B, whereas they were similar in SHD-A and TRAD (Tab. 3). However, the statistical analysis applied to the indices (abundance, S, H', and e') did not show significant differences between the three olive orchards (Kruskal-Wallis test, p > 0.05 level).

*Table 3* - Comparison of abundance (individuals/soil sample) and diversity of soil Collembola in the three orchards.

	SHD-A	SHD-B	TRAD
Abundance	63.67±28.7	53.67±11.0	103.33±23.4
$(\text{mean} \pm \text{SE}, \text{n} = 3)$			
S	12	11	13
Shannon H'	2.03	1.58	2.03
Simpson e'	0.85	0.73	0.83

These results indicate that all diversity indices measured cannot detect changes in communities of soil Collembola from olive orchards differing in their management systems. Differences in the relative abundances are related to Collembola's population dynamics and more in general with the considerable variability of various soil taxa, even in similar pedogenic conditions (Fierer, 2017), particularly evident at a very fine scale (O'Brien et al., 2016).

The one-way ANOSIM showed that the different management systems significantly influenced Collembola assemblages (Global R = 1, P = 0.03). SIMPER analysis identified an average similarity at the species level of 59.95 % among SHD-A and SHD-B samples. In contrast, the SHD-managed orchards diverged from TRAD via *P. quadrioculatus, F. penicula,* and *A. bougisi,* contributing most to the disparity. A subsequent CLUSTER analysis on the resemblance matrices indicated that the Collembola assemblages were grouped into two main clusters, the first including only TRAD and the second including both SHD-A and SHD-B, as a function of the management system factor (Fig. II).



Fig. II – CLUSTER diagram showing linkages of Collembola communities by different management systems (TRAD, SHD-A and SHD-B). Authenticated connections are based on the Bray-Curtis similarity matrix.

By Collembola classification into eco-morphological life form groups and subgroups, the two main groups of atmobionts and edaphobionts were present in TRAD, whit the first one scarcely represented in terms of abundance and absent in SHD (Tab. 4).

*Table 4* – Relative abundance of Collembola's different life form groups and subgroups across the three orchards.

GROUP	SUBGROUP	SHD-A	SHD-B	TRAD
Edaphobionts	Euedaphobionts	82,72%	64,60%	51,61%
Edaphobionts	Hemiedaphobionts	15,18%	32,92%	39,68%
Edaphobionts	Epigeonts	2,09%	2,48%	8,39%
Atmobionts	Xylobionts			0,32%

The Euedaphobionts subgroup is the most represented in abundance (Fig. III) and species number (Fig. IV) in all orchards. The SHD olive orchard's system features, such as heavy machines for harvesting, different cultivars, and high plant density per hectare, seem to favour the Collembola of the Euedaphobionts life form probably by reducing microhabitat diversity.



Fig. III – Relative abundance of Collembola's different life form groups across the three orchards.



Fig. IV – Species number for each life form group of Collembola across the three orchards.

The QBS-ar values ranging from 125 to 193 (Tab. 5) are according to those recorded in another study in olive orchards (Vignozzi *et al.*, 2019). The number of taxa recorded in each orchard is between 11 and 15. The highest QBS-ar value was recorded for TRAD (193). The SHD-A and SHD-B had similar values (125 and 128, respectively), indicating that the more intensive management system negatively affects the QBS-ar index values.

Crown	EMI (eco-	morphologi	cal index)
Group	SHD-A	SHD-B	TRAD
Acari	20	20	20
Chilopoda			20
Coleoptera	1	1	10
Collembola	20	20	20
Diplopoda	10		10
Diplura	20	20	20
Diptera	1		1
Diptera (larvae)	10		10
Hymenoptera	1	5	
Isopoda		10	10
Other holometabolic insects	1	1	1
Other holometabolic insects		10	10
(larvae)		10	10
Pauropoda	20	20	20
Pseudoscorpiones			20
Psocoptera	1	1	1
Symphyla	20	20	20
QBS-ar	125	128	193
N° of Taxa	12	11	15

*Table* 5 - QBS-ar values (resulting from the sum of the EMI scores) and the number of taxa for the three orchards.

## **Conclusive remarks**

In general, the composition of the Collembola community structure showed significant differences between the two olive grove systems, the super-high density and the traditional one; no relevant variations were found between SHD-A and SHD-B, indicating that the management system changes the Collembola community of the soil associated with olive groves, and also affects the QBS-ar index. This study represents an essential step in better understanding the Collembola assemblages as a function of different olive orchards systems. However, further studies are necessary to understand the impact of management on the soil Collembola community, the factors involved, and their interactions. Moreover, these investigations can improve our knowledge of the springtail faunas, as occurred for the Tullbergiidae *Metaphorura riozoi*, collected for the first time in Italy in the same olive orchards in which the present study was carried out (Giuga et al., 2022).

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# Chapter 4 - Impact of vineyards soil management practices on soil arthropods in Sicily

#### Abstract

Soil arthropod communities play an important role in all terrestrial ecosystems and can provide major services in agroecosystems; maintaining their communities is of economic and ecological importance. The objective of this study was to evaluate the effects of different cover crops on soil arthropods and soil Collembola communities and biological soil quality employing the QBS-ar index in Mediterranean vineyards located in Sicily. We also investigated the variations of the above parameters between rows and inter-rows within the same cover crop. Statistical analysis showed that the vineyard age and the sample position (row or inter-row) within the plots significantly affected the soil arthropods abundance. Soil Collembola community composition was not significantly influenced by the cover crop type or sampling positions, while Collembola assemblages followed the vineyard age and the sample position within the plot being consistently higher in the row than in the inter-row.

Keywords: Collembola, Vitis vinifera L., springtails, soil biodiversity

### Introduction

Human activities, such as the intensification of land use, play a critical role in soil functioning leading to the modification of soil abiotic properties and tremendous changes in the structure, composition, and diversity of the soil communities.

Although driven by multiple factors, land use change due to agricultural expansion is the primary driver of biodiversity loss (Cardoso et al., 2020; IPBES, 2019). Many farming practices, such as tillage (Roger-Estrade et al., 2010; Fiera et al., 2020; Gonçalves et al., 2020), the use of fertilizers and pesticides (Brühl & Zaller, 2019) and the homogenization of landscapes leading to the loss of semi-natural habitats tend to reduce biodiversity (Habel et al., 2019; Tilman et al., 2017; IPES-Food, 2016; Zabel et al., 2019). Biodiversity, especially soil-dwelling arthropods, of agroecosystems where intensification occurs is impoverished (Biaggini et al., 2007; Cotes et al., 2010; Ruano et al., 2004; Santos et al., 2007).

Soil health (Kibblewhite et al., 2008) and sustainability depend on the maintenance of the biodiversity-based soil functions (Altieri, 1999) that directly affect production (Dangles & Casas, 2019; Schowalter et al., 2018).

Soil arthropod communities can provide significant services in agroecosystems in terms of interactions with micro-organisms and other invertebrates and biocontrol (Daane and Johnson, 2010; Lavelle et al., 2006; Symondson et al., 2002), nutrient cycling and organic matter transformation (Brussaard et al., 1997; Seastedt & Crossley, 1984; Mocali et al., 2020), litter fragmentation (Hagvar, 2016), and improvement of soil structure (Reichle, 1977).

Collembola constitute a relevant part of soil arthropod communities and contribute to ecosystem functioning by directly and indirectly regulating soil microbial activity and nutrient cycling (Kaneda and Kaneko, 2011; Pieper and Weigmann, 2008). They enhance nitrogen mineralization, directly and indirectly, and thus can increase plant nutrient availability and plant growth (Filser, 2002); they also participate in the dispersal of active fungal spores and bacteria modifying the composition of the rhizosphere microbiome (Crowther et al., 2012; Soong et al., 2016), and can inoculate microbes on the matters to decompose then affecting carbon turnover (Chamberlain et al., 2006). Collembola indirectly contribute to soil structure dynamics and aggregate formation through mucilage secretion and their interaction with arbuscular mycorrhizal fungi (Siddiky et al., 2012). And they might also

indirectly reduce aphid reproduction depending on the plant host (Scheu et al., 1999; Schütz et al., 2008).

It follows that soil arthropods loss will have severe economic and ecological consequences (van der Sluijs, 2020) and that enhancing biodiversity in agricultural ecosystems would contribute to sustainability and productivity (Dainese, 2019; FAO, 2019; Jarvis et al., 2007).

Viticulture is a critical component of Mediterranean farmland (Raffa et al., 2022), which host the three top-producing countries – Italy, France and Spain – which account for almost half of the global wine production (OIV, 2016).

The exponential growth of environmental sustainability certification programs in recent years can undoubtedly be related to greater consumer awareness of the environmental sustainability of agricultural production, of which wine is at the forefront. Increasing the functional biodiversity in the vineyard is a new goal of the wine growing industry; more sustainable agricultural practices aimed at increasing sustainability and biodiversity conservation (Blaise et al., 2022) have been proposed within agroecosystems.

In addition, restoring soil health could confer a terroir effect, enhancing inter- and intra-site variability, which translates into a differential character in the selected wines (Mocali et al., 2020; Nerva et al., 2021; Zhou et al., 2021).

Some of these sustainable farming practices may include but are not limited to the reduction of the use of pesticides, the use of natural predators of pests, the inclusion of ecological infrastructures (e.g. woodlands, ground covers, etc.) adjacent to vineyards (OIV, 2018), the restoration of seminatural habitats (Winter et al., 2018).

Seeding plants to cover the ground is becoming increasingly common (Blaise et al., 2022). Integrating annual and perennial plant species with other than the cultivated species in vineyards in addition to protecting soil from erosion (Bidoccu et al., 2020; Novara et al., 2011; Rodrigo Comino et al., 2016), improves soil's capacity to deliver ecosystem services (Capó-Bauçà et al., 2019; Garcia et al., 2019; García-Díaz et al., 2018; Jian et al., 2020; Marques et al., 2010; Ruiz-Colmenero et al., 2013). The improvements consist of pest regulation (Blaise et al., 2021; Hofmann et al., 2017), refuge provision, and substrate and resources to sustain biodiversity (Eckert et al., 2019; Franin et al., 2016; Hendgen et al., 2018; Nicholls et al., 2008; Sáenz-Romo et al., 2019; Shapira et al., 2018; Schreck et al., 2012).

Although the importance of soil fauna is known, most vineyard studies have focused on aboveground factors, and the biological aspects and threats to the matrix that supports wine production are largely neglected (Renaud et al., 2004; Diti et al., 2020).

Many studies are dedicated to the diversity of microbes, invertebrates, vertebrates, and plants in vineyards (Paiola et al., 2020). However, only a few of these studies were conducted with soil-dwelling organisms.

Even further, several studies have demonstrated the positive effect of inter-row vegetation on grapevine epigean arthropod communities (Eckert et al., 2019; Fiera et al., 2020; Geldenhuys et al., 2021; Gonçalves et al., 2020; Judt et al., 2019), while none of them, to our knowledge, has investigated the soil arthropods nor the possible differences between rows and inter-rows. From that, the need to improve the understanding of vegetation effects on soil arthropods and to investigate more finely the response to managing vegetation cover in vineyards.

This work aimed to evaluate the effects of different soil management practices on soil arthropods and Collembola, communities in Mediterranean vineyards located in Sicily. Using taxonomic and eco-morphological approaches, we studied the response of arthropods to different cover crop management. We hypothesized that: (i) differences in cover crops are reflected in differences in soil arthropod communities, (ii) rows support greater abundance and diversity of soil arthropods than inter-rows, and (iii) also soil biological quality using QBS-ar index (Parisi, 2001; Parisi *et al.*, 2005) differs between rows and inter-rows.

# Material and methods

# Study area

The experiment was established in a nonirrigated organic wine grape vineyard in Camporeale municipality (Sicily, Italy). The area has a mean annual precipitation of 500-600 mm and a mean air temperature of 16-17°C (Portale SIT<agro />).

Three plots belonging to the same farm were selected to compare the soil collembola community between different interrow cover crops (CC) and year of planting: (i) Chardonnay planted in 2010, CC yearling mixture of common vetch (*Vicia sativa* L.), *Hedysarum coronarium* L., and oat (*Avena sativa* L.) (VSA); (ii) Catarratto planted in 1998, CC pigeon bean (*Vicia faba* L. var. *minor*) (FCA); (iii) Sauvignon planted in 2010, CC pigeon bean (*Vicia faba* L. var. *minor*) (FSA).

Rows were North East-South West oriented and manually cane-pruned (Guyot system: 8 and 2 buds per cane and spur, respectively). Vertical shoot-positioned canopies employed two double foliage wires and individual shoot positioning. No other canopy management practices were applied except topping at approximately 1.3 m shoot length/canopy height. Vines were subjected to standard pest management practices routinely used organic wine grape vineyards in the Camporeale area.

In the three plots we have chosen, vine spacing was as in Table 1. CC was sown in one out of two inter-rows in fall 2020 and 2021. Weeds were cut with a mower in mid-April, and no herbicides were applied. The maintenance practices began from mid-April to the end of September. Other cultural practices (soil, nutrition, irrigation, canopy, and pest management) were identical for all plots. Table 1 shows the main geographical and management features of the investigated plots.

,	, <b>,</b>	1	
Plot code	VSA	FCA	FSA
Coordinates	37°54'19.00"N 13° 4'19.74"E	37°54'21.53"N 13° 4'22.79"E	37°54'31.26"N 13° 4'15.45"E
Altitude a.s.l (m)	520	510	410
Soil Texture USDA*	Loamy Sand	Sandy Loam	Sandy Clay Loam
Grape cultivar	Chardonnay	Catarratto	Sauvignon
Rootstock	140 Ru	140 Ru	1103 P
Year of planting	2010	1998	2010
vine spacing (m)	2,40 between the rows 0,90 along the row	2,50 between the rows 1,10 along the row	2,40 between the rows 0,90 along the row
Green cover	Vicia sativa L. Hedysarum coronarium L. Avena sativa L.	Vicia faba minor L.	Vicia faba minor L.

Table 1. Location, characteristics, and vineyard features of the plots.

\* classified following the USDA soil texture triangle classification (Ditzler et al., 2017).

# Soil sampling design

An inter-row was selected in the inner area of each plot to minimize edge effects. We assessed the abundance of soil arthropods using cubic soil samples (with a dimension of about  $10 \times 10 \times 10$  cm). Three samples were collected along a linear transect in the row, between two consecutive vines, and three more in the middle of the adjacent inter-row, resulting in six soil samples per

plot/vineyard/year. The distance between soil samples within the inter-row and rows was 10 m. (Fig. 1).

The soil arthropods were collected on two dates (April 2021 and April 2022), resulting in 36 samples. Sampling was carried out in the first half of April, before the weeds were cut, in order to achieve maximum ground cover of the CC and minimise the mechanical soil disturbance effects of maintenance practices.



Figure 1. Study design: position of soil samples (white circles) (N = 6) in a plot.

# Soil Arthropods Community Analysis

To characterize soil arthropods, soil Collembola communities and the Biological soil quality, soil samples were transferred to the laboratory – being protected from thermal shock and avoiding evaporation – where the fauna was extracted using a modified Berlese-Tullgren funnel (Wallwork, 1976; Parisi, 2001; André et al., 2002; Parisi et al., 2005) and stored in 70 % alcohol. All collected microarthropods were counted and examined under a binocular stereo-microscope (Zeiss Wild M5A stereomicroscope,  $7-45\times$  magnification). The different taxa and biological forms based on morphological features indicating an adaptation to the edaphic environment were carried out following the QBS-ar (Soil Biological Quality-arthropod) method (Parisi et al., 2005; Menta et al., 2018).

Regarding Collembola, at least one specimen for each morpho-species was slide mounted in Hoyer's medium for subsequent optical microscope observation and identification. Mounted

specimens were examined using an Olympus BX51-TF microscope with a multi-viewing system and phase contrast, and a Zeiss «Axio Imager.A1» with differential interference contrast (DIC).

Collembola were identified at species level using the proper springtail taxonomy literature (Gisin, 1960; Dunger, 1994; Jordana *et al.*, 1997; Bretfeld, 1999; Potapow, 2001; Thibaud *et al.*, 2004; Dunger and Schlitt, 2011; Jordana, 2012). When attribution at the species level was impossible (e.g. immature stage), specimens were assigned to the higher taxa and included in the respective groups' total abundance. The examined specimens are deposited in the Museum of Zoology of the University of Navarra (MZNA).

Soil arthropods and soil Collembola communities were analysed according to the following population parameters: (i) taxa richness (S), (ii) Margalef index (d), (iii) Pielou's evenness (J), (iv) Shannon–Wiener (H'), (v) Simpson evenness (e'), (vi) overall abundance, and (vii) abundance at the life forms level.

# Statistical analysis

Soil arthropods community and, more detailed, Collembola community composition variability was assessed by a multivariate analysis of the species abundance based on the Bray-Curtis similarity index by performing a quadratic transformation of the data. To assess significant assemblage differences in soil arthropods community and Collembola community composition, a one-way Analysis of Similarity (ANOSIM) was conducted based on the different CC (mixture and pigeon bean), sampling position (row and inter-row) within each management system and sampling years (2021 and 2022) as factors and transformed species abundance data as the response variable. All analyses were performed using the PRIMER 6 v.6.1 software package (PRIMER-E Ltd., Plymouth). The species of soil Collembola shared among plots were obtained by a Venn diagram analysis using the software PAST (2020) (Hammer et al., 2001). To assess differences in the total number of collected arthropods and the QBS-ar values, a GLM was performed after a BoxCox transformation of data, including year, vineyard age, CC, and position of soil samples as fixed factors.

## **Results and Discussion**

12,703 arthropods were collected, of which 5054 and 7649 were in 2021 and 2022, respectively. A total of 22 taxa were identified in the soil samples (Table 2). Acari were the most abundant group (9648 individuals), followed by Collembola (1856 individuals), Formicidae (367 individuals) and Pauropoda (255 individuals).

			2	021					20	)22					
Таха	VS	SA	F	CA	F	SA	VSA		FCA		F٤	SA			
	i	r	i	r	i	r	i	r	i	r	i	r			
Acari	778	750	69	138	705	1040	588	1488	552	2150	680	710			
Amphipoda										1					
Araneae										2	1	7			
Chilopoda		1				2		1	1		2	4			
Coleoptera	4	9	6	11	8	9	13	15	15	4	11	5			
Collembola	211	395	34	69	157	209	20	59	168	106	332	96			
Diplopoda		1			1	6		1	2	1	1	5			
Diplura	3	4		2	1	13	1	1		1	3	1			
Diptera	1	5	1	2	3	4	9	1	1	4	4	7			
Diptera (larvae)	8		22	4	7	3		1	3			4			
Embioptera												12			
Formicidae	46	25	5	18	4		18	9	1	1	79	161			
Hemiptera	7	1	1	5	1			5	8	1	1	6			
Hymenoptera	1	1		1		1		1		3	3	1			
Isopoda		6			4	1	1	1			1				
Other holometabolic (larvae)	9	7	10	4	8	1	9	6	7	2	18	5			
Pauropoda	8	17	7	10	30	72	3	18	12	13	39	26			
Protura		2	1					1			1				
Pseudoscorpiones						1						2			
Psocoptera		1				2									
Symphyla	4	6		1	1	15	1	44		18	6	12			
Thysanoptera	3	4		4	1	1	3	4	3	1					
Total n° specimens	1083	1235	156	269	931	1380	666	1656	773	2308	1182	1064			

Table 2. Taxa abundance for each plot and among inter-rows (i) and rows (r) in 2021 and 2022.

Microarthropod abundance was higher in the rows than inter-rows in all plots and years, except for FSA in 2022. The density of microarthropods registered in 2022 was higher than in 2021.

Figure 2 shows the total soil microarthropods abundance recorded in the different samplings. In 2021, a higher density was registered in FSA and VSA inter-row and row samples, respectively. In all the other treatments, a higher abundance was recorded in 2022 compared to 2021. These annual changes in microarthropod abundance between years are also associated with shifts in microarthropod community composition (Figure 4).

Statistical analysis showed that the only factors which significantly affected the soil arthropod abundance were the vineyard age ( $F_{1,25} = 0.23$ , p < 0.01) and the sample position within the CC ( $F_{2,25} = 4.33$ , p < 0.01). Vineyard age and the sample position are chiefly related to the intense spatial variation of soil resources, which can generate hotspots of biological activity and abundance in soil, for example, on and around plant roots (Kuzyakov & Blagodatskaya, 2015) that have been shown to play a very active role in attracting soil fauna (Bonkowski et al., 2009). The mechanical disturbance of inter-row management and the root attraction could be additive.


Figure 2. Soil arthropod abundance (specimens per sample, mean  $\pm$  standard deviation, N = 3). Density of soil microarthropods measured as means ( $\pm$ SE) of individuals, (N)/1 dm<sup>3</sup> soil volume, determined for management and data sampling.

The biodiversity indexes and their comparisons are reported in Table 3-4. In 2011, the taxa richness and the Margalef index were higher in the rows; evenness and Shannon–Wiener index were more elevated in FCA while denoted a higher variation among inter-rows and rows. In 2022, the same numbers of taxa were registered in VSA and FCA, and the rest of the biodiversity indexes were higher in FSA; a different and relatively homogeneous distribution in the population structure from this plot determined higher values of Shannon–Wiener and equitability indexes. A high variation was registered among inter-rows and rows.

		2021				2022		
	VSA	FCA	FSA	-	VSA	FCA	FSA	
Ν	2318	425	2311		2322	3081	2246	
S	18	14	18		17	17	19	
d	2,19	2,15	2,20		2,06	1,99	2,33	
J	0,34	0,61	0,30		0,20	0,18	0,42	
H'	1,00	1,60	0,88		0,56	0,52	1,25	
e'	0,50	0,69	0,40		0,20	0,22	0,57	

**Table 3**. Taxa abundance (*N*), richness (*S*), and ecological indices values (d: Margalef; J: Pielou's evenness; *H*': Shannon–Wiener; *e*': Simpson evenness) for each plot in 2021 and 2022.

**Table 4**. Taxa abundance (N), richness (S), and ecological indices values (d: Margalef; J: Pielou's evenness; H': Shannon–Wiener; e': Simpson evenness) for each plot and among inter-rows (i) and rows (r) in 2021 and 2022.

	2021								2022						
	VSA		FCA		FSA			VSA		FCA		FSA			
	i	r	i	r	i	r		i	r	i	r	i	r		
Ν	1083	1235	156	269	931	1380		666	1656	773	2308	1183	1064		
S	13	17	10	13	14	16		11	17	12	15	17	17		
d	1,72	2,25	1,78	2,15	1,90	2,08		1,54	2,16	1,65	1,81	2,26	2,30		
J	0,36	0,36	0,70	0,59	0,32	0,32		0,24	0,18	0,36	0,13	0,42	0,42		
H'	0,92	1,02	1,62	1,50	0,84	0,88		0,58	0,52	0,89	0,34	1,20	1,20		
e'	0,44	0,53	0,73	0,67	0,40	0,41		0,22	0,19	0,44	0,13	0,59	0,52		

Soil arthropods community composition was not significantly influenced by the analysed factors: CC (mixture and pigeon bean), sampling positions (row and inter-row) and sampling years (2021 and 2022). Nevertheless, soil arthropods showed different relative abundance between plots, positions (inter-rows and rows) (Figure 3), and between years (Figure 4). Acari dominate in all plots on both sampling dates. Nevertheless, this taxon showed lower relative abundance in 2021 than in 2022 in VSA and FCA, while FSA showed an inverse trend.



**Figure 3.** Relative abundance of arthropods across the three plots and between inter-rows (i) and rows (r) in 2021 (on the left) and 2022 (on the right).



Figure 4. Relative abundance of arthropods across the three plots and between years.

# Soil biological quality

Taking into account the plots, without distinction between rows and inter-rows, the QBS-ar values in the dataset ranged from 152 to 234 (Figure 5), and are in line with those recorded in the same habitat by Costantini et al. (2015) and Gagnarli et al. (2015), and higher than those recorded from Ghiglieno et al. (2019) or Gonçalves et al. (2020).

The distribution of groups identified ranges between 14 and 19 and it's higher than the numbers found by Costantini et al. (2015) (2-17 groups), Gagnarli et al. (2015) (10-17 groups), and Ghiglieno et al. (2019) (3-14 groups).



Figure 5. QBS-ar values (resulting from the sum of the EMI scores) for each plot.

Considering rows and inter-rows separately, the QBS-ar values of the row are higher than the interrow in all plots and on both dates (Figure 6).



**Figure 6**. QBS-ar values (as resulting from the sum of the EMI scores) for each plot and among inter-rows (i) and rows (r).

Statistical analysis showed that the only factors which significantly affect the QBS-ar values are the vineyard age ( $F_{1,25} = 20.01$ , p < 0.01) and the sample position within the CC ( $F_{2,25} = 4.12$ , p = 0.03).

### Collembola communities

Overall, 1856 springtail individuals were collected, of which 242 were mounted for identification; 42 species or morphospecies, belonging to 14 families were identified (Table 5).

Family	Spacios	VS	SA	FCA		FSA	
Failing	Species	i	r	i	r	i	r
Dicyrtomidae	sp.1					1	
Entomobryidae	Entomobrya cf marginata		1				
Entomobryidae	Entomobrya cf schoeti			1			
Entomobryidae	Lepidocyrtus cf lignorum				3		
Entomobryidae	<i>Lepidocyrtus rapitalai</i> Giuga & Jordana, 2023			1		6	14
Entomobryidae	Pseudosinella cf decipiens						2
Entomobryidae	Pseudosinella cf francae				4		
Entomobryidae	<i>Pseudosinella fallax</i> (Börner, 1903)					6	21
Entomobryidae	Pseudosinella sp.1	2	24		1		5
Entomobryidae	Pseudosinella sp.2						1
Entomobryidae	Pseudosinella octopunctata Börner, 1901	11	19		8		
Entomobryidae	Pseudosinella cf occidentalis						1
Entomobryidae	Seira sp.						1
Hypogastruridae	Acherontiella bougisi Cassagnau & Delamare, 1955					3	1
Hypogastruridae	Ceratophysella gibbosa (Bagnall, 1940)	1					
Hypogastruridae	Ceratophysella succinea (Gisin, 1949)	22	89	42	13	31	5
Hypogastruridae	Hypogastrura sp.			1			
Hypogastruridae	<i>Xenylla marittima</i> Tullberg, 1869				2		
Isotomidae	Desoria cf neglecta		4	36	18	6	6
Isotomidae	Hemisotoma thermophila (Axelson, 1900)			1			
Isotomidae	Isotoma sp.	8					
Isotomidae	Isotomiella minor (Schäffer, 1896)		6	2		102	94
Isotomidae	Isotomiella paraminor Gisin, 1942				4		
Isotomidae	Isotomodes trisetosus Denis, 1923	1					
Isotomidae	Isotomurus cf palustris			1			
Isotomidae	Parisotoma notabilis (Schäffer, 1896)	5	14		4		
Katiannidae	Sminthurinus sp.			1		2	
Neanuridae	Pseucachorutes parvulus Börner, 1901		1				
Neanurinae	Deutonura sp.				1	1	
Neelidae	Megalothorax minimus Willem, 1900				1	1	1
Oncopoduridae	Oncopodura crassicornis Shoebotham, 1911						2
Onychiuridae	Mesaphorura critica Ellis, 1976	1	1			1	1
Onychiuridae	<i>Protaphorura armata</i> (Tullberg, 1869)	112	271			115	25
Onychiuridae	Protaphorura campata (Gisin, 1952)	21	1	21	65	96	30
Orchesellidae	Heteromurus major (Moniez, 1889)					57	12
Orchesellidae	Heteromurus nitidus (Templeton, 1836)			4		14	14
Orchesellidae	Orchesella cf balcanica			1		10	2
Sminthuridae	Sminthurus cf multifasciatus					2	
Sminthurididae	Sminthurides inaequalis Börner, 1903						2
Sminthurididae	Sminthurides schoetti Axelson, 1903						11
Sminthurididae	<i>Sphaeridia pumillis</i> (Krausbauer, 1898)	2	1	9	2	3	
Tullbergiidae	Tullbergiidae sp.1				1		

Table 5.	List of t	families	and spec	eies (or	morphosp	becies)	identified	d in the	three pl	lots.
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*Protaphorura armata* (Tullberg, 1869) was absent from FCA but was the most common species in 2021. The congeneric *P. campata* (Gisin, 1952) was the most common species in 2022.

Statistical analysis of the indices (abundance, S, d, H', J and e') applied to Collembola communities (Tables 6 and 7, Figs 7 and 8) did not show significant differences between VSA, FCA and FSA plots at the p > 0.05 level.

**Table 6**. Soil Collembola abundance (N), richness (S), and ecological indices values (d: Margalef; J: Pielou's evenness; H': Shannon–Wiener; e': Simpson evenness) for each plot and among inter-rows (i) and rows (r) in 2021 and 2022.

	2021								2022							
	VSA		FC	CA	FS	FSA		VSA		FCA		FSA				
	i	r	i	r	i	r		i	r	i	r	i	r			
Ν	171	390	9	37	141	165		15	42	112	90	316	86			
S	9	8	4	8	11	13		5	6	11	8	15	17			
d	1,56	1,17	1,37	1,94	2,02	2,35		1,48	1,34	2,12	1,56	2,43	3,59			
J	0,56	0,43	0,92	0,88	0,63	0,73		0,67	0,62	0,65	0,46	0,73	0,82			
H'	1,22	0,89	1,27	1,82	1,51	1,87		1,08	1,11	1,56	0,96	1,97	2,31			
e'	0,55	0,47	0,78	0,82	0,72	0,78		0,56	0,60	0,74	0,47	0,82	0,87			

**Table 7**. Soil Collembola abundance (N), richness (S), and ecological indices values (d: Margalef; J: Pielou's evenness; H': Shannon–Wiener; e': Simpson evenness) for each plot and among inter-rows (i) and rows (r) in 2021 and 2022.

	_	2021		2022						
	VSA	FCA	FSA	VSA	FCA	FSA				
Ν	561	46	306	57	202	402				
S	13	10	17	8	17	20				
d	1,90	2,35	2,80	1,73	3,01	3,17				
J	0,43	0,87	0,68	0,67	0,56	0,73				
H'	1,09	2,00	1,93	1,40	1,57	2,17				
e'	0,50	0,84	0,78	0,71	0,72	0,85				



**Figure 7.** Soil Collembola abundance (specimens per sample, mean  $\pm$  standard deviation, N = 6) for each plot. Density of soil Collembola measured as means ( $\pm$ SE) of individuals, (N)/1 dm<sup>3</sup> soil volume, determined for management and data sampling.



**Figure 8.** Soil Collembola abundance (specimens per sample, mean  $\pm$  standard deviation, N = 3) for each plot and among inter-rows (i) and rows (r). Density of soil Collembola measured as means ( $\pm$ SE) of individuals, (N)/1 dm<sup>3</sup> soil volume, determined for management and data sampling.

Soil Collembola community composition was not significantly influenced by the analysed factors: CC (mixture and pigeon bean), sampling positions (row and inter-row) and sampling year (2021 and 2022). Nevertheless, species composition differed between plots (Figure 9) and inter-rows and rows (Figure 10).

Species associated with each plot ranged from 11.9% (VSA) to 28.6% (FSA) of their Collembola communities; 14.3% of soil Collembola were shared among the three plots (Figure 9). The soil

Collembola shared among inter-rows and rows. The three plots ranged from 17.4% (FCA) to 53.3% (VSA) (Figure 10).



Figure 9. Venn diagram showing the common and exclusive Collembola species of the three plots.



**Figure 10**. Venn diagram showing the common and exclusive Collembola species of inter-rows (i) and rows (r) in the three plots.

The CLUSTER analysis on the similarity matrix indicated that the Collembola assemblages grouped into three main clusters, following the vineyard age more than the sample positions (Fig. 11); a higher similarity was recorded between the two ten years old vineyards (VSA and FSA) that resulted moore similar in comparison to the 20 years old vineyard FCA.



**Figure 11**. CLUSTER diagram showing linkages of Collembola communities by different management systems. Linkages shown are based on the Bray-Curtis similarity matrix.

### Conclusion

The vineyard age and the sampling position (row or inter-row) within the plots significantly affected the soil arthropods abundance and QBS-ar values, which seem to be negatively affected by the mechanical disturbance of inter-row management and positively affected by the proximity of plant roots. The vineyard age also affected Soil Collembola assemblages.

Neither the Soil arthropods community nor the Collembola community composition was significantly influenced by cover crops, sampling positions, and sampling years.

Although the diversity indices are less sensitive to detect changes in soil arthropod and soil Collembola communities, the rows support higher diversity and abundance than reported by other authors. Studies aimed at evaluating the biodiversity of the vineyard soil fauna should consider these inhomogeneities between rows and inter-rows.

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