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# Influence of farming practice, crop type and soil properties on the abundance of Enchytraeidae (Oligochaeta) in Greek agricultural soils

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#### Abstract

Little information is available on the distribution of soil organisms such as enchytraeids in Greek agricultural soils. Therefore the aim of this work was to study their population densities under different farming practices, in different regions, and in relation to physico-chemical soil properties such as texture, pH, organic matter content, C:N ratio, and total copper content in soil. Soil samples were taken from 380 sites located in 18 regions all over Greece, but only data from 120 sites in 9 regions were appropriate for statistical evaluations. At each site soil samples were taken once, transported to the laboratory and kept there at 20 °C and at a moisture of 40–50 % WHC for six weeks. Enchytraeids were extracted from the soils by a wet extraction method and counted. Species composition was determined in a few samples, revealing species of the genera Fridericia and Enchytraeus. Enchytraeids were absent from many soil samples which may be caused by the fact that many soils in Greece have a xeric moisture regime, conditions that are unfavourable to soil organisms. Highest abundances (about 14 000 ind. m<sup>-2</sup>) were recorded at olive groves and vineyard sites in mainland regions. Lowest abundances were recorded on islands such as Santorini, where no enchytraeids were detected in 13 out of 17 samples. Statistical analyses revealed significant differences of enchytraeid abundance among crop types (higher in olive than in vineyards or citrus) but not among farming practices or soil properties, including copper content. Sites with the same crop located in different regions differed in enchytraeid abundance in the case of olive groves and vineyards but not in citrus orchards. Total copper content was significantly higher in citrus orchards than in the other two crop types, but the determined copper levels were generally below those considered to harm oligochaetes. Further sampling using standard methods while addressing the species level are needed to reveal details of enchytraeid distribution, species composition and their ecological role in Greek soils.

Keywords: monitoring, regions, organic matter content, copper

## 1. Introduction

In contrast to other Mediterranean countries such as Turkey or Italy (Rota 1994, 1995), very little information is available for the distribution of Enchytraeidae (Oligochaeta) in Greek soils. With the exception of a few data about enchytraeids in Greek agricultural soils (Vavoulidou et al. 1998) no data are available that relate their distribution to soil types, farming practices or crops. Therefore enchytraeid population densities were studied under different farming practices, in different regions, and in relation to physical-chemical soil properties such as particle size, organic matter content and C:N ratio. In addition, total copper content in soil was considered, to assess possible effects of prolonged fungicide treatment on enchytraeids (see Vavoulidou et al. 2005). Since it is known from studies in other European countries that enchytraeids react sensitively to environmental changes, they can be used as bioindicators for assessing soil quality (Römbke 1995, Jänsch et al. 2005). Soil samples were taken in 1996–2007 as part of various research projects related to soil quality indicators, assessment of the implementation of organic agricultural practices in Greece and soil mapping.

The aim of this study was to produce research data (1) for a use of enchytraeids as potential bioindicators of soil conditions across Greece, (2) to gather preliminary information on the spatial distribution and ecological requirements of enchytraeids, and (3) to assess the influence of farming practices on enchytraeid abundance.

#### 2. Materials and methods

Soil samples were collected once from more than 380 sites in 18 regions across Greece (Fig.1). Of these, data sets from 120 sites located in 9 regions were analysed here (Tab. 1), being the most suitable for rigorous statistical testing. These regions are (number codes in brackets as given in Fig. 1): Attika (6); Western Greece (7b); Achaia (9); Ilia (10); Messinia (11); Arkadia (12); Argolis (13); Chania, Crete (14); Santorin (16). They are located in the southern part of the Greek mainland (in particular the Peloponnes) and the islands of Santorin and Crete. Sampling began in 1996 and continued up to 2007, covering the whole country. It took place mainly during late winter until the beginning of spring since in this period climatic conditions are favourable for enchytraeid worms. At each site samples were categorised according to the farming system (organic, conventional, fallow land) and the crop type: vineyards (mainly Peloponnese and Crete), citrus (Western Greece, Peloponnese and Crete) or olive groves (all over the country). Soil sampling for the determination of physicochemical soil properties was conducted using a riverside auger to a depth of 0-30 cm. Each sample was a composite sample of 3 to 5 subsamples and was analysed for texture (% clay), soil organic matter (% OM), pH, C:N-ratio and total copper (Cu) content (mg kg-1 soil dry weight). The results of these determinations are summarised in the Annex. Most soils sampled are characterized by fine texture (i.e. high clay content), neutral or alkaline in pH, often with low C:N-ratios and frequently poor in organic matter. The climate in Greece is characterised by hot, dry summers and winter rainfall (late September to May), with mean annual rainfall generally less than 800 mm except in the west of the country. Rains can be very intensive, particularly in the driest areas.



Fig. 1 Sampling regions. Samples of regions 6, 7b, 9–14 and 16 are analysed in this contribution.

For enchytraeid sampling, top soils without indication of disturbance were selected. A cylindrical core (5.6 cm, 25 cm<sup>2</sup>) was used to collect 4 samples from the topsoil layer to a depth of 5–10 cm. In the laboratory samples were placed in glass dishes (Weck glass) and incubated at 20 °C with sufficient soil moisture (40–50 % WHC). Crushed organic material was also placed at the soil surface for food. Objective of the incubation was to allow hatching of young worms from cocoons. After 6 weeks of incubation, the enchytraeids were extracted by wet extraction (Graefe in Dunger & Fiedler 1997), then stained (rose Bengal), and finally counted using a stereomicroscope. Classification of age stages as well as species-level determination was not performed due to the lack of personnel and specialists. However, in a few samples the species composition was determined.

Abundance of enchytraeids (ind. m<sup>-2</sup>) in Greece, grouped by region, crop type (citrus, olive and vineyard) and farming system (organic, conventional and fallow). Number of investigated sites per group are given in brackets, together with the raw data in case of only one or two sites investigated per group, or with the range and the mean  $\pm$  standard deviation of the raw data in cases of more than two sites per group; n.a.: no data available. Code for the regions: 6 Attiki; 7b Western Greece; 9 Achaia; 10 Ilia; 11 Mesinia; 12 Arkadia; 13 Argolis; 14 Chania, Crete; 16 Santorini; see also Fig. 1. Tab. 1

Crop and					Region code					
farming system	10	11	12	13	14	16	9	7b	6	Group Total
Citrus	400; 5800 (2)	n.a.	n.a.	1200- 2000; $1600 \pm$ 327 (4)	0-8800; 2929 ± 3970 (7)	n.a.	n.a.	$\begin{array}{c} 0-5800;\\ 1700\pm\\ 2153\ (6)\end{array}$	$\begin{array}{c} 0-2200; \\ 450\pm 871 \\ (6) \end{array}$	$\begin{array}{c} 0-8800;\\ 1840\pm2581\ (25)\end{array}$
organic	5800 (1)	n.a.	n.a.	1600; 2000 (2)	$\begin{array}{c} 0-8500;\\ 3500\pm4444\ (3)\end{array}$	n.a.	n.a.	1200; 2000 (2)	0; 0 (2)	$\begin{array}{c} 0-8500;\\ 2310\pm2759\ (10)\end{array}$
conv	n.a.	n.a.	n.a.	1600 (1)	$300-8800; 3333 \pm 4744 (3)$	n.a.	n.a.	0; 1200 (2)	0; 100 (2)	$\begin{array}{c} 0-8800;\\ 1613\pm2965\ (8)\end{array}$
fallow	400 (1)	n.a.	n.a.	1200 (1)	0 (1)	n.a.	n.a.	0; 5800 (2)	400; 2200 (2)	$\begin{array}{c} 0-5800;\\ 1429\pm2080~(7)\end{array}$
Olive	$\begin{array}{c} 0-400;\\ 80\pm179\\(5)\end{array}$	0-14300; 5700 $\pm 4978 (15)$	2000–5700; 3500 ± 1947 (3)	400; 800 (2)	0-9900; 4300 ± 2909 (12)	n.a.	n.a.	n.a.	$\begin{array}{c} 0-4000;\\ 1550\pm1821 \ (6)\end{array}$	0–14300; 3686 ± 3908 (43)
organic	0; 400 (2)	3900-12300; $6880 \pm 3440$ (5)	2000 (1)	400 (1)	2100-9900; $5750 \pm 3207$ (4)	n.a.	n.a.	n.a.	$\begin{array}{c} 0-4000;\\ 1333\pm2309\ (3)\end{array}$	0−12300; 4013 ± 3740 (16)
conv	0 (1)	$\begin{array}{l} 0-14300 \ ; \ 5225 \\ \pm \ 6252 \ (4) \end{array}$	5700 (1)	n.a.	$\begin{array}{c} 0-9000;\\ 3520\pm3336~(5)\end{array}$	n.a.	n.a.	n.a.	700 (1)	0−14300; 3742 ± 4242 (12)
fallow	0; 0 (2)	$\begin{array}{c} 0-14100; \ 5033\\ \pm \ 5862 \ (6) \end{array}$	2800 (1)	800 (1)	$\begin{array}{c} 1900{-}5000;\\ 3667\pm1595\;(3)\end{array}$	n.a.	n.a.	n.a.	900; 3700 (2)	$0-14100; 3293 \pm 4049 (15)$

cont.	
Tab. 1	

Crop and					Region code					
farming system	10	11	12	13	14	16	9	7b	6	Group Total
Vineyard	n.a.	n.a.	$\begin{array}{l} 600 - 14500; \ 6687 \\ \pm \ 5086 \ (15) \end{array}$	0; 800 (2)	n.a.	$\begin{array}{c} 0-5600;\\ 465\pm1363\ (17)\end{array}$	0-800; 233 ± 267 (12)	n.a.	$\begin{array}{c} 0-5800;\\ 1683\pm2277\ (6)\end{array}$	0−14500; 2344 ± 4023 (52)
organic	n.a.	n.a.	$\begin{array}{l} 600-13000; 5467\\ \pm 5475 \ (6)\end{array}$	800 (1)	n.a.	0-1200; $300 \pm 502$ (6)	0-400; $200 \pm 231$ (4)	n.a.	0; 5800 (2)	0−13000; 2211 ± 3892 (19)
conv	n.a.	n.a.	$1800-14400; 5700 \\ \pm 5939 (4)$	n.a.	n.a.	0-5600; 933 ± 2286 (6)	0-400; $200 \pm 231$ (4)	n.a.	0; 2300 (2)	0−14400; 1969 ± 3748 (16)
fallow	n.a.	n.a.	$\begin{array}{l} 4200{-}14500; 8940 \\ \pm 4130 \ (5) \end{array}$	0 (1)	n.a.	$\begin{array}{c} 0-500;\\ 100\pm224~(5)\end{array}$	$\begin{array}{c} 0-800;\\ 300\pm 383 \ (4)\end{array}$	n.a.	0; 2000 (2)	0–14500; 2847 ± 4576 (17)
Total										0–14500; 2720 ± 3772 (120)

The data set presented in the Annex was used for statistical analysis (Statistica, version 8.0, StatSoft Inc., Tulsa, OK, USA). The design of the data set was not fully factorial with regard to all three characteristics (region, crop type and farming system). None of the three crop types (or the three farming types) was present in all regions. Analysis in a three-way ANOVA was therefore not possible, but differences among regions were analysed in one-way ANOVAs within crop types. The data set is, however, fully factorial with regard to crop type and farming system, which allows a two-way ANOVA analysis. Since variance homogeneity was achieved by data transformation, the fact that the data set is rather unbalanced with regard to crop type (25 data sets for citrus in contrast to 43 and 52 for olive and vineyards, respectively) is not considered as impacting the analysis. With regard to farming system, the data set is roughly balanced (41 fallow, 45 organic and 36 conventional). Enchytraeid abundance data were square root transformed (sqrt (ind. m<sup>-2</sup> + 0.4)) to achieve compliance with assumptions of ANOVA (normal distribution, variance homogeneity).

### 3. Results and discussion

Extracting and counting worms after incubation of samples is a novel sampling technique adapted to climates largely hostile for enchytraeids. The idea of incubation is to prolong the period favourable for enchytraeids: maturation of juveniles increases the percentage of identifiable specimens, and hatching of young worms from cocoons increases the number of countable worms (eggs and cocoons cannot be extracted). However, comparability of this study with other investigations that use standard techniques (e.g., ISO 2007) is limited, since incubation may alter abundance and species composition. On the other hand, these alterations have probably not influenced the overall outcome of this study, since all samples were treated in the same way.

Abundance of enchytraeids across all 120 data sets ranged from 0 ind.  $m^{-2}$  to 14 500 ind.  $m^{-2}$  with a mean of 2720 ind.  $m^{-2}$  (Tab. 1). In the following, the influence of crop type and farming system, regions, different soil properties and copper content will be analysed.

## 3.1. Analysing influence of crop type and farming system

A two-way ANOVA of transformed abundance with crop type and farming system as main factors (across all regions) revealed significant difference among crop types, but not among farming systems (crop type:  $F_{2,111} = 3.58$ , p = 0.031; farming system:  $F_{2,111} = 0.27$ , p = 0.762; crop\*farming:  $F_{4,111} = 0.013$ , p = 0.971). The lack of difference due to the farming system may be attributed to the fact that changes in management practices were too recent to influence enchytraeid abundances. Conversions to organic practice have largely taken place in the past 10 years and farms still in conversion have been included as organic. Irrespective of the farming system, enchytraeid abundance is higher in olive orchards than in vineyards and citrus orchards (Fig. 2).



Fig. 2 Abundance of enchytraeids in different crop types and farming systems, as means and 95 % confidence intervals of sqrt-transformed data ( $\sqrt{(ind. m^2 + 0.4)}$ )

#### 3.2. Analysing the difference among regions

Potential differences among regions were subsequently analysed within the three different crop types, averaging across the farming systems, since they had no apparent impact on enchytraeid abundance within these crop types. Within vineyards, a significant difference among regions was detected ( $F_{4,47} = 17.9$ , p < 0.0001), with abundance being highest in region 12 (Arkadia) (Fig. 3). None of the factors investigated in this study explain the higher number of enchytraeids in this region – in the northern part of the Peloponnes – compared to numbers in the other four vineyard regions studied. Olive orchards also differed significantly among regions ( $F_{5,37} = 4.36$ , p = 0.003), but in this case there were several regions with high numbers, one of them again region 12 (Arkadia), and one region (10, Ilia) with a very low abundance of enchytraeids (Fig. 4). Within citrus orchards, no difference among regions was detected ( $F_{4,20} = 0.996$ , p = 0.433).



Fig. 3 Abundance of enchytraeids in vineyards of different regions (averaged across farming systems), as means and 95 % confidence intervals of sqrt-transformed data ( $\sqrt{(ind m^{-2} + 0.4)}$ ). Region codes as given in legend of Tab. 1.



Fig. 4 Abundance of enchytraeids in olive groves of different regions (averaged across farming systems), as means and 95 % confidence intervals of sqrt-transformed data ( $\sqrt{(ind m^{-2} + 0.4)}$ ). Region codes as given in legend of Tab. 1.

#### 3.3. Analysing the influence of organic matter content

Regarding organic matter (OM) content, two data points were clearly identified as outliers (Grubbs test, p < 0.001) with values of 12.1 % and 8.0 %. The respective data sets were excluded in all analyses including OM as factor. The organic matter content differed significantly both among crops types and among farming systems (crop:  $F_{2,107} = 22.9$ , p < 0.0001; farming:  $F_{2,107} = 6.7$ , p = 0.002; crop\*farming:  $F_{4,107} = 1.4$ , p = 0.253). OM was generally highest at fallow sites and lowest particularly in non-fallow vineyards (Fig. 5). Organic matter content did not correlate with the abundance of enchytraeids across the entire data set (n = 116, Pearson R = 0.105, p = 0.263). OM was also not significant as a covariate when analyzing the effect of crop type and farming system on enchytraeid abundance (OM:  $F_{1,106} = 0.006$ , p = 0.936). Hence, the difference in OM content among crops and farming system cannot explain the observed difference in enchytraeid abundance among crop types.



Fig. 5 Content of organic matter (%) in different crop types under different farming systems, as means and 95 % confidence intervals.

## 3.4. Analysing the influence of pH, C:N ratio and soil texture

Enchytraeid abundance was not correlated with soil pH across all sites (Spearman Rank correlation, p = -0.093). Neither C:N ratio nor soil texture as represented by the percentage of clay were correlated with transformed enchytraeid abundance (Pearson R = -0.025, p = 0.791 and. Pearson R = 0.162, p = 0.076, respectively. Therefore, none of these three soil characteristics are further considered here.

#### 3.5. Analysing the influence of copper content

The total copper content differed significantly among crop types independently of the farming system (crop:  $F_{2,88} = 8.74$ , p = 0.0003; farming:  $F_{2,88} = 0.53$ , p = 0.593; crop\*farming:  $F_{4,88} = 0.17$ , p = 0.954). The copper content was particularly increased in the soil of citrus orchards (Fig. 6), which differed significantly from the other two crop types in copper content (Scheffe posthoc test; citrus/vineyard: p = 0.0006; citrus/olive: p = 0.0032; olive/vineyard: p = 0.938). However, total copper content was not correlated with the transformed abundance of enchytraeids across all sites (Pearson R = 0.013, p = 0.897) and also not significant when considered as covariate in a two-way ANOVA with crop type and farming system as main factors ( $F_{1,87} = 2.27$ , p = 0.964).



Fig. 6: Total copper content (mg kg<sup>-1</sup> soil dry weight) in different crop types under different farming systems, as means and 95 % confidence intervals.

Copper is often used as a fungicide in many crops, particularly in grapes (Strumpf et al. 2002). Due to accumulation over time in Western and Central European vineyards, its further use is intensively discussed within the European Union. In early 2009 the EU decided that the application of copper will be restricted to greenhouses in the future. This decision was partly based on the fact that copper is highly toxic to enchytraeid worms, not only in laboratory tests (Jänsch et al. 2007), but also in the field (Maraldo et al. 2006). However, while in the laboratory a NOEC (No-observed-effect-concentration) value of 105 mg kg<sup>-1</sup> soil dry weight (dw) was determined, the enchytraeid field study was performed with higher concentrations

 $(395 + 890 \text{ mg kg}^{-1} \text{ soil dw})$ . In any case, the total soil copper content can be considered as a stress factor for these worms. However, no significant correlation was found here between the copper content measured in Greek agricultural soils and the number of enchytraeids. This result is probably due to the fact that mean total copper contents in soils of different crop types were determined as 54.6, 35.7, and 34.0 mg Cu kg<sup>-1</sup> soil dw for citrus orchards, olive groves, and vineyards, respectively. Values above 100 mg kg<sup>-1</sup> were found only in 2 % of the soil samples. Therefore, the copper content of almost all Greek soils studied is within the range considered to be safe by the German Soil Protection Law (depending on the soil type: 20–60 mg/kg soil dw; BBodSchV 1999) and also lower than the content considered harmful for oligochaetes (about 55 mg kg<sup>-1</sup>) (Römbke et al. 2006). Copper accumulation in soil due to the use of copper fungicides has not been reported in Greek soils (Vavoulidou et al. 2005).

### 3.6. Species composition of selected samples

The most common enchytraeid species found in some samples belong to the genera *Fridericia* and *Enchytraeus*, they are as follows (in brackets, the respective region where they were found is given):

Enchytraeus bigeminus Nielsen & Christensen, 1963 (Attika)

Enchytraeus buchholzi Vejd. sensu lato (Santorin; Iraklio, Crete)

Enchytraeus coronatus Nielsen & Christensen, 1959 (Lamia; Arkadia, Peloponnese)

Fridericia berninii, Dózsa-Farkas, 1988 (Santorini)

Fridericia cusanica Schmelz, 2003 (Santorini)

Fridericia granosa Schmelz, 2003 (Iraklio, Crete)

*Fridericia pretoriana* Stephenson, 1930 (= *F. caprensis* Bell, 1947) (Attika, Voiotia and Lamia)

*Fridericia berninii* and *F. pretoriana* are typically Mediterranean species, not recorded from temperate regions further north. The recently described *F. granosa* and *F. cusanica* were known so far only from northern Germany (Schmelz 2003). The three *Enchytraeus* species are widely distributed and typical of agricultural soils, but *E. bigeminus* is rarely recorded from temperate regions; it seems that this species, which reproduces mainly by fragmentation, is more common in warmer climates (Schmelz, pers. comm.). Taxic diversity is probably higher than recorded here, but in-depth taxonomic studies are required for any further statement regarding species number and composition.

## 4. Conclusion

Judging from this pilot study, soils in Greece do not show significant enchytraeid activity (abundance) during the late winter / early spring season. Farming practice does not appear to considerably affect population abundance in arable soil, while the crop type can significantly influence enchytraeid numbers. Despite the co-occurrence of highest enchytraeid densities and highest contents of organic matter in olive groves (Fig. 2 and Fig. 5), soil properties such as OM, pH, C:N ratio and texture did in general not influence enchytraeid numbers significantly. Although xerothermic conditions in Greece limit the survival and activity of enchytraeids, the existence of small enchytraeid populations may be reason enough for a systematic research. Further sampling using standard methods (i.e. ISO 2007) while

addressing the species level are needed to reveal details of enchytraeid distribution, species composition and their ecological role in Greek soils.

### 5. References

- BBodSchV (1999): Bundes-Bodenschutz- und Altlastenverordnung (BBodSchV) vom 12. Juli 1999. BGBL I, Nr. 36: 1554–1582.
- Dunger, W. & H. J. Fiedler (eds) (1997): Methoden der Bodenbiologie, 2nd ed. Gustav Fischer, Jena: 539 pp.
- ISO (International Organization for Standardization) (2007): Soil Quality—Sampling of Soil Invertebrates Part 3: Sampling and Soil Extraction of Enchytraeids. ISO 23611–3, Geneva, Switzerland.
- Jänsch, S., J. Roembke, & W. Didden (2005): The use of enchytraeids in ecological soil classification and assessment concepts. Ecotoxicology and Environmental Safety **62**: 266–277.
- Jänsch S., J. Römbke, H-J. Schallnaß & K. Terytze (2007): Derivation of soil values for the path 'soil soil organisms' for metals and selected organic compounds using species sensitivity distributions. – Environmental Science Pollution Research 14: 308–318.
- Maraldo, K., B. Christensen, B. Strandberg & M. Holmstrup (2006): Effects of copper on enchytraeids in the field under differing soil moisture regimes. Environmental Toxicology and Chemistry **25**: 604–612.
- Römbke, J. (1995): Enchytraeen (Oligochaeta) als Bioindikator. UWSF Zeitschrift für Umweltwissenschaften und Schadstoffforschung 7: 246–249.
- Römbke, J., S. Jänsch, H-J. Schallnaß & K. Terytze (2006): Bodenwerte für den Pfad 'Boden Bodenorganismen' für 19 Schadstoffe. Bodenschutz 11: 112–116.
- Rota, E. (1994): Enchytraeidae (Oligochaeta) of western Anatolia: taxonomy and faunistics. Bollettino di Zoologia 61: 241–260.
- Rota, E. (1995): Italian Enchytraeidae (Oligochaeta). I. Bollettino di Zoologia 62: 183-231.
- Schmelz, R. M. (2003): Taxonomy of *Fridericia* (Oligochaeta, Enchytraeidae). Revision of species with morphological and biochemical methods. – Abhandlungen des naturwissenschaftlichen Vereins in Hamburg (Neue Folge) 38: 488 pp.
- Strumpf, T., B.-D. Traulsen & W. Pestemer (2002): Verfügbarkeit von Kupfer in landwirtschaftlich genutzten Böden mit hohen Kupfer-Gehalten. I. Eine Bestandsaufnahme (Availability of Copper in Arable Soils with High Copper Contents. I. A Status Review). – Nachrichtenblatt Dt. Pflanzenschutzdienst 54: 161–168.
- Vavoulidou, E., N. J. Sidirias, J. Römbke, D. Mpilasis & R. Tsigou (1998): Effect of three different soil cultivation and fertilization treatments on earthworms and enchytraeids. – Newsletter of Enchytraeidae 6: 91–100.
- Vavoulidou, E., A. Charoulis, E. Avramides, P. Papadopoulos, A. Dimikrou & S. Konstandinidou (2005): Copper content in agricultural soils related to cropping systems in different regions of Greece. – Communications in Soil Science and Plant Analysis 36: 759–773.

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Region code (Fig.1)	Crop type	Farming system	Enchytraeid abundance (ind m <sup>-2</sup> )	Total copper content (mg kg <sup>-1</sup> soil dw)	рН	C:N ratio	% clay	Organic matter content (%)
6	vineyard	fallow	0	34.5	7.5	9.59	37	4.3
6	vineyard	conventional	0	19.5	7.6	8.12	43	1.4
6	vineyard	organic	0	28.9	7.5	7.89	41	1.7
6	vineyard	fallow	800	31.3	7.6	10.55	49	3
6	vineyard	conventional	400	44.3	7.6	9.02	45	1.4
6	vineyard	organic	400	34.5	7.7	7.57	51	1.5
6	vineyard	fallow	400	25.5	7.5	8.38	31	2.6
6	vineyard	conventional	0	32.9	7.6	4.64	29	0.8
6	vineyard	organic	400	29.3	7.7	7.3	35	1.7
6	vineyard	fallow	0	24.2	7.6	6.96	46	1.2
6	vineyard	conventional	400	22	7.5	7.98	50	1.1
6	vineyard	organic	0	19.5	7.6	7.38	46	1.4
7b	citrus	fallow	5800	32.6	7.6	9.43	33	1.3
7b	citrus	conventional	1200	43	7.4	5.8	29	1.2
7b	citrus	organic	2000	43.2	7.4	8.22	28	1.7
7b	citrus	fallow	0	62.8	7.5	10.15	59	4.2
7b	citrus	conventional	0	103	7.4	9.67	29	3.5
7b	citrus	organic	1200	77.4	7.5	7.73	45	2.2
9	olive	fallow	900		6.6	7.87	49	1.9
9	olive	conventional	700		7.5		35	2.6
9	olive	organic	0	36.9	6.8	11.32	35	4
9	vineyard	fallow	0	19.7	7.5	10.05	23	2.6
9	vineyard	conventional	0	38.8	7.7	4.09	57	0.6
9	vineyard	organic	5800	53.3	7.7	9.62	53	3.4
9	citrus	fallow	400	43.5	7.6	9.24	34	4.3
9	citrus	conventional	0	55.7	7.4	6.74	38	2.5
9	citrus	organic	0	42.5	7.6	6.79	40	2.4
9	olive	fallow	3700	10.6	6.7	9.94	42	2.4
9	olive	organic	0	55.2	6.6	10.88	26	3
9	olive	organic	4000		6.5	9.36	30	2.5
9	vineyard	fallow	2000	35	7.3	12.68	36	4.7
9	vineyard	conventional	2300	69.1	5.5	8.48	44	1.9
9	vineyard	organic	0	64.2	5.9	12.76	36	2.2
9	citrus	fallow	2200	42.2	7.6	10.27	44	5.4
9	citrus	conventional	100	65.8	7.5	8.12	42	2.8
9	citrus	organic	0	84.2	7.7	7.73	40	2.4

Annex List of the characteristics of 120 data sets analysed in the present study.

Region code (Fig.1)	Crop type	Farming system	Enchytraeid abundance (ind m <sup>-2</sup> )	Total copper content (mg kg <sup>-1</sup> soil dw)	рН	C:N ratio	% clay	Organic matter content (%)
10	olive	fallow	0	26.5	7.6	9.49	31	1.8
10	olive	conventional	0	31.8	7.1	7.85	39	2.3
10	olive	organic	400	22.7	7.4	7.25	35	2.5
10	citrus	fallow	400	103	7.4	9.06	24	2.5
10	citrus	organic	5800	45.3	7.5	8.82	24	1.9
10	olive	fallow	0	39.4	7.6	10.88	44	4.5
10	olive	organic	0	53.2	7.6	11.6	42	2.5
11	olive	organic	3900				42	
11	olive	fallow	3100	51	7	10.19	50	2.9
11	olive	fallow	0	107	5.6	6.96	42	2.1
11	olive	conventional	0	28.4	4.2	9.35	28	2.9
11	olive	fallow	10600		7.3	8.44	50	8
11	olive	conventional	3600	29.1	7.2	8.73	54	6.4
11	olive	organic	7000	32.2	6.4	8.58	56	3.7
11	olive	conventional	3000	22.5	5.4	12.93	30	3.9
11	olive	conventional	14300		4.9	9.23	30	3.5
11	olive	fallow	900	29.7	5.8	10.09	24	2
11	olive	organic	12300	32	6.4	10.83	26	2.8
11	olive	fallow	14100	21.7	5.8	10.77	27	3.9
11	olive	organic	7300	30	6.5	9.35	24	2.9
11	olive	fallow	1500		5.8	14.92	19	5.4
11	olive	organic	3900	29	5.6	9.84	21	2.8
12	vineyard	fallow	14500		7.8	6.86	62	1.3
12	vineyard	conventional	14400		7.4	5.8	46	1
12	vineyard	organic	13000	48	7.4	9.67	56	1.5
12	vineyard	fallow	4200	45.2	5.9	7.18	34	1.3
12	vineyard	conventional	1800	32.9	5.8	5.62	30	1.6
12	vineyard	organic	11900	37.5	5.5	6.11	32	1
12	vineyard	fallow	9700	40	7.5	14.02	29	2.9
12	vineyard	conventional	4600		7.2	3.99	23	1.1
12	vineyard	organic	1900	45	6.8	6.28	25	1.3
12	vineyard	fallow	5600	56.5	7.6	10.22	53	3.7
12	vineyard	conventional	2000	68	6.3	7.46	53	1.8
12	vineyard	organic	600	59.8	7.5	7.73	57	2.2
12	vineyard	fallow	10700		5.8	11.84	26	5

Annex	cont.
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Region code (Fig.1)	Crop type	Farming system	Enchytraeid abundance (ind m <sup>-2</sup> )	Total copper content (mg kg <sup>-1</sup> soil dw)	рН	C:N ratio	% clay	Organic matter content (%)
12	vineyard	organic	2700	68	5.4	5.8	41	0.8
12	vineyard	organic	2700		5.1	8.7	37	1.2
12	olive	fallow	2800		7.8	6.72	33	4.4
12	olive	conventional	5700	50.1	7.6	9.12	35	2.2
12	olive	organic	2000		7.8	165.73	43	4
13	vineyard	fallow	0	62	7.5	34.48	46	4.4
13	vineyard	organic	800	47.5	7.4	21.55	45	1
13	citrus	conventional	1600	85	7.5	28.92	44	2.6
13	citrus	organic	2000	61.9	7.6	23.71	39	2.2
13	olive	fallow	800	24.3	7.5	31.03	35	6.3
13	olive	organic	400	46.9	7.5	30.81	35	4.2
13	citrus	fallow	1200	66.1	7.2	18.96	25	2.2
13	citrus	organic	1600	69.8	7.2	26.48	21	4.3
14	olive	fallow	4100	36.7	6.9	7.57	31	3
14	olive	conventional	3600	36.7	6.2	5.1	29	2.9
14	olive	organic	2100	36	7.8	5.67	31	2.2
14	olive	fallow	1900		7.5	10.31	41	4.8
14	olive	conventional	9000		7.4	8.23	43	2.2
14	olive	organic	5800	34	7.4	6.72	47	2.2
14	olive	conventional	2500	25.2	7.6	7.3	41	3.3
14	olive	organic	9900		7.6	7.3	41	2.9
14	olive	fallow	5000	20.7	7.4	15.26	24	12.1
14	olive	conventional	2500	33	7.5	10.19	37	3.6
14	citrus	conventional	900	26.5	7.6	8	32	2
14	citrus	organic	2000	68.7	7.1	9.16	28	3
14	olive	conventional	0	45.2	6	11.6	34	3.1
14	olive	organic	5200	29.6	7.2	10.26	36	2.3
14	citrus	fallow	0	11	7.5		46	
14	citrus	conventional	300	29.8	7.4	8.94	52	3.7
14	citrus	organic	0	45.2	7.5	9.82	34	3.3
14	citrus	conventional	8800	16.6	6.9	8.7	24	2.7
14	citrus	organic	8500	40.1	7.3	7.52	26	2.4
16	vineyard	organic	0	11	5.3	5.8	12	0.6
16	vineyard	fallow	0	9.4	7.2	8.12	17	1.4
16	vineyard	conventional	0	9.8	7.2	13.81	16	0.5
16	vineyard	organic	0	37.3	7.3	13.71	14	1.3

Region code (Fig.1)	Crop type	Farming system	Enchytraeid abundance (ind m <sup>-2</sup> )	Total copper content (mg kg <sup>-1</sup> soil dw)	рН	C:N ratio	% clay	Organic matter content (%)
16	vineyard	fallow	0	12	6.9	17.4	15	4.5
16	vineyard	conventional	0	11.2	5.9	1.78	14	0.6
16	vineyard	organic	600		6.9	55.24	16	0.8
16	vineyard	fallow	0	10	6.7	9.02	15	1.4
16	vineyard	conventional	0	25	5.1	9.12	17	1.1
16	vineyard	conventional	0		3.3	11.23	13	3
16	vineyard	organic	0	8.6	7	9.77	16	1.6
16	vineyard	fallow	0	8.9	6.7	3.31	12	0.6
16	vineyard	conventional	5600		4.1	2.49	10	0.6
16	vineyard	organic	1200		5.6	8.29	12	0.5
16	vineyard	fallow	500		7	8.87	15	1.3
16	vineyard	conventional	0		6.5	11.6	14	0.8
16	vineyard	organic	0	14.2	5.3	13.71	14	1.3

Annex cont.