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Soil Biological Quality Index based on earthworms (QBS-e). A new way to use earthworms as bioindicators in agroecosystems

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ABSTRACT

Traditionally, earthworms are considered one of the most frequently used bioindicators to evaluate the sustainability of soil use. Therefore a new index, called QBS-e (Soil Biological Quality Index based on earthworms) has been provided in order to improve the monitoring of the soil's biological fertility in the rural environment, as well as for use by non-experts in earthworm taxonomy. We present two case studies to test the application of this new practical tool: one in horticultural agroecosystems and the other in vineyards in North-Eastern Italy, and we compare the QBS-e method with the traditional method of earthworm diversity analysis. We analysed the differences in earthworm fauna between organic and conventional agroecosystems to assess if some particular agronomical practices affect earthworms. The results obtained with the two methods are comparable: this seems to demonstrate the effectiveness of using the QBS-e index in order to save time and costs in soil monitoring programmes. In addition, we propose a simple software to calculate the QBS-e index value and to help with the attribution of the correct ecological category to the sampled specimens.

1. Introduction

1.1. Need to evaluate soil quality

Nowadays agriculture is facing the unprecedented challenge of feeding a rapidly growing world human population, while simultaneously reducing its environmental footprint (Bennett et al., 2014; Godfray et al., 2010; Gomiero et al., 2011; Liu et al., 2015). Since cultivated areas are subjected to serious problems such as soil erosion, loss of fertility, soil tiredness, salinization and agricultural-industrial pollution problems (Karaca et al., 2011), the sustainability of crop production in agriculture is becoming a more and more pressing issue, whereas in the past the focus has solely been on increasing short-term yields (Gliessman, 2007; Gomiero et al., 2011; Liu et al., 2015; Paoletti et al., 1992). In this scenario the availability of resources allowing an easy and rapid assessment of agroecosystem condition is becoming of primary importance in order to plan appropriate interventions and monitor the efficiency of ecosystem services (Gardi et al., 2009; Hole et al., 2005; Paoletti, 1999a, 1999b; Paoletti et al., 2011).

With soil quality being fundamental in agriculture, bioindicators to evaluate the sustainability of soil use were the main focus of several types of research in recent decades (Culman et al., 2010). Several biological indicators of soil health have been proposed also at a microbiological level, such as soil microbial biomass and activity, as well as soil microbial respiration (Anderson, 1982; Jenkinson and Ladd, 1981; Sparling, 1997), soil enzyme activities (Dick, 1997; Karaca et al., 2011), general soil microflora, including eubacteria, archaebacteria, fungi and algae, (Roper and Ophel-Keller, 1997), but also, specifically, plant root pathogens (Hornby and Bateman, 1997). Overall soil microfauna, consisting of protozoa, nematodes and small-sized collembola and mites, has further been proposed as a bioindicator of soil health (Gupta and Yeates, 1997) and the Maturity Index, in particular, was conceived in order to utilize nematode population data to explain the condition of a soil ecosystem (Bongers, 1990; Yeates, 1994; Yeates and Bongers, 1999). Ruff (1998) designed one more Index based on another group of soil microfauna, the Gamasina mites (Mesostigmata). Van Straalen (1997) proposed to use the community structure of soil arthropods as a bioindicator of soil health, considering the intricate relations between arthropods and their niches, by analysing, for example, species composition, life-history diversity or feeding type diversity.

Parisi and colleagues (Blasi et al., 2012; Parisi, 2001; Parisi et al., 2005; Rudisser et al., 2015) proposed an index of soil quality evaluation

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Abbreviations: QBS-e, Soil Biological Quality Index based on earthworms; EMI, EcoMorphological Index scores; OM, Organic Matter; ANE, Anecic or Deep-burrower; END, Endogeic; EPI, Epigeic; COP, Coprophagic; HYD, Hydrophilic; Im, Immature; Ad, Adult

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(QBS-ar and QBS-c) based on the soil mesofauna as an indicator of soil functionality.

In particular the QBS index has four properties that a useful index should possess. First of all, the need to use functional information rather than only detailed taxonomy. Several authors demonstrated that indexes based just on species' (or other taxonomical groups') richness is reductive and leads to a loss of information (De Bello et al., 2010; Vandewalle et al., 2010). Therefore it is useful to associate with each taxonomical group, its ecological features (i.e. environmental needs, life cycle, trophic level) (Obrist and Duelli, 2010). Secondly, it is important that the method can be easily adopted also by non-specialists (personnel not formally trained in taxonomy) (Oliver and Beattie, 1996). Specifically, QBS-ar and QBS-c are based on high-level systematic taxa so as to allow their attribution also to non-experts (Parisi, 2001; Parisi et al., 2005). Thirdly, it is fundamental to express the result of monitoring in terms easily comprehensible (Buchs, 2003). For this purpose, the final value of the QBS index is in reference to the soil quality: the higher the value, the better the soil conditions (Parisi et al., 2005). Lastly it is applicable for large-scale monitoring (Rudisser et al., 2015).

However, even though the use of the QBS-ar index favours practicality in soil quality evaluation, on the other hand some critical elements remain. Firstly, the organisms on which the QBS-ar is based need to be extracted using the Berlese-Tullgren extractor (such as the one modified according to Paoletti et al., 1991) and subsequently observed under the stereomicroscope. This procedure requires instrumentation which is typically out of the reach of farmers or field operators. Secondly, avoiding species identification leads to a loss of detailed information (Obrist and Duelli, 2010; Oliver and Beattie, 1996). Finally, the QBS-ar is based solely on the presence/absence of different organisms, to which an EcoMorphological (EMI) score is attributed, ignoring the abundance of individuals belonging to that group (Yan et al., 2012). Introducing the abundance parameter in the QBS- ar index would be difficult, since mesofauna organisms are very small and often very numerous, therefore the count of abundance could be time consuming.

1.2. Why use earthworms as soil quality bioindicators?

Earthworms belong to macrofauna (4–200 mm in size) but some species can reach the dimension attributed to megafauna (> 200 mm) (Bachelier, 1986) and are considered soil engineers, as they are able to modify soil structure and features by their etho-physiological action (Blouin et al., 2013; Lavelle et al., 2014; Gavinelli et al., 2017). It is possible to recognise three types of effects of earthworm activity on soil (Blouin et al., 2013; Brown et al., 2000; Cunha et al., 2016; Dell'Agnola and Nardi, 1987; Lee, 1985; Lavelle, 1996; Lavelle et al., 1995; Lavelle et al., 2007; Lavelle et al., 2016; Le Bayon and Milliret, 2009; Le Bayon et al., 2017; Paoletti, 1999a,b; Pérès et al., 1998; Stirling, 2001; Zangerlé et al., 2011), which are:

- the *physical* effects, which result from the digging of burrows and cast production. Burrows and casts can improve soil porosity (micro- and macro-pores), influencing soil aggregation structure. Moreover, burrows provide a pathway for the movement of surface water and large soil particles from the surface to deeper layers and easy access for plant roots to penetrate the soil. These effects can prevent or reduce soil anoxia conditions, allowing the correct action of aerobic microorganisms. Casts consist of mixed inorganic and organic materials that are voided after passing through earthworm gut. They contribute to paedogenesis, soil profile development and structure.
- the *chemical* effects, which consist of chemical weathering produced by earthworms, or microorganisms stimulated in their gut, or by a synergic action of both organisms. With their trophic activity, earthworms help the decomposition of soil litter, producing humified OM horizon. Furthermore, earthworm casts enrich the soil with macronutrients (especially N) and consequently improve it for plant growth.
- the *biological* effects, which consist mainly in interactions (symbioses) with soil microorganisms, such as bacteria and fungi, including VAM (vesicular-arbuscular mycorrhizae) (Rabatin and Stinner, 1988), by ingesting them together with litter and contributing to their metabolic activation and dispersal. Furthermore, their activity results in an increase in the surface area of organic substrates available for microbial activity.

Earthworms are usually divided into different ecological categories (Bouché, 1972; Edwards, 1998; Lee, 1985; Paoletti, 1999b; Paoletti et al., 2013; Sims and Gerard, 1985):

- **Epigeic earthworms**, dorsally pigmented, living and feeding in the litter or in A01 horizon of soil profile, with scarce digging capacity (i.e. *Lumbricus castaneus*);
- **Endogeic earthworms**, usually less pigmented, living and feeding between A02 and A1 horizons, able to dig mainly horizontal burrows (i.e. *Aporrectodea caliginosa*);
- **Anecic and/or deep-burrower earthworms**, even larger in size than other ecological categories, can reach A2 and B soil horizons, are able to dig vertical burrows up to a few meters in depth (i.e. *Lumbricus terrestris*), but often rise to the surface to feed on litter (i.e. *Octodrilus complanatus*);
- **Coprophagic earthworms**, living and feeding in manure or compost and closely associated with a high content of raw OM (i.e. *Eisenia fetida*);
- **Hydrophilic earthworms**, living and feeding in damp soils, river bottom and shallow water-table soils (i.e. *Eiseniella tetraedra*).

Among bioindicators in agriculture, earthworms are one of the most frequently used to evaluate soil fertility and the sustainability of soil use (Blakemore and Paoletti, 2006; Falco et al., 2015; Kingston, 2001; Lavelle et al., 2007; Paoletti, 1999a; Paoletti et al., 1991; Paoletti et al., 2007; Paoletti et al., 2013; Peigné et al., 2009; Pfiffner and Mader, 1997). Earthworms are important in the agroecosystem not only from an environmental point of view, but also for their implication in crop production. In fact, Van Groeningen and colleagues (2014), reviewing data reported in other studies, such as Brown et al. (1999) and Scheu (2003), highlighted that earthworm presence can significantly increase crop yield by +25%, aboveground biomass by +23%, belowground biomass by +20% and total biomass by +21%.

Given these premises, we propose an index similar to the one of Parisi (2001), called QBS-e (Soil Biological Quality Index based on earthworms) (Paoletti et al., 2013). The QBS-e index aims to assess the soil health based on the monitoring of the earthworm community hosted within, considering that earthworms are recognised as good bioindicators, and they are also, in general, well known by farmers (unlike other groups of soil organisms which are little appreciated by farmers such as microorganisms, micro- or meso-fauna). This index facilitates the estimation of the sustainability of soil management practices, and it can be used also by non-experts, directly in the field. However, there can be some limits to QBS-e application in drylands or particularly sandy soils, since earthworms can barely survive in these conditions.

1.3. Research objectives

The main objectives of the present study are:

(1) to validate the QBS-e index, published in Italian in 2013, with additional sets of data: two case studies, horticultural agroecosystems and vineyards;
(2) to compare, in monitoring programmes of soil sustainability management and agronomical practices, the most used and resource-consuming analysis of earthworm diversity, species and abundance (taxonomical approach), with the information associated with ecological categories and the QBS-e index (functional approach).

2. Material and methods

2.1. Earthworm sampling

Hand sorting is the traditional active collection of earthworms from standard soil volumes (Jiménez et al., 2006; Paoletti et al., 1991; Raw, 1960; Valckx et al., 2011). In detail, this technique consists of extracting a soil bulk (30 × 30 × 20 cm in this study) with a spade fork. Afterwards, a visual examination of soil bulk takes place for 15 min upon a white cloth and each earthworm is picked up. In order to collect deep burrower species as well, an effective recommendation is the use of an irritant suspension (Bouché, 1972; Lee, 1985) poured into the soil. The mustard powder water suspension acts as an expellant for earthworms and it is a natural substance without toxic or dangerous consequences for the operator and the environment (Chan and Munro, 2001; Pelosi et al., 2009; Valckx et al., 2011). For these reasons, it was adopted in this work.

Two samplings/year were made, in spring and in autumn, which, in temperate regions, are the best periods to collect earthworms because of good soil moisture and moderate temperatures. In each sampled field, seven random hand sorting points were analysed, avoiding field marginal areas. The water suspension of mustard powder (*Sinapis alba* L.) with concentration of 30 g/l previously prepared, was spread on the 30 × 30 cm soil surface before hand-sorting.

First of all, we proceeded with the classical method of determination of each specimen at species level (taxonomical approach), viable solely by experts in taxonomy and that requires the use of the stereomicroscope, and then we focused on a more speditive method, the application of the QBS-e index (functional approach – explained below) (Paoletti et al., 2013), in order to facilitate the use of earthworms as indicators of soil quality, since it does not need neither the use of the stereomicroscope, nor to be an expert in earthworm taxonomy, therefore it can be a suitable tool also for farmers and agronomists.

The interactive LOMBRI software (Paoletti and Gradenigo, 1996) was the key tool for the first step of species determination, in addition to literature (Bouché, 1972; Edwards, 1998; Sims and Gerard, 1985).

2.2. Case studies

2.2.1. Case study n°1: Horticultural agroecosystems

The first case study was carried out in North-Eastern Italy, in the provinces of Venice and Treviso (Veneto region, Fig. 1). The area is largely modified by human activities: within a 1 km radius from the centre of each field, arable land use covers approximately 51% of the surface, 25% is characterised by semi-natural habitats (woodlands, grasslands, hedgerows) and approximately 20% by urban habitats (towns, residential dwellings, streets, industrial areas) (Fusaro et al, 2016).

Two crops were studied: Treviso Red chicory in 2012 and White cabbage in 2013. A total of ten fields was monitored: five under organic management (certified at least since 2007) and five conventional ones. The minimal distance between differently managed fields (organic and conventional) ranged from 200 m to 6900 m. The two management systems differed particularly in the frequency and invasiveness of tillage practices and in the supply of chemicals (Table 1). Each farmer, both organic and conventional, practiced rotation among different horticultural crops.

The meteorological conditions during the sampling years were marked by a significant difference in rainfall. 2012 was characterized by drought with an annual mean precipitation of 771 mm, more than 100 mm lower than the reference period (1994–2011). The following year was characterized by wetter conditions: the annual mean precipitation was 1119 mm, more than 200 mm higher than the reference period (1994–2012) (ARPAV data, www.arpa.veneto.it) (Fusaro et al., 2016).

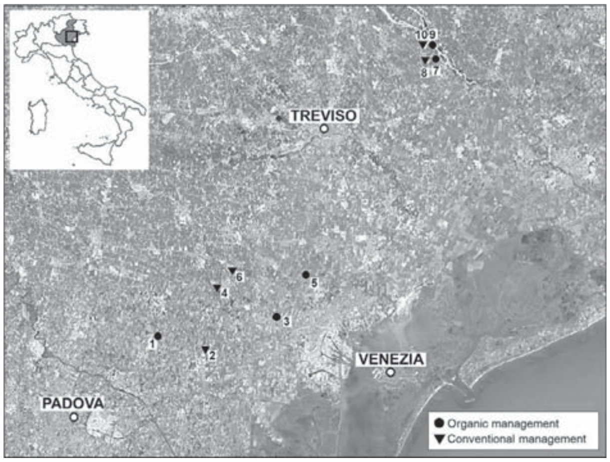

Fig. 1. Localization of studied sites in North-Eastern Italy (case study n°1: horticultural agroecosystems). Image elaborated from Google maps (Fusaro et al., 2016).

Table 1
Different agronomical practices adopted in the two types of management of the horticultural agroecosystems. See the reference numbers in Fig. 1.

Agronomical practices/ Fields	Conventional					Organic				
	2	4	6	8	10	1	3	5	7	9
Herbicides	Yes	Yes	Yes	Yes	Yes	No	No	No	No	No
Green manure	No	No	No	No	No	Yes	Yes	Yes	Yes	No
Rotary tillage	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Pesticides	Yes	Yes	Yes	No	No	Yes	No	Yes	Yes	No
Ploughing (35–40 cm depth)	Yes	Yes	Yes	No	No	No	No	No	No	No
Polyculture	No	No	No	No	Yes	Yes	Yes	Yes	Yes	Yes
Harrowing	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No

2.2.2. Case study n° 2: Vineyards

The second case study was carried out in North-Eastern Italy, in the provinces of Vicenza and Padova (Veneto Region, Fig. 2). The area is composed mainly of semi-natural and natural cover: within a 1 km radius from the centre of each field, arable land use covers approximately 34% of the surface, 57% is characterised by semi-natural habitats (26% woodlands, 31% grasslands and hedgerows) and approximately 9% by urban habitats (towns, residential dwellings, streets, industrial areas).

Vineyards were studied in 2013 and 2014. Twelve fields located in the Berici and Euganean Hills were monitored: four conventionally and

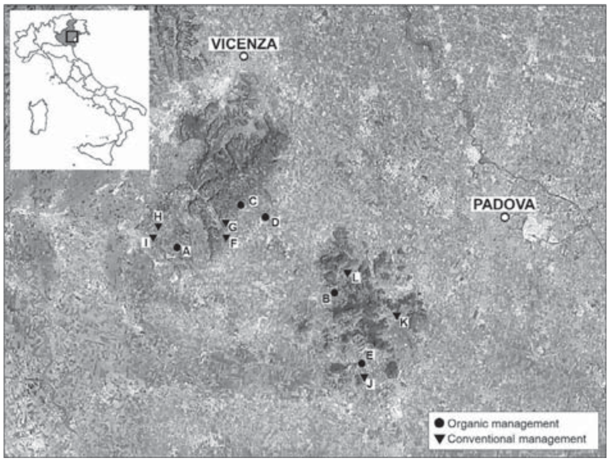

Fig. 2. Localization of studied sites in North-Eastern Italy (case study n°2: vineyards). Image elaborated from Google maps.

Table 2
Different agronomical practices adopted in the two types of management of the vineyards. See the reference letters in Fig. 2.

Agronomical practices/ Fields	Conventional							Organic				
	F	G	H	I	J	K	L	A	B	C	D	E
Excavation	Yes	Yes	No	No	No	No	Yes	Yes	No	Yes	No	No
Ripper	No	No	No	No	Yes	No	Yes	Yes	No	No	Yes	Yes
Harrowing	Yes	Yes	No	No	Yes	Yes	No	Yes	Yes	Yes	No	Yes
Mowing	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Green manure	No	No	No	No	No	No	No	Yes	Yes	No	No	Yes
Fertilization	No	No	Yes	No	No	Yes	Yes	No	No	No	No	Yes
Pesticides	Yes	Yes	No	No	Yes	No	Yes	No	No	Yes	No	No
Herbicides	No	No	Yes	Yes	Yes	Yes	Yes	No	No	No	No	Yes
Fungicides	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

two organically managed vineyards in the Berici Hills, together with three organically and three conventionally managed ones in the Euganean Hills. The two management systems differed particularly in the practice of green manure and in the frequency of chemical supply of both pesticides and herbicides (Table 2).

As regards the meteorological conditions, both 2013 and 2014 were wet years characterized by an annual mean precipitation of 1415 mm and 1348 mm respectively. Precipitations were quite uniform for all sites which were object of study (ARPAV data, www.arpa.veneto.it).

2.3. Data analysis

Two levels of data analysis were performed: the first concerning the description of earthworm diversity in terms of species richness and community composition, and the second concerning their functionality in the ecosystem. Both levels regarded the comparison between organic and conventional agroecosystem management.

2.3.1. Description of earthworm diversity (taxonomical approach)

Data on the species composition of the earthworm fauna were processed by the ordination data technique of Non-metric Multidimensional Scaling (NMDS), in order to reduce the data dimensionality (Shaw, 2003). NMDS is appropriate to elaborate ecological data coming from populations with non-normal and discontinuous distributions (Shaw, 2003; Mouhmadou et al., 2013).

Since ordination techniques do not directly provide probability levels, to objectify the interpretation of the plots, the hypotheses-testing principle was applied to the coordinates of the samples, referred to axis 1, on the new data space calculated by the software (Gotelli and Ellison, 2004; Shaw, 2003). Therefore in this study the new coordinates of the samples referred to the main axis (1) of the NMDS plot were used to perform the most commonly used classical cluster analysis (with Ward's method) applied to a matrix of Euclidean distances (Shaw, 2003), in order to represent a dendrogram of relations among the samples. So samples were grouped according to similarity: the ones positioned closer in the dendrogram were more similar with respect to earthworm community. Hence ordination and classification methods were used to extrapolate the existence of relations between earthworm community composition and agroecosystem management.

To highlight the relation between the community structure in ecological categories and the agronomical practices adopted by farmers, the Principal Component Analysis biplot (PCA) was applied. This plot directly allows the operator to visualize the properties strongly associated with each observation since proximity implies close association: so the biplot suggests tendencies between two data sets (Shaw, 2003).

2.3.2. The QBS-e index (functional approach)

The QBS-e index (Paoletti et al., 2013) is based on the attribution of an ecological category to each sampled earthworm among five categories: endogeic, epigeic, deep-burrower, coprophagic, and hydrophilic, established on the ecology, ethology and anatomic features of each living specimen, and the age recognition between immature and adult (without or with clitellum). The complete list of Italian earthworm species from Blakemore 2008, with the specification of a proper ecological category, can be consulted in Appendix Table A.

Table 3
EcoMorphological (EMI) scores attributed to each ecological category and age (Paoletti et al., 2013).

Ecological category	Age	EMI score
Hydrophilic (HYD)	Immature (Im)	1
Hydrophilic (HYD)	Adult (Ad)	1
Coprophagic (COP)	Immature (Im)	2
Coprophagic (COP)	Adult (Ad)	2
Epigeic (EPI)	Immature (Im)	2.5
Endogeic (END)	Immature (Im)	2.5
Epigeic (EPI)	Adult (Ad)	3
Endogeic (END)	Adult (Ad)	3.2
Anecic/Deep-burrower (ANE)	Immature (Im)	10
Anecic/Deep-burrower (ANE)	Adult (Ad)	14.4

An EcoMorphological score (EMI) was attributed to each ecological category and age (Table 3). The lowest EMI score (1) was attributed to hydrophilic species, since their living conditions (high level of watertable) are not compatible with agriculture (water stagnation that originates soil anoxia and scarce OM degradation, excluding water rice cultivation). An EMI score of 2 was attributed to coprophagic species since they can be spread in the agroecosystem with organic fertilization based on manure, compost or vermicompost, but they have low survival rates in the soil, where the OM amount is scarce if compared to that of the compost heap. Epigeic, endogeic and deep burrower species can live in agroecosystems as well as in more natural environments. The EMI score attribution to these ecological categories was not arbitrary, taking into account only knowledge on their ecological role and etho- physiological functions, but also specific data on earthworm body mass. Since a larger earthworm can affect the soil to a great extent with its physiological activity (i.e. the amount of ingested soil, of casts, burrow dimensions, etc.), body mass data referred to Italian species were considered. The numerical calculation of the EMI score was based on studies chosen because reporting the presence of the three main ecological categories (epigeic, endogeic and deep-burrower), contemporaneously, in different agroecosystemical situations (Ernst and Emmerling, 2009; Paoletti et al., 1998). These data concern density (ind/m^2) and fresh weight (g/m^2) for each one of these ecological categories. The mean values of weight (g) of an hypothetical individual belonging to each ecological categories (estimation) was calculated, being aware that in each category can be included different species. Then the relative ratio in weight was calculated between an average adult endogeic individual and an average adult epigeic individual and between an average adult deep-burrower individual and an average adult epigeic individual, since the epigeic one resulted to have the lesser weight.

A slight difference in mean body mass was found between endogeic and epigeic specimens, and so 3, 2 and 3 are the EMI scores attributed respectively, while the same EMI score (2,5) was assigned to immature specimens of both categories. Arguing this score attribution from an ecological point of view, the presence of epigeic species indicates good conditions of soil litter (organic soil layers), while endogeic species finding implies the upper organo-mineral soil layers are not so frequently disturbed (Bouché, 1972; Coleman and Wall, 2015; Edwards, 1998; Kiyasudeen et al., 2015; Le Bayon et al., 2017; Sims and Gerard, 1985). The higher EMI score (14, 4) was conferred to adult anecic/deep-burrower individuals, which have a lower reproductive rate, a larger body dimension (mean 4, 8 times larger than endogeic and epigeic specimens), and a more incisive influence in soil structure, affecting a great part of soil profile. In fact their presence implies an optimal soil condition, with a well-structured soil profile, a scarce or null disturbance of soil horizons and a good and deep circulation of water and air, therefore making the soil an hospitable environment also for other edaphic organisms (Bouché, 1972; Gavinelli et al., 2017; Lamparski et al. 1987; Le Bayon et al., 2017). The EMI score assigned to immature deep burrower individuals (10) is higher in respect to the score attributed to other immature individuals because of the low reproductive rate typical of deep-burrower species, but it is lower in respect to the score of adult individuals since their physiological activity is less effective (Fernandez et al., 2010).

The following formula is used to calculate the index value:

$$\begin{aligned} \text{QBS}-\text{ e} = {} & (\text{HYD Im, Ad score}*\text{N}) + (\text{COP Im, Ad score}*\text{N}) \\ & + (\text{EPI Im score}*\text{N}) + (\text{END Im score}*\text{N}) + (\text{EPI Ad score}*\text{N}) \\ & + (\text{END Ad score}*\text{N}) + (\text{ANE Im score}*\text{N}) \\ & + (\text{ANE Ad score}*\text{N}) \end{aligned}$$

where N = n° individuals/m^2, therefore it is important to determine the density of each ecological category in order to compare data.

A few species supposedly belonging to the endogeic ecological category, can have a big size and dig vertical burrows, features which are quite different from the other endogeic species. These species could show clear deep-burrowing habits, typical of the anecic/deep-burrower ecological category. An example, found in this research, is *Perelia gestroi*. These particular species should be attributed to the anecic/deep-burrower category in the calculation of the QBS-e index, to better define the soil biological quality. In order to attribute the correct ecological category to these few particular specimens, it could be useful to follow the identification key section presented in the QBS-e calculator software, answering questions concerning ethology, ecology and anatomic features of the earthworm.

To conclude the evaluation, it is necessary to refer the QBS-e value to a Soil Quality Class, according to Table 4.

In order to express with an increased resolution the soil evaluation of the widest Soil Quality Classes (Sufficient, Decent and Good), we suggest the QBS-e index users to add a "−" or a "+" after the Soil Quality Class score, if the calculated value is between a range of 50 points after and before the thresholds of the classes (i.e. if the QBS-e value is 645 the evaluation will be "3^-", if the QBS-e value is 973 the evaluation will be "3^+").

2.4. Statistical tests

One-way ANOVA was performed to test the variance within and between the two groups of fields under different management. The assumptions of ANOVA (homoscedasticity and normality) were verified and data transformations were applied, if needed. If the assumptions were not met, the non-parametric Kruskal-Wallis test was applied. The Monte Carlo permutation test was performed to test the statistical difference between agroecosystem managements by using the coordinates of samples on PCA axis 1. Software PAST version 3.15 was used to perform statistical analyses.

Table 4
Soil quality classes based on the QBS-e index value (Paoletti et al., 2013).

QBS-e value	Soil Quality Class (agroecosystem, semi-natural environment)
QBS-e > 1000	Excellent (4)
600 < QBS-e < 1000	Good (3)
300 < QBS-e < 600	Decent (2)
100 < QBS-e < 300	Sufficient (1)
0 < QBS-e < 100	Poor (0)

2.5. A new software to calculate earthworm density and QBS-e index value

In order to quickly calculate the QBS-e value, a software with guidelines and support service is available, on request at no cost, by writing to this e-mail address: qbse.index.help@gmail.com. The software is for WIN only.

The software is composed of different sections:

- a "MAIN MENU" section introduces the tools;
- an "INTRODUCTION" section contains instructions on how to run the software;
- an "IDENTIFICATION KEY" section is useful to check the ecological category for each collected specimen by answering dichotomous questions, based on anatomical, ecological and ethological features, so as to attain the correct ecological category, then by clicking on the different ecological category names, a photo of a representative species will appear along with a brief description;
- a "SPECIES LIST" section helps to check the correct ecological category for each species belonging to the Italian fauna;
- a "CALCULATOR" section is the application to evaluate the analysed sites. Here it is possible to calculate earthworm density (individuals/m^2) and QBS-e values, according to the number of replicates (hand-sorting points in the studied site – it is recommended to analyse at least 3 hand-sorting points/site). Moreover, a table with the Soil Quality Class Values allows the soil quality to be assessed. In "COLLECTED SPECIMENS" enter the total number of specimens distributed according to the proper ecological category and age (adult, immature). In "COLLECTING DATA" enter the total number of replicates made in the site object of study and the side length latus in centimetres in "SAMPLING REPLICATE" (Fig. 3).

In case of recovery of an immature specimen, to which it is impossible to attribute a specific ecological category, it is recommended that the most likely ecological category is attributed, according to the most abundant one assigned observing the other adult specimens.

To calculate the value of earthworm density (individuals/m^2) and to get the QBS-e value, just click on the buttons with the same names.

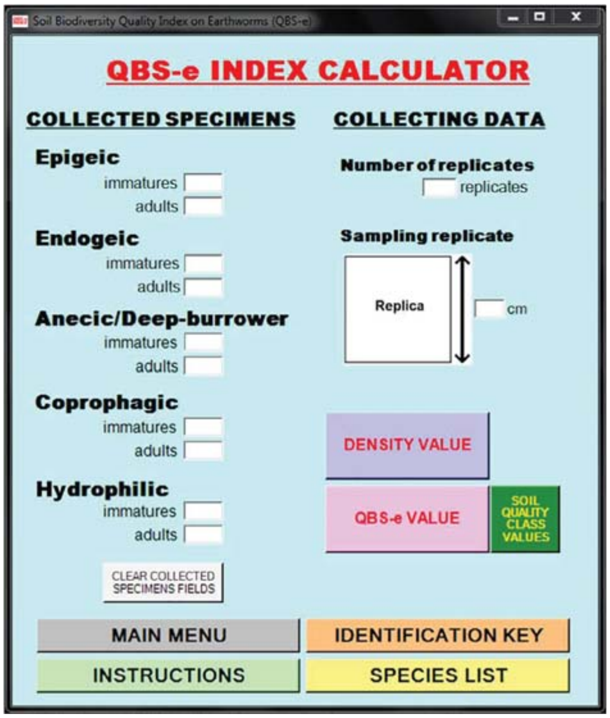

Fig. 3. An example of a screenshot of the QBS-e index calculator software.

Table 5
Mean values of earthworm species (ind/m^2/sampling) in organic and conventional soils. Ecological categories: END: endogeic; EPI: epigeic; ANE: anecic/deep-burrower; COP: coprophagic; HYD: hydrophilic. Age: Ad: adult; Im: immature. Significance: *: $p < 0.05$; **: $p < 0.01$; n.s.: $p > 0.05$.

Species (ecological category-age)	Organic	Conventional	Significance
	[Mean (S.E.)]	[Mean (S.E.)]	
Allolobophora cfr. *chlorotica* (END Im)	5.5 (4.4)	2.6 (1.5)	n.s.
Allolobophora chlorotica (END Ad)	39.8 (16.1)	4.0 (2.4)	n.s.
Allolobophora/Aporrectodea sp. (END Im)	119.8 (19.2)	18.3 (6.5)	**
Aporrectodea caliginosa (END Ad)	38.7 (11.6)	8.4 (3.0)	*
Aporrectodea cfr. *caliginosa* (END Im)	26.0 (7.0)	14.2 (8.7)	n.s.
Aporrectodea cfr. *georgii* (END Im)	0.05 (0.05)	/	n.s.
Aporrectodea cfr. *handlirschi* (EPI Im)	0.6 (0.4)	/	n.s.
Aporrectodea cfr. *jassyensis* (END Im)	1.8 (1.8)	/	n.s.
Aporrectodea cfr. *rosea* (END Im)	9.4 (7.6)	/	**
Aporrectodea cfr. *sineporis* (EPI Im)	2.4 (2.4)	/	n.s.
Aporrectodea handlirschi (EPI Ad)	1.3 (1.3)	0.1 (0.1)	n.s.
Aporrectodea jassyensis (END Ad)	5.0 (1.9)	0.7 (0.7)	n.s.
Aporrectodea rosea (END Ad)	8.9 (6.5)	1.1 (0.6)	n.s.
Aporrectodea sineporis (EPI Ad)	5.5 (5.0)	/	n.s.
Dendrobaena byblica (END Ad)	0.1 (0.1)	/	n.s.
Dendrobaena veneta (COP Ad)	0.1 (0.1)	/	n.s.
Eisenia fetida (COP Ad)	0.1 (0.1)	/	n.s.
Eiseniella tetraedra (HYD Ad)	1.7 (1.5)	0.2 (0.1)	n.s.
Eiseniona sp. (EPI Im)	0.2 (0.2)	/	n.s.
Lumbricus castanues (EPI Ad)	0.4 (0.4)	/	n.s.
Lumbricus cfr. *castaneus/rubellus* (EPI Im)	0.2 (0.1)	/	n.s.
Lumbricus cfr. *terrestris* (ANE Im)	0.1 (0.1)	/	n.s.
Lumbricus rubellus (EPI Ad)	0.1 (0.1)	0.1 (0.1)	n.s.
Lumbricus sp. (EPI Im)	0.5 (0.3)	0.1 (0.1)	n.s.
Microscolex sp. (END Im)	0.4 (0.4)	/	n.s.
Octodrilus cfr. *complanatus* (ANE Im)	0.6 (0.6)	/	n.s.
Octodrilus cfr. *lissaensis* (END Im)	0.1 (0.1)	/	n.s.
Octodrilus complanatus (ANE Ad)	1.2 (1.0)	/	n.s.
Octodrilus lissaensis (END Ad)	0.1 (0.1)	0.1 (0.1)	n.s.
Octodrilus sp. (ANE Im)	2.8 (2.2)	/	n.s.
Octodrilus transpadanus (END Ad)	0.4 (0.2)	/	n.s.
Octolasion lacteum (END Ad)	0.1 (0.1)	/	n.s.
Octolasion sp. (END Im)	0.1 (0.1)	/	n.s.
Perelia nematogena (END Ad)	0.4 (0.4)	0.1 (0.1)	n.s.
Proctodrilus antipae (END Ad)	0.4 (0.4)	/	n.s.

Each value is expressed in decimal format. The calculator provides the use of the integer or floating point numbers. Please use "." as a decimal separator. A "CLEAR COLLECTED SPECIMENS FIELDS" button is set to clear all entries given in the "collected specimens" fields.

In order to obtain the overall SOIL QUALITY CLASS VALUE, by clicking on the button of the same name, the software provides a table with five quality classes in descending order, linked to the QBS-e value ranges.

3. Results

3.1. Case study n°1: Horticultural agroecosystems

3.1.1. Earthworm diversity (taxonomical approach)

The mean number of earthworm species was significantly higher in organically managed soils: 11.0 (± 1.9) species in respect to 4.8 (± 1.3) species found in conventionally managed soils ($p < 0.05$, One-way ANOVA).

As regards species determination, Table 5 shows the differences between the two types of management.

Differences in soil management seem to affect the abundance of some species, such as *A. caliginosa* and *A. rosea* that are endogeic species, living in the first ten-fifteen centimetres of soil depth.

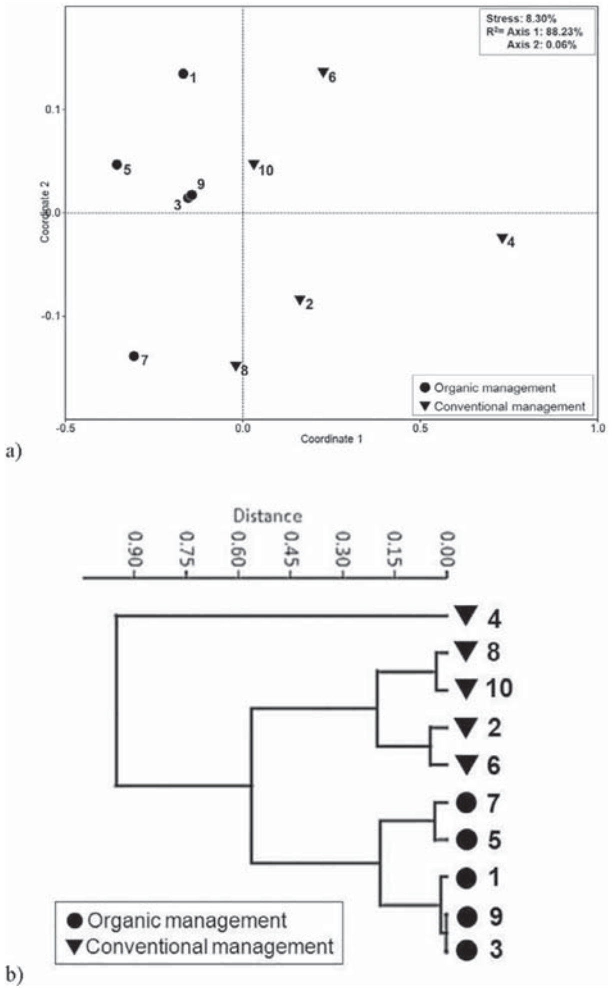

Fig. 4. a) NMDS of soil earthworm communities data (species ind/m^2/sampling), Bray-Curtis similarity measure. b) Classical cluster analysis of NMDS scores on axis 1 data of earthworm communities, Ward's method- Euclidean similarity measure. Conventional fields: 2, 4, 6, 8, 10. Organic fields: 1, 3, 5, 7, 9.

In order to visualize relationships of similarity among field samples, an NMDS ordination was performed (Fig. 4a).

By considering earthworm communities (species and their relative abundances), it was possible to distinguish the two different agroecosystem management systems. In fact, in the cluster diagram (Fig. 4b) there is a significant separation into two groups, mainly according to management type ($p < 0.05$, One-way ANOVA).

3.1.2. Ecological categories and the QBS-e index (functional approach)

The earthworm community structure of organic and conventional fields was described in terms of ecological categories and age of earthworms (Table 6).

The overall amount of earthworms was significantly higher in organic fields and moreover all ecological categories were present in these fields in comparison with the conventional ones, which hosted only 3/5 categories. In particular, there were statistically more endogeic earthworms in organic soils.

The following ordination plot shows mean data of ecological categories for each field (Fig. 5).

Table 6
Mean values (ind/m^2/sampling) of earthworms divided according to the five ecological categories and to age. Significance: *: $p < 0.05$; **: $p < 0.01$; n.s.: $p > 0.05$.

Community structure parameters		Organic [mean (S.E.)]	Conventional [mean (S.E.)]	Significance
Ecological categories	Anecic/Deep-burrower	0.4 (0.3)	/	n.s.
	Endogeic	58.2 (9.6)	15.0 (4.4)	**
	Epigeic	2.5 (1.1)	0.2 (0.2)	n.s.
	Coprophagic	0.1 (0.03)	/	n.s.
	Hydrophilic	0.8 (0.7)	0.1 (0.1)	n.s.
Age	Adult	23.8 (7.6)	5.4 (2.0)	*
	Immature	38.2 (5.7)	9.8 (3.2)	**
Total		61.9 (11.0)	15.3 (4.6)	**

The PCA shows how the major part of sample variance (60.2%) is visualised on axis 1, and along this axis, a separation of samples into two groups is evident: organic samples on the right side and conventional ones on the left. The overall presence of earthworm ecological categories was significantly different in the two types of agriculture ($p < 0.05$, One-way ANOVA).

In order to deepen the effects of specific agronomical practices on the earthworm community adopted by farmers, a PCA was performed (Fig. 6).

The presence of all ecological categories was promoted especially by the adoption of fertilization with green manure and crop diversification (polyculture); instead, some more invasive agronomical practices such as ploughing, chemical weed control with herbicides, as is typically used in conventional farms, and rotary tillage seem to negatively affect earthworms. In general, in horticultural agroecosystems, the agronomical practices adopted in organic farms would significantly encourage the presence of all ecological categories of earthworms ($p < 0.01$, Monte Carlo permutation test).

The QBS-e index analysis is shown in Fig. 7.

The mean QBS-e value was significantly different according to agroecosystem management: 173.8 (± 31.1) for organic fields, that corresponds to a sufficient soil quality class, and 41.7 (± 12.4) for conventional fields, that corresponds to a poor soil quality class ($p < 0.01$, One-way ANOVA).

In conclusion, as the earthworm diversity analysis was able to separate the two types of management of horticultural agroecosystems, also the QBS-e index application was able to highlight this difference.

3.2. Case study n°2: Vineyards

3.2.1. Earthworm diversity (taxonomical approach)

In organic vineyards the mean number of earthworm species was 7.6 (± 1.1), while in conventional vineyards it was 5.4 (± 1.4): there was no significant difference between the two types of management ($p > 0.05$, One-way ANOVA).

As regards species determination, Table 7 shows results of differences between the two types of managed soils.

There was no difference in abundance of any one earthworm species when comparing organic and conventional vineyards.

In order to visualize relationships of similarity among earthworm communities, an NMDS ordination was performed (Fig. 8a).

It was not possible to distinguish between different agroecosystem management systems by considering species abundance and community structure parameters, and it was the same when earthworm communities (all species and their relative abundances) were considered. In fact, in observing the cluster diagram (Fig. 8b) there is no clear separation into two groups according to management type.

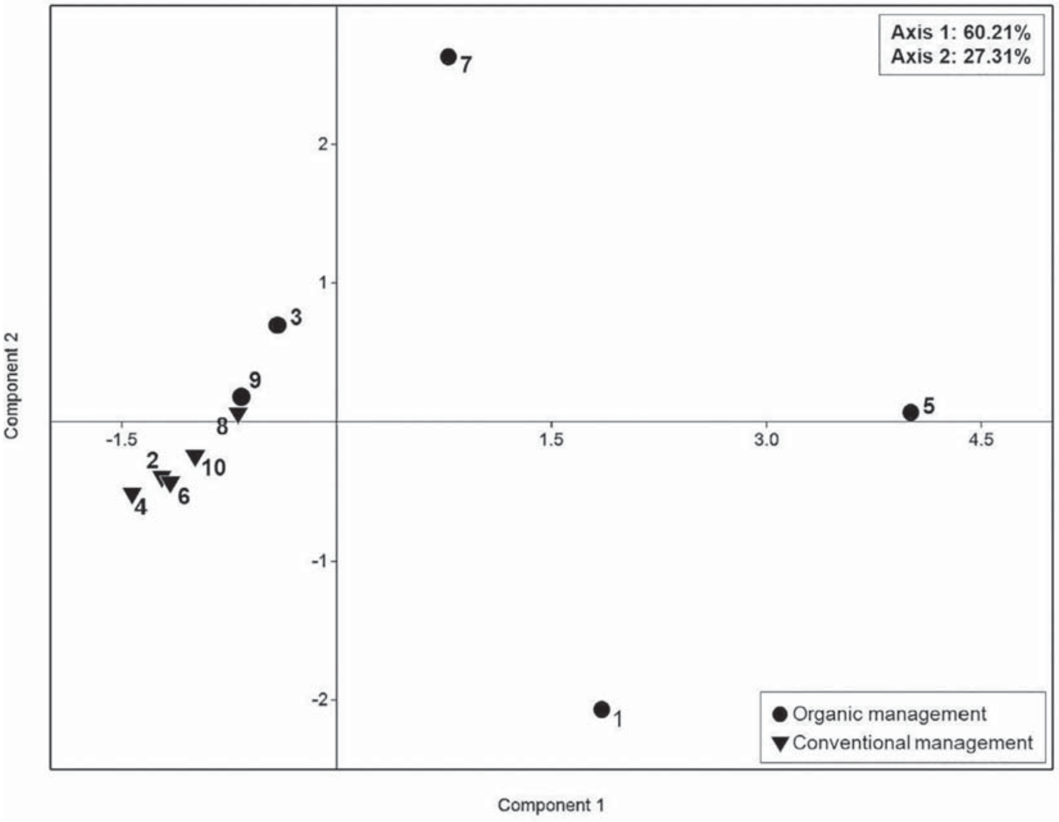

Fig. 5. PCA elaborated with normalised data from earthworm ecological categories (ind/m^2/sampling). Conventional fields: 2, 4, 6, 8, 10. Organic fields:1, 3, 5, 7, 9.

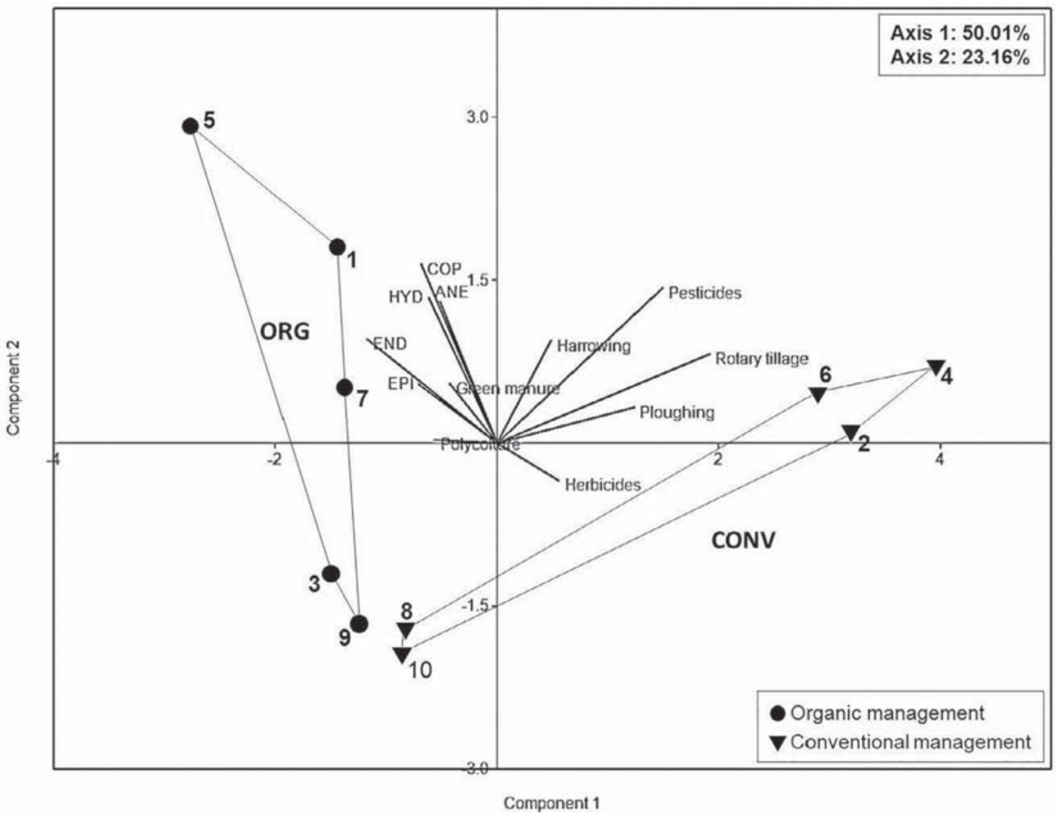

Fig. 6. PCA elaborated with data on the abundance of ecological categories and data on the agronomical practices adopted by farmers. END: endogeic; EPI: epigeic; ANE: anecic/deep-burrower; COP: coprophagic; IDR: hydrophilic. Conventional fields: 2, 4, 6, 8, 10. Organic fields: 1, 3, 5, 7, 9.

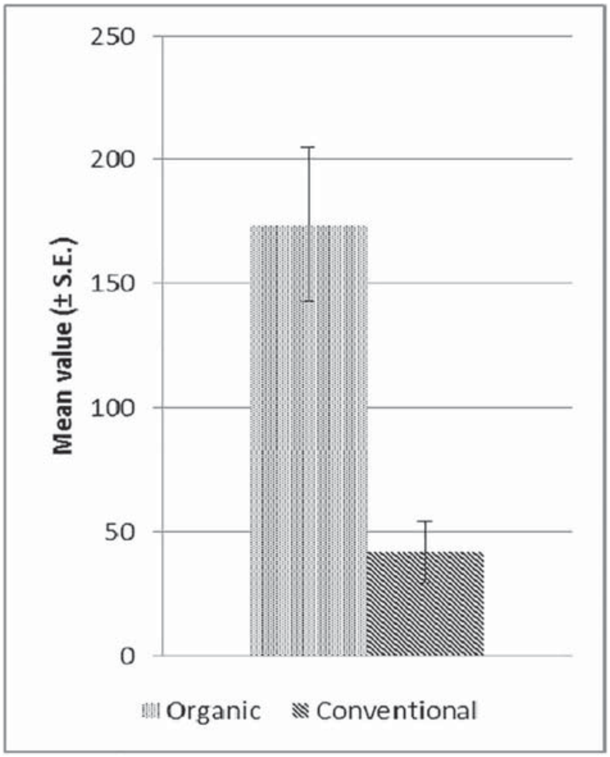

Fig. 7. Mean values of the QBS-e index calculated for differentially managed horticultural agroecosystems.

3.2.2. Ecological categories and the QBS-e index (functional approach)

The earthworm community structure of organic and conventional vineyards was described in terms of ecological categories and age of earthworms (Table 8).

Considering the parameters of ecological categories and age, again there were no differences in abundance between organic and conventional vineyards.

The following PCA plot shows mean data of ecological categories for each vineyard (Fig. 9).

As regards earthworm ecological categories data, it was not possible to separate the vineyards according to different management systems.

In order to deepen the effects on earthworm community structure of specific agronomical practices adopted by farmers, a PCA was performed (Fig. 10).

Unlike the case of horticultural agroecosystems, in vineyards there seems to be no single agronomical practice or overall soil management system that predominantly influences any ecological category ($p > 0.05$, Monte Carlo permutation test), even if a weak trend could be seen: fertilization with green manure could encourage the presence of deep-burrower earthworms, as occurred in the first case study.

The QBS-e index analysis is shown in Fig. 11.

The mean QBS-e value was not significantly different according to vineyard management: 116.3 (± 37.4) for organic vineyards, that corresponds to a sufficient soil quality class, and 60.3 (± 22.8) for conventional ones, that corresponds to a poor soil quality class ($p > 0.05$, One-way ANOVA).

Concluding this study case, as the earthworm diversity analysis was not able to distinguish the two types of farming systems, also the QBS-e index application was not able to do so: again the taxonomical approach in earthworm analysis is concordant with the index approach.

Table 7
Mean values of earthworm species (ind/m^2/sampling) in organic and conventional vineyards. Ecological categories: END: endogeic; EPI: epigeic; ANE: anecic/deep-burrower. Age: Ad: adult; Im: immature. Significance: n.s.: $p > 0.05$.

Species (ecological category-age)	Organic	Conventional	Significance
	[Mean (S.E.)]	[Mean (S.E.)]	
Allolobophora cfr. *chlorotica* (END Im)	4.8 (2.4)	1.5 (0.7)	n.s.
Allolobophora chlorotica (END Ad)	8.4 (4.6)	3.6 (1.2)	n.s.
Allolobophora/Aporrectodea sp. (END Im)	0.8 (0.3)	1.4 (0.7)	n.s.
Aporrectodea caliginosa (END Ad)	0.6 (0.6)	1.2 (0.7)	n.s.
Aporrectodea cfr. *caliginosa* (END Im)	/	0.5 (0.3)	n.s.
Aporrectodea cfr. *jassyensis* (END Im)	0.1 (0.1)	/	n.s.
Aporrectodea jassyensis (END Ad)	0.2 (0.1)	0.2 (0.2)	n.s.
Aporrectodea rosea (END Ad)	/	0.1 (0.1)	n.s.
Dendrobaena octaedra (END Ad)	0.1 (0.1)	0.1 (0.1)	n.s.
Lumbricus cfr. *rubellus* (EPI Im)	0.8 (0.8)	0.6 (0.6)	n.s.
Lumbricus rubellus (EPI Ad)	1.4 (1.4)	0.6 (0.5)	n.s.
Lumbricus sp. (EPI Im)	0.6 (0.6)	1.3 (1.3)	n.s.
Microscolex cfr. *dubius* (END Im)	0.1 (0.1)	/	n.s.
Microscolex cfr. *phosphureus* (END Im)	0.2 (0.2)	0.6 (0.4)	n.s.
Microscolex phosphureus (END Ad)	0.1 (0.1)	0.6 (0.6)	n.s.
Octodriloides phaenohemiandrus (END Ad)	0.1 (0.1)	/	n.s.
Octodriloides phaenohemiandrus (END Im)	0.4 (0.4)	/	n.s.
Octodrilus cfr. *complanatus* (ANE Im)	/	0.4 (0.4)	n.s.
Octodrilus cfr. *pseudocomplanatus* (ANE Im)	1.0 (0.8)	/	n.s.
Octodrilus cfr. *transpadanus* (END Im)	0.1 (0.1)	/	n.s.
Octodrilus complanatus (ANE Ad)	0.8 (0.5)	0.1 (0.1)	n.s.
Octodrilus complanatus (ANE Im)	2.1 (1.8)	1.1 (1.1)	n.s.
Octodrilus lissaensis (END Ad)	/	0.1 (0.1)	n.s.
Octodrilus pseudocomplanatus (ANE Ad)	0.2 (0.2)	0.04 (0.04)	n.s.
Octodrilus transpadanus (END Ad)	0.1 (0.1)	/	n.s.
Octodrilus/Octodriloides sp. (Im)	1.7 (1.1)	0.4 (0.3)	n.s.
Octolasion cfr. *lacteum* (END Im)	/	0.04 (0.04)	n.s.
Octolasion lacteum (END Ad)	/	0.04 (0.04)	n.s.
Perelia cfr. *gestroi* (ANE Im)	1.2 (1.1)	0.5 (0.3)	n.s.
Perelia gestroi (ANE Ad)	0.2 (0.2)	0.2 (0.2)	n.s.
Proctodrilus antipae (END Ad)	0.1 (0.1)	0.1 (0.1)	n.s.
Proctodrilus cfr. *antipae* (END Im)	0.6 (0.5)	0.1 (0.1)	n.s.

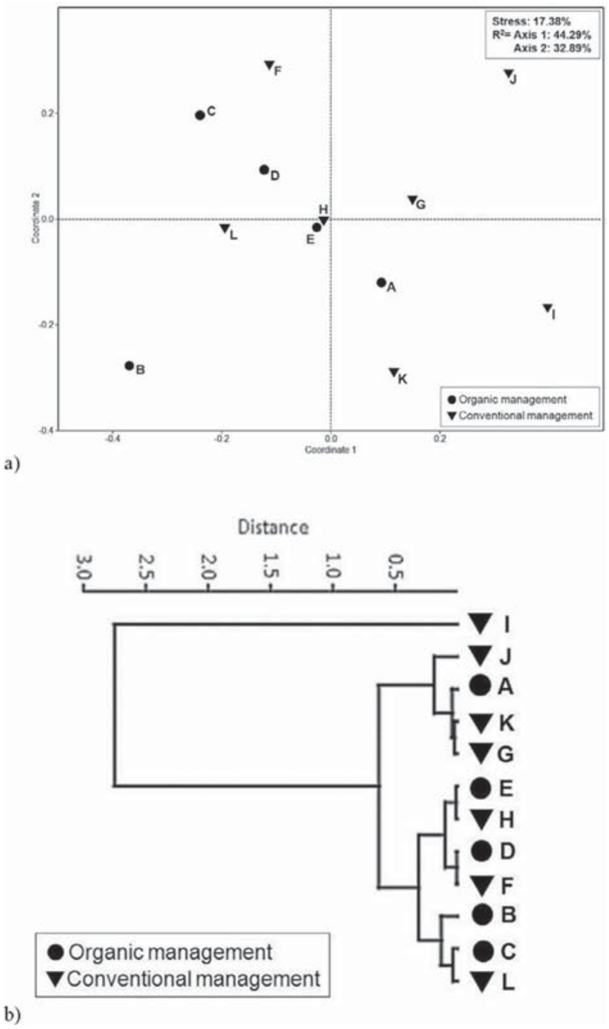

Fig. 8. a) NMDS of data concerning soil earthworm communities (species ind/m^2/sampling), Bray-Curtis similarity measure. b) Classical cluster analysis of NMDS scores on axis 1 data of earthworm communities, Ward's method-Euclidean similarity measure. Conventional vineyards: F, G, H, I, J, K, L. Organic vineyards: A, B, C, D, E.

4. Discussion

4.1. General considerations about earthworm communities

The earthworm community presented in this paper seems to have a rather large species richness, if compared with other Italian studies (i.e. 7 species/m^2 in Paoletti et al., 2010; an average of 4,5 species/m^2 in cultivated fields in Paoletti, 1999a), while it has quite similar results if compared with other European studies (i.e. from 1 species/m^2 in a 10-year old vineyard to 9 species/m^2 in a permanent meadow in Cluzeau et al. 1987).

With regards to data about earthworm densities, these seem quite low if compared to an average of 80 ind/m^2 calculated for cultivated fields (Paoletti, 1999a) and 68 ind/m^2 calculated for vineyards of Champagne (Cluzeau et al., 1987) , but are comparable with other studies citing organic and conventional cultivated fields (i.e. 20 ind/m^2 in organic fields and 14 ind/m^2 in conventional ones (Paoletti et al., 2010)).

4.2. Case study n°1: Horticultural agroecosystems

A first consideration in applying the QBS-e index to this case study is that, in general, the final QBS-e value was particularly low (that corresponded to a sufficient soil quality class for organic fields and a poor soil quality class for conventional ones): this can be due to the type of agroecosystem analysed. In fact, horticultural agroecosystems hosted annual crops and are highly and frequently disturbed by agronomical practices independently of the type of management label (organic or conventional). Nevertheless, the QBS-e index application was so sensitive as so to be able to distinguish between the two farming systems, as the application of the taxonomical approach was also able to do. Therefore both approaches of analysis (taxonomical and functional) gave a concordant result. This means that the QBS-e index, simpler to use, reliable and sensitive, could be a useful tool to explore and monitor earthworm fauna by saving costs in taxonomical analyses and by obtaining a final result which is easier to interpret also for stakeholders, since it provides the soil quality classes.

Concerning relationships between earthworm density and farming system, several studies affirmed different results. Contrary to our results, by considering just the abundance of earthworms, Bengtsson and colleagues (2005) found no significant difference between organic and

Table 8
Mean values (ind/m^2/sampling) of earthworms divided according to the three ecological categories and to age. Significance: n.s.: $p > 0.05$.

Community structure parameters		Organic [mean (S.E.)]	Conventional [mean (S.E.)]	Significance
Ecological categories	Anecic/Deep-burrower	5.5 (2.9)	2.3 (1.4)	n.s.
	Endogeic	16.7 (6.3)	10.1 (3.2)	n.s.
	Epigeic	2.9 (2.9)	2.6 (2.3)	n.s.
Age	Adult	12.4 (5.4)	6.9 (2.1)	n.s.
	Immature	14.4 (4.8)	8.4 (2.9)	n.s.
Total		26.7 (9.0)	15.3 (4.8)	n.s.

conventional farming systems and also Pankhurst et al. (1995) noticed that earthworms tended to show inconsistent responses across a range of soil management practices. Instead Irmler (2010), by analysing changes in earthworm populations during conversion from a conventional to an organic farming system, found that earthworm abundance increased in 2–4 years from 0.2 up to 4.5 ind/m^2, highlighting the better conditions in organic farming systems that allow an increase in density for these important soil bioindicators.

With regards to earthworm ecological categories and their role in the agroecosystem, some evidence, raised by laboratory trials, found that endogeic earthworms reduce the competition in plant-plant interaction between the intercropped annual species (Coulis et al., 2014) and they play an important role in symbiosis with microorganisms (Brown et al., 2000; Lavelle et al., 1995) in phosphorus availability for plant growth (Coulis et al., 2014; Le Bayon and Milliret, 2009). Moreover, it was demonstrated that *A. rosea* and *A. caliginosa* faeces enhance nitrate uptake (Dell'Agnola and Nardi, 1987) thereby improving natural soil fertilization. Therefore it is reasonable to infer that the organically managed soils studied in this work that present a higher abundances of these particular species are naturally well fertilised, have better functioning macronutrient cycles and allow an easier growth for crop plants. With regards to the presence of different ecological categories, since recent organic matter is buried in the soil, whereas deep soil is brought to the soil surface by the deposition of casts above-ground, particularly by deep-burrowing (anecic) species (Blouin et al., 2013; Brown et al., 2000), the importance of the presence of species belonging to this ecological category is evident and, in this study, these were found only in organically managed soils. Furthermore, Valckx and colleagues (2010) found that erosion rates decreased exponentially as a function of anecic earthworm biomass and they underlined the need to promote appropriate soil ecosystem management guidelines by farmers to support populations of anecic species, such as non-inversion tillage or direct drilling. Our results emphasize these management guidelines since Fig. 6 clearly shows how ploughing, for example, an inversion tillage practice, disturbs the presence not only of anecic/deep-burrower species but also of all the other ecological categories. In fact, also Cluzeau and colleagues (1987) pointed out that ploughing helps in the struggle against weeds, but it destroys the upper soil horizons, where a large part of its biological activity is concentrated, and earthworms,

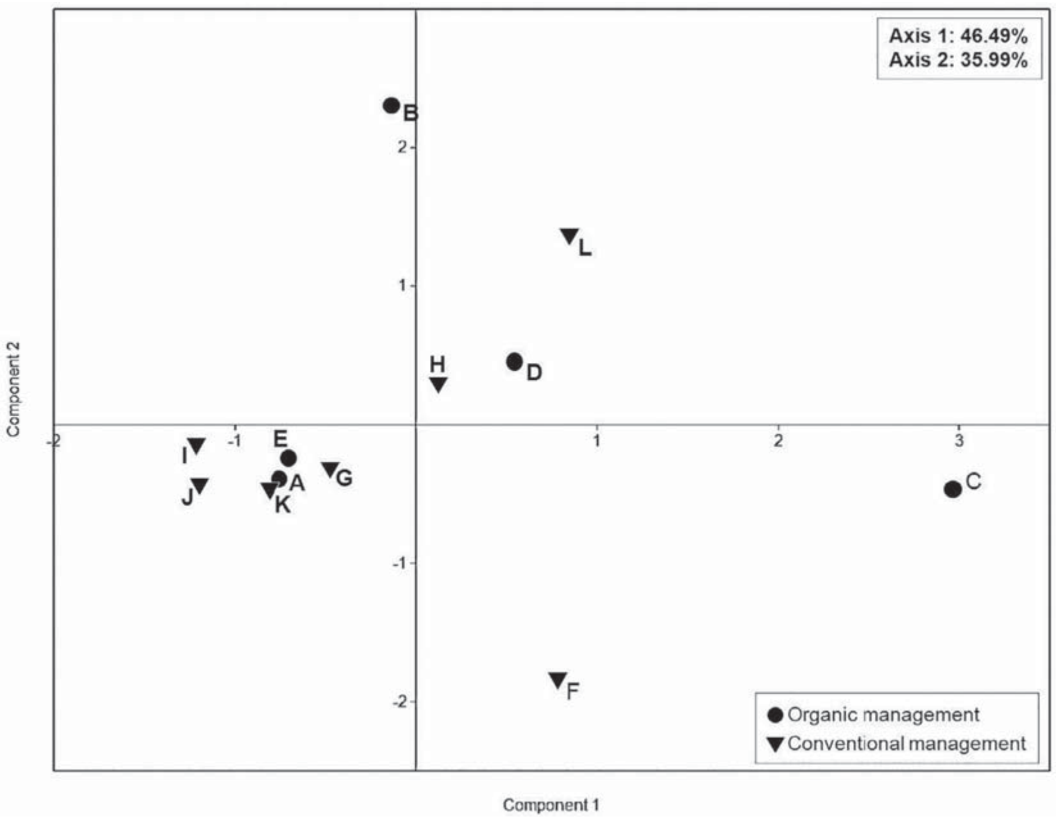

Fig. 9. PCA elaborated with normalised data from earthworm ecological categories (ind/m^2/sampling). Conventional vineyards: F, G, H, I, J, K, L. Organic vineyards: A, B, C, D, E.

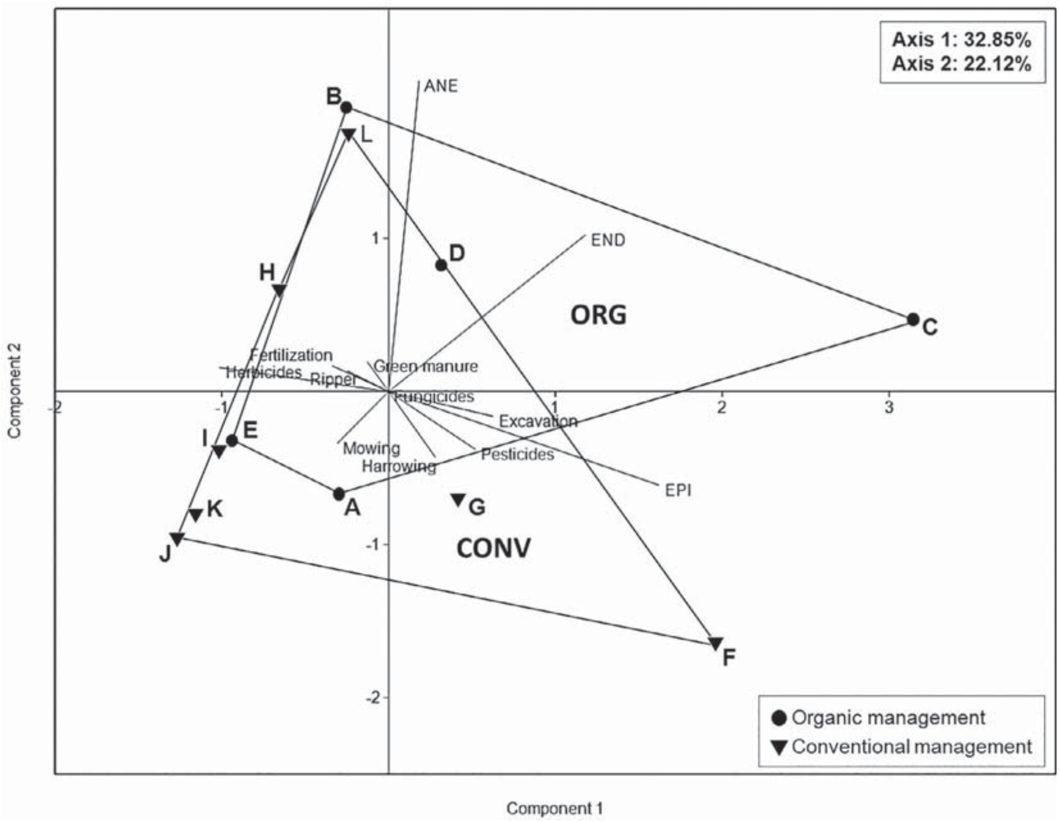

Fig. 10. PCA elaborated with data on the abundance of ecological categories and data on the agronomical practices adopted by farmers. END: endogeic; EPI: epigeic; ANE: anecic/deep-burrower. Conventional vineyards: F, G, H, I, J, K, L. Organic vineyards: A, B, C, D, E.

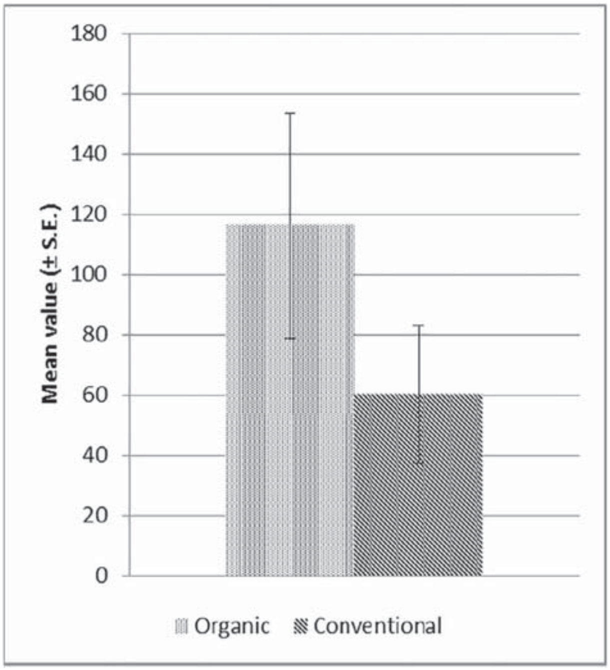

Fig. 11. Mean values of the QBS-e index calculated for differentially managed vineyards.

that are good bioindicators, are negatively affected by this agronomical practice.

Continuing with the eco-physiological functions produced by deep-burrowing species in the soil, Blouin et al. (2013) specified that they can dig semi-permanent vertical burrows at depths down to 1-m and therefore efficiency in water drainage is likely to be increased. Also Valckx et al. (2010) stressed how earthworms and in particular anecic species, such as *Lumbricus terrestris*, significantly reduce runoff and soil loss in arable land. They may also have other effects in improving soil structure. In fact, in spite of the huge deposition of casts at the soil surface, most anecic and endogeic species probably deposit their casts primarily below ground, which markedly affects bulk density and soil aggregation (Blouin et al., 2013; Brown et al., 2000; Lavelle et al., 1995). Generally trying to quantify earthworm eco-physiological effects, particularly of epigeic and anecic species, the contribution of earthworms to the burial of surface litter (leaves, twigs and other vegetal material) at some sites may reach 90–100% of the litter deposited annually on the soil surface by the above-ground vegetation from both natural vegetation or crops (Knollenberg et al., 1985), representing up to several tonnes/ha/year of OM useful to improve soil fertility (Blouin et al., 2013).

It is important to characterize the community according to age, because adults are able to reproduce if there are appropriate conditions, they represent a more stable environment that allowed immatures to become adults and often they are larger than immature individuals (more biomass) and therefore their physiological influence on soil is more incisive, while the presence of immatures indicates that there had been good conditions for earthworm reproduction (Pérès et al., 1998). We found that adult and immature earthworms were more abundant in organic soils. Similarly Hole et al. (2005) indicated a general trend for higher earthworm abundance (adults and immatures) under organic management, and with greater detail further studies (Pfiffner and Mader, 1997) found a higher number of earthworm species, a higher density and more anecic and immature earthworms under organic management, regardless of crop type within the rotation.

As concerns the presence of earthworms and farming practices, some studies pointed out that organic management typically promotes large application rates of organic manure or high-quality crop residues, providing excellent conditions for earthworm activity (van Groeningen et al., 2014). In these agronomic systems, earthworm activity might be crucial in closing the yield gap with conventional agriculture and, for this reason, they highlighted that future research in these systems should focus on management strategies in order to increase earthworm populations. Our study not only investigated differences between different management systems (organic and conventional), but also revealed effects of different management practices on earthworm ecological categories: green manure fertilization and crop diversification (polyculture), associated to a more considerable presence of all ecological categories, are among the agronomical practices adopted in organic farms and therefore these results can be favourable in the direction advocated by van Groeningen et al. (2014) in order to reduce the yield gap between the two production systems.

4.3. Case study n°2: Vineyards

The application of the QBS-e index to the case study n°2 obtained, again, a similar result to the analysis of earthworm diversity. Also for another type of agroecosystem, the vineyard, the taxonomical approach to the earthworm community analysis was concordant with the functional approach, based on their ecological categories and that does not need species determination, confirming the possibility of using the QBS-e index in order to save resources (time, costs, expertise).

Organic farming systems can positively affect species richness in annual crops (case study n°1) or grassland, where the intensity of disturbance caused by management practices is particularly high (Bengtsson et al., 2005; Hole et al., 2005), but the response of biodiversity to organic farming may not be the same in perennial systems, such as vineyards, because the intensity of disturbance differs in these systems (Bruggisser et al., 2010). In fact, even though the answer differs depending on the biodiversity group studied, by also analysing earthworms in this research it emerged that the biodiversity - disturbance relation might be ruled by the *Intermediate Disturbance Hypothesis* (Grime, 1973; Svensson et al., 2007), according to which diversity is linked to disturbance in a non-linear relation and particularly low disturbance is associated with competitive exclusion by the dominant species, high disturbance with the few stress-tolerant species, while intermediate disturbance (of perennial agroecosystems- like case study n°2) seems to favour the coexistence of the former and the latter (Bruggisser et al., 2010; Townsend and Scarsbrook, 1997), making the biodiversity difference between organic and conventional management not univocal. Therefore as shown by the results obtained by Bruggisser and colleagues (2010), according to which organic farming did not promote diversity or abundance of plants, grasshoppers, and spiders in vineyards, likewise our results could suggest that the same situation seems to occur also for earthworm diversity and abundance in vineyards, possibly explained by the Intermediate Disturbance Hypothesis.

Another noteworthy consideration is that vineyards seem to have strong negative impacts on earthworm communities, independently of the agroecosystem management. This could be due to some remnant practices in organic farms, such as the application of copper as a fungicide (Table 2), that seriously and negatively affect the presence of earthworms (Kovacic et al., 2013; Eijsackers et al., 2005).

5. Conclusions: Advantages of using the QBS-e index

(1) The QBS-e index is based on the presence of earthworms, soil organisms widely known by farmers, technicians and agronomists and appreciable by eye without the use of a stereomicroscope.

This study confirms that earthworms are good bioindicators of the sustainability of soil management practices, also in disturbed habitats such as annual horticultural agroecosystems; a different result is shown in vineyards, where some practices adopted in both types of management, such as the application of fungicides (i.e. copper-based products), prevented us from distinguishing organic farms from conventional farms.

(2) By using this index and the dedicated software, it is possible to assess and monitor the soil quality autonomously, even without taxonomical expertise.

In fact, with this new index based on earthworm ecological categories and not on species, the operator can obtain the same result as by analysing the community composition: this was confirmed in both case studies. In the first one (horticultural agroecosystems) organically managed fields totalized a higher QBS-e value than conventionally managed fields, while in the second one there was not a significant difference between organically and conventionally managed vineyards.

(3) The index provides soil quality classes, to express the result of monitoring in a more comprehensible way also for non-experts and stakeholders.

Therefore we propose this index as a useful tool to express earthworm presence and their functionality in agroecosystems and to monitor soil health, in order to save time and costs in monitoring programmes.

There is an increasing need for more and more effective tools to investigate and communicate the influence of land use on soil biota, with the aim of transforming agricultural production from one of the greatest threats to global biodiversity and ecosystem services to becoming a major contributor towards ecosystem integrity (Scherr and McNeely, 2008): the QBS-e index could be effective for this purpose.

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Appendix A

Table A
List of the Italian earthworm species. *data according to Omodeo, M. Matajur (from Paoletti et al, 2013; Blakemore, 2008). HYD: hydrophilic; COP: coprophagic; EPI: epigeic; END: endogeic; ANE: anecic/deep–burrower.

Genus	Species	Ecol. Cat.	Pigment	Body shape	Ecological features	Adult length (cm)	Adult diameter (mm)
Allolobophora	*andreinii*	ANE	Slate grey	Cylindrical	Mediterranean scrub	12–18	7–10
Allolobophora	*chlorotica*	END	Lime green, yellowish or flesh-coloured	Cylindrical with flattened clitellum	Lawn, field, meadow, weak digger	3.5–8.5	3–5
Allolobophora	*cuginii*	END	Absent	Cylindrical	–	7.5	4
Allolobophora	*leoni*	END	Absent	–	Clay soil, forest, crop field	9–13.5	3.5–6.5
Allolobophora	*cupulifera*	END	Absent	Cylindrical with flattened caudal part	–	4.5–5	2–2.5
Allolobophora	*icterica*	HYD	Yellowish	–	Damp soil next to water	5–14	3–4
Allolobophora	*jassyensis*	END	Absent	Cylindrical, with flattened caudal part; 10–13 segments divided into two parts, beyond into three parts	–	6–11	3–3.5
Allolobophora	*rubicunda*	EPI	Red	Cylindrical, sub trapezoid caudal part shape	Degraded lawn	8–10	3–5
Allolobophora	*smaragdina*	END	Emerald green	Cylindrical, slightly depressed lower part	Spruce and mixed forest, litter, decaying wood	4–8	6
Allolobophora	*terrestris*	END	From dark brown to black dorsally, light brown ventrally	Cylindrical in front, trapezoid behind	Mountain lawn, meadow	12–22	5–6
Allolobophoridella	*eiseni*	EPI	Crimson red	Cylindrical	Litter, below cow dung, bark, decaying wood	3–5	3–4
Amynthas	*corticis*	HYD	Grey	Cylindrical, slightly tapered at the far end	Semiaquatic, riverbank	6–16	3–4
Aporrectodea	*caliginosa*	END	From light brown to dark brown, almost black dorsally	Cylindrical in front, trapezoid behind	Damp meadow, lawn, pasture, crop field, riverbank	6–18	2.5–5.5
Aporrectodea	*georgii*	END	Absent	Cylindrical, blunt caudal part, dorsally extended anal fissure	Lawn, meadow, forest, crop field	3.5–7	2.5–5
Aporrectodea	*handlirschi*	EPI	Absent; light red	Slender, trapezoid-shaped caudal part	Wood, lawn, meadow, very damp soil	3.5–9.5	2.5–4.5
Aporrectodea	*rosea*	END	Always absent	Cylindrical rather stocky	Lawn, meadow, crop field, pasture	2–5	1.5–3.5
Aporrectodea	*sineporis*	EPI	Brick red	Cylindrical, slightly quadrangular	Litter	3–4	3
Criodrilus	*lacuum*	HYD	From dark green to black	Quadrangular, dorsal furrow, dorsal anus	Submerged mud in river bed; green spindle-shaped 4–5 cm long cocoons	6.5–14 (up to 32)	4–5
Dendrobaena	*alpina*	EPI	Very light pink	Cylindrical	Alpine pasture, wood litter, under bark	3.5–7.5	3–4.5
Dendrobaena	*attemsi*	EPI	Light red, dorsal light stain around sperm pores	Cylindrical	Litter, crop field margin, under bark	2–6	2–2.5
Dendrobaena	*byblica*	END	Scarce flesh-colored	Octagonal section	Litter, crop field margin, under bark	2.6–5	2.4–3.2
Dendrobaena	*cognettii*	EPI	Reddish in front	Cylindrical, flattened anterior part	Wood litter	1.1–3	1–1.5
Dendrobaena	*hortensis*	EPI	Crimson red with light bands	Flattened	Very damp soil, organic matter, often in caves	2.6–6	2.3–2.5
Dendrobaena	*octaedra*	EPI	Dark purple-brown	Octahedral section	Litter, under bark, decaying wood, cow dung	1.6–4	1.5–4
Dendrobaena	*pantaleonis*	EPI	Very light pink	Slender, polygonal section	Wood litter	1.8–4	1.2–2.6
Dendrobaena	*schmidti*	EPI	Dark purple-brown	Slightly prism-shaped	–	3.2	3
Dendrobaena	*veneta*	COP	Purple-reddish bands, light intersegmental wrinkles	Flattened, squat	Damp organic matter-rich soil, manure and compost yard, garden, under bark	4–9	4.5–5.8
Dendrodrilus	*rubidus rubidus*	EPI	Red, darker at the far ends	Cylindrical, flattened	Mountain lawn, meadow, wood litter and under bark	2.4–4	2–3.5

(*continued on next page*)

Table A (*continued*)

Genus	Species	Ecol. Cat.	Pigment	Body shape	Ecological features	Adult length (cm)	Adult diameter (mm)
Dendrodrilus	*rubidus subrubicundus*	EPI	Crimson red, sometimes scarce	Cylindrical, flattened	Lawn, pasture, litter, decaying wood, not in the mountains	3.5–6.5	3–3.5
Diporodrilus	*pilosus*	END	Absent	Cylindrical, squat	Lawn, wood	6–11	6.5–9
Eisenia	*fetida*	COP	Purple red with bands	Cylindrical, with flattened clitellum and caudal part	Strongly linked to manure yard; maybe originally under the bark	5–12	2–4
Eisenia	*spelaea*	COP/HYD	Absent; whitish; brick red with bands	Flattened	Sub aquatic, mud, guano, also outside caves	7–11	5.6–6
Eiseniella	*neapolitana*	HYD	Greenish, flesh-colored- orange clitellum	Quadrangular with dorsal furrow	Stagnant water bodies	4–8	2.5–3.5
Eiseniella	*tetraedra*	HYD	Brown-ocher	Quadrangular	Sub aquatic, river banks, damp moss	1.2–6	1.5–3
Eophila	*asconensis*	ANE	Absent	Cylindrical, club-shaped posterior part	Deep digger; wood, fallow land	6.5–13	4–5
Eophila	*tellinii*	ANE	Purple-brown transverse rings	Cylindrical, club-shaped posterior part	Deep burrower; often tunnel entrance under big stones	17–36 (up to 60–80)	12–16
Eophila	*crodabepis*	ANE	Purple-brown transverse rings	Cylindrical, club-shaped and depressed at posterior part	Deep burrower; often tunnel entrance under big stones	24–60	10
Eumenescolex	*gabriellae*	EPI	Red, scarce	Cylindrical, thinner anterior part	Only in Mediterranean wood litter, crop field	4–7	2.1–3.5
Eumenescolex	*gabriellae gallurae*	EPI	Red, scarce	Cylindrical, thinner anterior part	–	4–7	2.1–3.5
Helodrilus	*oculatus*	HYD	Absent	Cylindrical	Aquatic, putrid mud	3.5–8	1.5–2
Helodrilus	*patriarchalis*	HYD	Absent	Cylindrical	Aquatic, submerged anoxic mud	6–13	4–6
Hormogaster	*pretiosa*	ANE	Silver, steel grey	Cylindrical, club-shaped anterior part	Oak woods, ruderal sites	24–34 (up to 50)	12–18
Hormogaster	*redii*	ANE	Light brown, slate grey	Cylindrical, club-shaped anterior part	Euryecious, it can tolerate extreme conditions of humidity and salinity	12–30	9–13
Hormogaster	*samnitica*	ANE	Light brown-grey	Cylindrical, club-shaped anterior part, flattened clitellum	Euryecious, it can tolerate extreme conditions of humidity and salinity	8.5–30	6–11
Lumbricus	*castaneus*	EPI	Red, dorsally purple-brown	Almost cylindrical, club-shaped anterior part	Above all in mixed wood litter	3–5	2.5–4
Lumbricus	*meliboeus*	EPI	Red, dorsally purple-brown	Club-shaped anterior part, flattened posterior part	Litter, under stones, mediocre digger	5–7	3–5
Lumbricus	*rubellus*	EPI	Red, dorsally purple-brown	Club-shaped anterior part, flattened posterior part	Litter, under stones, mediocre digger, lawn, wood	3.8–12	3.5–5.5
Lumbricus	*terrestris*	ANE	Red-brown dorsally, yellowish ventrally	Club-shaped anterior part, flattened posterior part	Garden, pasture, wood margin, pre -Alps wood	7–20	7–8
Microeophila	*alzonai*	COP	Ash grey	Cylindrical, segments from 16 to 23 and post-clitellum with two–three rings	Caves, guano	6	2.5
Microeophila	*marcuzzii*	END	Absent	Cylindrical, swollen anterior part	–	3.5	3–4
Microscolex	*dubius*	END	Absent	Cylindrical	Anthropophilic, garden, scarce in mountains	3–10	1–3
Microscolex	*phosphoreus*	END	Absent	Cylindrical	Anthropophilic, garden, scarce in mountains	1–3.5	1–1.5
Murchieona	*minuscula*	END	Absent, whitish	Cylindrical	Weak digger, crop field, plain wood	1.2–2.5	1–1.8
Ocnerodrilus	*occidentalis*	HYD	Red (living), absent (dead)	Cylindrical	Aquatic, submerged mud	1.5–3	1
Octodriloides	*boninoi*	ANE	Brown, brown-reddish, no pigment in intersegmental spaces	Club-shaped anterior part	Pre-Alps, alpine, wood, pasture	13–24	10–12
Octodriloides	*kamnensis*	END	Brown	–	–	9 (5.2–7 *)	6
Octodriloides	*minor*	END	Light brown	Cylindrical	–	10–11	5.5–6
Octodriloides	*omodeoi*	END	Grey	–	–	5.5–6	0.5–0.6
Octodriloides	*phaenohemiadrus*	END	Red-brown	–	Wood	4.1–11.5	3–6
Octodriloides	*pseudokovacevici*	END	Red-brown	–	–	4.5–6.8	4–5
Octodriloides	*eubenhami*	END	Red-brown	–	–	4.3–6.5	4–5

(*continued on next page*)

Table A (*continued*)

Genus	Species	Ecol. Cat.	Pigment	Body shape	Ecological features	Adult length (cm)	Adult diameter (mm)
Octodriloides	*hemiandrus*	ANE	Pale red	–	Mediterranean wood	4.6–6.8	4.5–5
Octodrilus	*argoviensis*	END	Absent	–	–		
Octodrilus	*complanatus*	ANE	From brown to slate grey	Cylindrical, club-shaped anterior part	Widely distributed, crop fields margins near woodlands, flatlands and high hills	11–22	7–10
Octodrilus	*croaticus*	HYD	Dark purple	Cylindrical, blunt posterior part	Flood plain, river bank, wood	3.5–5.2	3.5–5
Octodrilus	*lissaensis*	END	Light red	Cylindrical, thinner caudal part	Humic soil in wood, lawn, pasture	3.3–6	3.5–4.5
Octodrilus	*mimus*	ANE	Brown, with darker rings	–	Mixed wood, crop field margin; 8–20 cm high cylindrical castings	14.3–19.5	10
Octodrilus	*pseudocomplanatus*	ANE	From brown-reddish to slate grey	Cylindrical, club-shaped anterior part	Hill, mountain, wood, pasture	10–18	6.6–10
Octodrilus	*ruffoi*	END	Brown- reddish	–	–	5–8	5–7
Octodrilus	*transpadanus*	END	Brown	–	–	5–7	4–5
Octolasion	*cyaneum*	END	Absent	Cylindrical, flattened clitellum and slightly flattened posterior part	–	8–14	6–8
Octolasion	*lacteum*	END	Absent; brown dorsally (living)	Cylindrical, flattened clitellum	Lawn, wood, crop field, weak burrower	2.7–16	2.5–5
Perelia	*nematogena*	END	Pink, orange-pink clitellum	Cylindrical	Fallow land, crop field	8–10	5
Perelia	*schneideri*	END	Absent	–	Degraded lawn	4.3–7.2	2.5–3
Perelia	*bouchei*	END	Absent	Cylindrical, squat, blunt caudal far end	Damp sand, slope, often associated to *H. redii*	3.3–4.4	3.5–4.5
Perelia	*gestroi*	ANE[*]	Absent but with traces in transverse bands	Cylindrical, swollen anterior part	Wood, deep burrower	9–12	4–7
Pietromodeona	*januaeargenti*	ANE	Absent	Cylindrical, club-shaped posterior part	Deep burrower; wood	7–12	3.5–5
Pontodrilus	*litoralis*	HYD	Red-brown first segments	Cylindrical	Intertidal zone	8–10	4–4.5
Proctodrilus	*antipae*	END	Absent	Thinner posterior part	Crop field, pasture	2.5–6	2–4
Prosellodrilus	*festae*	END	Absent	Cylindrical	Wood, crop field	3–6	2
Scherotheca	*simplex*	ANE	Absent	–	Deep burrower	10.5–11.2	5–7
Scherotheca	*dugesi*	ANE	Brown	Cylindrical, swollen anterior part, club-shaped posterior part	Wood	20–30	10–15
Scherotheca	*targionii*	ANE	Slate grey	Cylindrical, flattened caudal part	–	30	5

[*] Perelia gestroi, due to the absence of pigmentation and the average body length could be classified as an endogeic, but it is a deep-burrower species (see more details in the text).

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