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Short term recovery of soil physical, chemical, micro- and mesobiological functions in a new vineyard under organic farming

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Abstract

Deep earthwork activities carried out before vineyard plantation can severely upset soil profile properties. As a result, soil features in the root environment are often much more similar to those of the underlying substratum than those of the original profile. The time needed to recover the original soil functions is ecologically relevant and may strongly affect vine phenology and grape yield, particularly under organic viticulture.

The general aim of this work was to investigate soil resilience after vineyard pre-planting earthworks. In particular, an old and a new vineyard, established on the same soil type, were compared over a five year period for soil chemical, physical, micro and mesobiological properties.

The investigated vineyards (*Vitis vinifera* L., cv. Sangiovese) were located in the Chianti Classico district (Central Italy), on stony and calcareous soils and were not irrigated. The older vineyard was planted in 2000, after slope reshaping by bulldozing and back hoe ploughing down to about 0.8–1.0 m. The new vineyard was planted in 2011, after equivalent earthwork practices carried out in the summer of 2009. Both vineyards were organically managed and fertilized only with compost every autumn (1000 kg ha^{-1} per year). The new vineyard was cultivated by periodic tillage, while the old vineyard was managed with alternating grass-covered and tilled inter-rows.

Soil samples were collected at 0–15 cm depth from the same plots of the new and old vineyards, during the springtime from 2010 to 2014. The old vineyard was sampled in both the tilled and the grass-covered swaths.

According to the results from physical and chemical analyses, the new vineyard, during the whole 2010–2014 period, showed lower TOC, N, C / N and EC values, along with higher silt and total $CaCO_3$ contents than the old vineyard, suggesting still evolving equilibrium conditions. The microarthropod analysis showed significantly different abundances and communities' structures, in relation to both vineyard and time, increasing with rain precipitations in the old vineyard. Though the euedaphic forms, well adapted to soil life, were always rare. Microbiological analysis revealed a differ-

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ent structure of eubacterial communities between old and new vineyard in the whole period. However, the DGGE similarity values of such communities increased of about 2.5 % per year, suggesting that at least 3 years more are needed to compare intra- and inter-specific diversity of the two vineyards.

In conclusion, the consequences of deep earthworks on soil chemical, micro and mesobiological properties were still evident after four years from planting, indicating that more time is necessary for the recovery of soil functions, probably longer than that needed to obtain an economic grape production.

1 Introduction

Soil is an essential factor in terroir expression, having a unique role in water and nutrient supply that strongly relates to the vine growth and quali-quantitative yield performance (Vaudour, 2002; Van Leeuwen et al., 2004). A soil management that ensure proper soil physical conditions, organic matter turnover, adequate and balanced nutrient availability and biological diversity is, therefore, important to maintain adequate soil functionalities and high-quality wine productions (Van Leeuwen and Seguin, 2006; White, 2003). Most vineyards are established after the soil has been treated by deep tillage, to break and loose the soil and underlying rock, create a workable planting bed and incorporate the residues from the preceding cultivation and/or organic fertilizers. Slope reshaping activities may also be implemented to overcome slope limitations, by means of heavy machinery that moves the soil from the upper to the lower slope positions, or create terraces (Bazzoffi et al., 2006; Ramos and Casasnovas, 2007). Earthwork practices, when applied without taking into account the site-specific soil and environment conditions, may severely impact soil quality, threatening soil productive potential and ecosystem functions (Le Bissonnais et al., 2002; Costantini and Barbetti, 2008; Martínez-Casasnovas and Ramos, 2009; Garcia-Ruiz, 2010). This is of particular concern in hillside areas, under tillage practices that involve stripping or overturning the soil profile, which results in the upsetting of soil layers and outcropping of the underly-

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ing unweathered rock or sediment. The process may lead to higher soil susceptibility to erosion and intense physical, chemical and biological modifications in the root environment, e.g. mixing of soil horizons, alteration of soil structure and hydrology, loss of organic matter, modification in soil pH, organic matter depletion, enrichment of salt concentration and calcium carbonate content, reduction of soil depth, water retention capacity, nutrient availability, and biological activity and diversity (Ramos and Martinez-Casasnovas, 2006; Le Bissonnais et al., 2007; Bazzoffi and Tesi, 2011; Costantini et al., 2012; Seddaiu et al., 2013; Sharp-Heward et al., 2014). The degree to which soil quality is altered by earthworks depends upon the soil type, climate and management practices.

The inherent ability of a soil to counteract degradation and restore new equilibrium conditions, in which productive performances and ecosystem functioning are not significantly different from those before disturbance, is known as "soil resilience" (Lal, 1997). Soil resilience is a soil-specific attribute of great ecological relevance, depending on a complex dynamic interaction of soil physical, chemical and biological processes (Seybold et al., 1999; Blanco and Lal, 2008), that may strongly affect not only soil health, but also vine phenology and grape yield (Rawnsley, 2014).

However, the recovery of soil functions assumes a specific meaning when applied to vineyard plantation on lands of ancient agricultural use, like most of those interested by viticulture in Europe. Since only a marginal proportion of the new vineyards is planted on non-agricultural lands, the time needed to reach a new equilibrium should be assessed with reference to the same land use.

Organic farming is deemed to improve soil conditions in vineyards, and speed-up the recovery time in new vineyards, through the improvement of soil biological fertility (Huber et al., 2003; Reinecke et al., 2008; Probst et al., 2008). Furthermore, the organic treatments act both directly and indirectly, as they contribute to the preservation of more favourable moisture conditions for soil biological activity. Nevertheless, organic viticulture may have limitations on the recovery of some soil functions, in particular,

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nitrogen nutrition of vines in very poor soils, like those interested by bulldozing and scalping (Costantini et al., 2013).

Monitoring the degree of soil degradation and resilience over time requires the use of suitable soil quality indicators. These are traditionally based on a variety of soil chemical, physical and biological properties; soil organic matter, aggregate stability, microbial respiration, biological activity and diversity are some of the most frequently considered, for their multifunctional importance in soil ecosystem services and their highly sensitive response to soil perturbation.

The structure and functions of microbial communities are key drivers of soil biogeochemical cycles and general soil quality (Nannipieri et al., 2003), therefore the use of proper microbiological indicators is essential to assess their role in soil resilience (Bloem et al., 2006).

Although microbiological indicators have been applied since many years, recently, some new methods have been proposed in the field of soil mesobiological indicators, involving the characterization of soil microarthropod communities. In fact, these organisms are recognized to play an important role in soil formation as well as in soil organic matter transformation, nutrient cycling, C accumulation and plant and microbial diversity conservation, representing a useful and effective indicator for soil quality (Parisi, 2001; Parisi et al., 2005).

Our research was based on the monitoring of soil functions over time by means of chemical, micro and mesobiological indicators, with the aim to assess the time required for a vineyard soil under organic farming to recover its functions after pre-planting earthworks.

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2 Materials and methods

2.1 Site characteristics and experimental design

The surveyed vineyards belong to a premium wine farm, falling within the Chianti Classico district, in the northern part of the Siena Province (Tuscany, Central Italy; 43°23′19″ N, 11°26′66″ E). The vines (*Vitis vinifera* L. cv. Sangiovese) are grown on the interfluve and seepage slope of a hill (about 400 m a.s.l.) dominated by clayey-calcareous flysch lithotype, with stony and calcareous soils classified as Cambic Skeletic Calcisol (Loamic, Aric) (IUSS Working Group WRB, 2014).

Climate is Mediterranean sub-oceanic (Costantini et al., 2013), characterized by cool and rainy winters, with minimum monthly average air temperatures close to 0 °C, but hot summers, with a large number of days experiencing maximum temperature above 30 °C (on average, 8.3 days in June, 17.5 days in July, 17.3 days in August, and 2.8 days in September). According to the long-term average data, the annual precipitation was 800 mm, concentrated in autumn and springtime, the potential evapotranspiration (ET0) from April to September was 850 mm (Hargreaves and Samani, 1982), and the Winkler index was 1.856 Degree Days. The climate data were collected from the weather station located close to the experimental site.

The overall area, extending to approximately 40 ha, consisted of two zones (Fig. 1a, b, c): one covered by a 14 year old vineyard, planted in 2000 after slope reshaping by bulldozing and back hoe ploughing down to about 0.8–1.0 m; the other by a new vineyard established in 2011 after equivalent earthworks, carried out in the summer of 2009. Here, an old vineyard had been present until 1990, followed by a set aside up to 2009.

The old vineyard was managed with alternating grass-covered (G) and tilled inter-rows (T), while the new vineyard was entirely cultivated by periodic tillage, according to the farm strategy to maintain the soil surface free from weeds until the establishment of the new vines and the start of commercial level of grape production.

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For soil study and classification, within each vineyard four soil profiles were dug close to the experimental plots; in the old vineyard, two of them were dug in the grass-covered inter-rows and the other two in the tilled inter-rows. The soil before the earthworks made in 2009 was genetically similar to the one obtained after the ploughing. The main difference was the presence of a sharp but irregular lithological contact with the underlying rock within one meter depth. During the earthworks, the rock was broken and the biggest pieces carried out from the surface to build a stone wall.

Both vineyards were managed organically, applying with 1.0 Mg ha^{-1} compost per year in autumn. The compost had the following properties: total N = 3.6 %, organic N = 2.8 %, total OC = 33.4 %, C / N = 9.3, humic + fulvic acids = 15.2 %, total P (P_2O_5) = 3.3 %, total K = 0.28 % (s.s).

The results here presented concern the first five monitoring years, running from 2010 to 2014. Within each vineyard, four 10 m^2 georeferenced plots were selected (referred to as P1–P4 in the new vineyard and P5–P8 in the old vineyard (Fig. 1a) for soil chemical, physical, and biological monitoring. Each plot was sampled during the spring season in four different sites, using specific sampling procedures according to the analysis to be performed. The sites were the same for the whole duration of the experimentation. The old vineyard plots included both grass-covered (P5 and P7) and tilled inter-rows (P6 and P8). However, it needs to be pointed out that the effects of these two managements on this extremely poor soil were heterogeneous, with differences statistically not significant for all analyzed soil properties (Fisher test: $P < 0.05$). Actually, natural weed development often occurred also on tilled inter-rows in the periods before sampling, resulting in a variable degree of grass covering. Therefore, experimental data obtained from the grass-covered and tilled inter-rows of the old vineyard were pooled together for the statistical analysis.

Moreover, since experimental data were lacking for soil microarthropod analysis in 2010 and soil properties of the old vineyard in 2011, due to the lacking of soil samples, the comparison between the new and old vineyards could not be done for the mentioned variables and years.

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Neither vine phenology nor production were recorded during the five years; however, only in 2013 and 2014 very few and little clusters started to be produced sparse in the new vineyard, which in any case were not suitable for harvest or monitoring the grape production.

2.2 Soil physical and chemical analysis

For soil physical and chemical monitoring, each experimental plot was sampled by digging four 15 cm depth pits, from which disturbed soil samples were collected. The samples from the different sampling points were mixed thoroughly to provide a single composite sample per plot.

Before laboratory analyses, the samples were air-dried and sieved through a 2 mm mesh. For C and N determination, sub-samples were ground and homogenized to 0.5 mm. Soil physical and chemical analyses included: particle size, pH, electrical conductivity, total and active carbonates, total organic carbon and total nitrogen. Specifically, soil texture was determined using the sedigraph method (Andrenelli et al., 2013). Total organic C (TOC) and total N (TN) were measured by dry combustion on a Thermo Flash 2000 CN soil analyzer. To this aim, 70 mg soil were weighed into Sn-foil capsules to determine the total C (organic C + mineral C) and N contents. Separately, 20 to 40 mg soil were weighed into Ag-foil capsules, pre-treated with 10 % HCl until complete removal of carbonates and then analysed for total C content (corresponding to the whole OC content). The total equivalent $CaCO_3$ content was calculated from the difference between the total C measured before and after the HCl treatment (Sequi and De Nobili, 2000).

Active lime was determined according to the Drouineau method; the procedure involved reaction of the soil with 0.1 M ammonium oxalate for 2 h under agitation, followed by the determination of unreacted oxalate by back-titration with 0.1 M $KMnO_4$ (Loeppert and Suarez, 1996). Soil pH was measured potentiometrically in a 1 : 2.5 soil-water suspension. Electrical conductivity was measured in a 1 : 2 soil-water extract, after 2 h

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shaking, overnight standing and filtration. The main soil properties at the beginning of the study are reported in Table 1.

2.3 Soil microbiological analysis

Estimation of organic C mineralisation was performed by measuring C-CO_2 production [mg (C-CO_2) kg soil^{-1} day^{-1}] by soil in a closed environment (Isermeyer, 1952) after 1 day.

The structure of microbial communities was determined by means of denaturing gradient gel electrophoresis (DGGE), a PCR-based molecular technique which has been widely used in microbial ecology for the rapid evaluation of soil microbial community structure of multiple soil samples (Muyzer and Smalla, 1998; Nannipieri et al., 2003). Soil (0.5 g) DNA was extracted by the bead-beating method using FastDNA SPIN Kit and the FastPrep instrument (Bio 101, USA). The eubacterial community structure was determined by amplifying the 16S rRNA genes, using the primer set GC-968f (5′-CGC CCG GGG CGC GCC CCG GGC GGG GCG GGG GCA CGG GGG GAA CGC GAA GAA CCT TA-3′) and 1401r (5′-GCG TGT GTA CAA GAC CC-3′) designed by Felske and Akkermans (1998). Soil template DNA was amplified with a mix containing 1 U Go Taq Flexi (PROMEGA), 6.25 pM of primers, 6.25 mM deoxyribonucleotide triphosphates, 1.5 mM $MgCl_2$ and 25X reaction buffer in a final reaction volume of 25 µL. The PCR was then performed with a I-Cycler thermalcycler (BIORAD) with the following temperature cycle: 94 °C denaturation for 90 s, 56 °C annealing for 30 s, and 72 °C extension for 45 s, followed by 33 cycles at 95 °C for 20 s, 56 °C for 30 s, and 72 °C for 45 s, and a final extension at 72 °C for 7 min. PCR products were checked on 1 % agarose gel by electrophoresis.

The DGGE analysis was performed with the INGENY phor-U System (Ingeny International, The Netherlands) on a 6 % polyacrylamide gel (acrylamide/bis ratio, 37.5 : 1), under denaturation conditions (urea, 7 M; 40 % formamide with a denaturing gradient ranging from 42 to 58 %); the gels were run in 1X Tris-acetate-EDTA buffer at 75 V for 16 h at 60 °C and were stained with 12 mL of 1X Tris-acetate-EDTA buffer containing

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1.2 µL of SYBR Green I (dilution, 1 : 10 000) for 30 min in the dark. Visualization and digital pictures were performed with a ChemiDoc System (Bio-Rad).

The diversity indices and the band number of DGGE's patterns were calculated using Gel Compare II software v 4.6 (AppliedMaths) as described by Fabiani et al. (2009).

2.4 Soil biological quality index (QBS-ar)

Soil microarthropod communities were studied according to the procedure described by Parisi et al. (2005). Generally, the application of microfauna-based indicators of soil quality have been often limited by the difficulties in classifying organisms to the species level. To overcome this limitation, Parisi et al. (2005) introduced a new approach, based on the use of a simplified Eco-Morphological Index (EMI) for the determination of the "Soil Biological Quality" of arthropods index (QBS-ar). This index is based on the concept that the higher soil quality, the higher will be the number of microarthropod groups adapted to the soil habitat. The degree of microarthropod adaptation is defined by specific morphological characters; in particular, more adapted organisms will typically show reduced pigmentation and visual apparatus, loss or reduction of wings, reduced appendages and streamlined body form (Parisi, 2001). Each biological form (morpho-type) isolated from the soil can be classified to the order level and is eco-morphologically scored. The scoring is proportional to organism adaptation degree, ranging from 1 (surface-living organisms) to 20 (deep-living organisms). The sum of all EMI values for a given soil sample provides its QBS-ar index. Once determined, the QBS-ar values were used to define "soil biological quality class", according to the classification by D'Avino (2002). In particular, each class was identified by a number, ranging from 0 to 7, which increases with increasing complexity and the adaptation degree of soil microarthropod communities as expressed by the QBS-ar ("class 0": absence of edaphic groups and occurrence of only surface-living arthropods and/or Holometabola larvae; "class 7": occurrence of at least three edaphic groups, including Protura and/or edaphobiont Coleoptera and QBS > 200).

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Soil microarthropod communities were also characterized quantitatively, by measuring the abundance of the main arthropod groups and the respective relative frequencies.

All biological determinations were performed once a year, from 2011 to 2014, collecting 1/3 dm^3 soil cores from 4 replicated zones within each vineyard. The samples were treated for the extraction of microarthropods using a Berlese-Tullgren selector; all microarthropods collected were identified to the order level using a stereo microscope.

2.5 Statistical analysis

Differences in soil properties between the new and the old vineyards were analyzed statistically by the non-parametric Kruskal-Wallis test, to avoid inaccuracies due to variance heterogeneity and non-normality patterns in data distribution (Statsoft STATISTICA v. 7; SPSS v. 15.0). Soil QBS-ar data were analyzed using the Mann-Whitney rank test (SPSS v. 15.0; $P = 0.05$).

In order to evaluate the resilience of the new vineyard, a Principal Component Analysis (PCA) was performed. For each experimental year, the analysis was run on the matrix correlation, therefore, without variable standardisation. The results are reported graphically as variance of cases and variable biplots. Furthermore, a separate PCA was done on the whole 2010–2014 dataset, with and without the inclusion of climate variables. As previously mentioned in the Sect. 2.1, most of soil chemical and microbiological data were not available in 2011 for the old vineyard; therefore, in order to perform the PCA, the old vineyard dataset was completed by replacing the missing value of each variable with the average of that variable across all other trial years in the same experimental condition. This procedure is justified by the fact that PCA was mainly aimed at interpreting the phenomenon under study through new latent components resulting from the correlation among variables, and not to classify the values itself of the variables (ISTAT, 2000).

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3 Results

3.1 Climatic conditions during the trial

The trends of rainfall and temperature recorded during the monitoring period are shown in Fig. 2 with the respective long-term average trends.

In 2010, the temperature and rainfall values were close to the long-term means. Starting from 2011, the area was affected by highly variable annual precipitation, often with marked differences from the long-term means. In particular, 2011 was characterized by below-average rainfall over almost the whole year and strong drought conditions in August and September. 2012 had above-average rainfall in spring and autumn, with an intense drought period in June–July. 2013 was moderately drought in August, with above-average precipitation during winter-spring and in autumn. Finally, 2014 experienced above-average winter rainfall and moderate drought conditions in July.

3.2 Soil physical and chemical properties

Soil texture was quite stable over time, in fact, the clay and sand contents in each vineyard did not vary significantly from the beginning to end of the trial. Nevertheless it revealed some significant differences between the two vineyards in the less-than 0.05 mm size particle fraction, with the new vineyard featuring a significantly higher silt content (47.3 against 41.2 %) and a lower clay content (23.7 against 31.1 %). Accordingly, soil texture classification varied from “clay loam” in the old vineyard to “loam” in the new vineyard.

Almost all selected soil chemical properties followed temporal fluctuations (Fig. 3), with similar patterns in the two vineyards, thus suggesting the influence of common variability factors. Over the five year monitoring period, the new vineyard averaged lower TOC and TN amounts, with higher $CaCO_3$ and pH values. The best discriminating soil variable was $CaCO_3$ content, with differences falling in the ranges of 25–69 and 38–67 % for the total and the active pools, respectively. Soil TOC content averaged

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higher values in the old vineyard over the whole monitored period (+33 %), though the differences were statistically significant only in 2010 and 2012.

From 2010 to 2012, the two vineyards had similar soil pH values (8.2). In the following years, the new vineyard showed slight but significant pH increases, while the old vineyard confirmed a substantial stability.

3.3 Soil microbial activity and diversity

The DGGE fingerprints showed complex banding patterns, indicating a high bacterial diversity, with clear distinction between the two vineyards in each sampling year. The cluster analysis designated two distinct clusters for the old and the new vineyard (Fig. 4) with varying degree of similarity over time. These differences indicate a clear effect of pre-planting earthworks on the composition of soil bacterial communities in the new vineyard, due to the redistribution of bacterial communities across the soil profile caused by the mixing of soil horizons (Eilers et al., 2012; Fierer et al., 2003). It's interesting to note that the similarity between the two main clusters increased from 2010 (79 %) to 2014 (86 %), thus suggesting a slow but constant increase of similarity between soil bacterial communities of the two vineyards.

The diversity indices displayed temporal variability, with unstable differences between the new and the old vineyard (Fig. 5). The latter had similar (2010 and 2014) or higher (2012 and 2013) Shannon index than the former. The Simpson index showed not significant differences for almost the whole experimental period, except for 2013, when it averaged a higher value in the new vineyard; however, it decreased with time in both vineyards. The number of bands significantly differed between the old and the new vineyard (except in the year 2010), confirming a different structure of bacterial communities; moreover, in contrast to the Simpson index, it increased with time (Fig. 5). Although poorly significant, the results of microbial respiration after 24 h (Fig. 6) in the new vineyard showed lower values as compared to the old one (about 46.6 %), except for 2013.

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3.4 Soil mesobiological quality

As concerns microarthropod communities, more than three thousand organisms were extracted from soil samples over the entire experimental period. On the whole, arthropod abundance was relatively low in both vineyards, however it averaged higher values in the old vineyard (though differences were statistically significant only in 2011 and 2013), following an increasing trend with time until the end of the trial (Fig. 7a).

During the first three years, the relative distribution of the main mesofauna groups (mites, springtails and "other arthropods") was characterized by a large dominance of mites (over 50 %), with a higher frequency in the old vineyard (Fig. 7b). In contrast, in the last year, the frequency of collembola was remarkably higher compared to that of the other groups and the relative frequency of mites was higher in the new than in the old vineyard. The "other arthropods" always represented a very small component of mesofauna community.

According to the criteria proposed by D'Avino (2002), soil quality as evaluated by the QBS-ar index was always higher in the old vineyard (Mann-Whitney test: $U = 58$; $P = 0.008$) (Fig. 7c). The highest values of soil QBS-ar were measured in 2014, in the old vineyard (old vineyard: QBS-ar = 204, *n* taxa = 18; new vineyard: QBS-ar = 171, *n* taxa = 12). During the first three years, the QBS-ar values in the new vineyard were typical of low-quality soils (class II–III, *n* taxa = 2–5); in the same period, higher QBS-ar values were registered in the old vineyard (class IV–VI, *n* taxa = 6–12). The considerable increase of QBS-ar index registered in the last experimental year in both vineyards (class VI in the new vineyard; class VII in the old vineyard) was mainly due to the presence of euedaphic forms (Protura, Symphyla, Diplura, Pauropoda, Coleoptera).

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4 Discussion

4.1 Soil physical and chemical properties

Earthwork operations carried out before planting the new vineyard caused the upsetting of the soil layers and a surface enrichment of the silt-sized mineral particles originating from the mechanical grinding of the sedimentary marly rock of substratum. The overturning action of tillage caused a relatively higher $CaCO_3$ level in the surface layer, which combined with a lowered soil buffering capacity due to the organic matter depletion, may account for the tendency of soil pH in the new vineyard to increase with time.

The results indicate that in the new vineyard soil chemical conditions are still evolving and different from those of the old vineyard. It is difficult to foresee the time required to have similar soil $CaCO_3$ values in the two vineyards, and even whether it will be ever possible, since lime dynamics may vary greatly, depending on factors controlling the dissolution/precipitation reactions and physical redistribution within the soil profile, such as, in particular, climate (temperature, precipitations), water and dissolved CO_2 availability, soil surface and subsurface hydrology, organic matter content, biological activity and soil management (Lamb, 1990; Egli and Fitze, 2001). On the other hand, also the old vineyard looks far from being in a steady state. Actually, it is interesting to note that both vineyards experienced a decrease of $CaCO_3$ content over time. This can be, at least in part, attributed to modifications in soil carbonate equilibrium by intensified leaching processes, caused by above-average rainfall occurred during the last three years of the experimental period (Fig. 2).

As to soil OC status, this depends upon the balance between degrading and restorative processes, which are strongly affected by the management system employed. In our case, both vineyards had a poor soil OM level, like most vineyards in the area under the same management (Costantini et al., 2013). The level was lower in the new vineyard, as a result of tillage-based management of the soil surface, which limited the potential for OM accumulation. To this respect, it must be considered that plant

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residues are here the main source of soil OM, and that the whole residue biomass provided by the young vines in the new vineyard is lower, due to the still reduced plant development.

Soil TN followed similar trend as TOC (TN vs. TOC: $R^2 = 0.800^{**}$), averaging lower contents in the new vineyard. The outcomes confirm the crucial role played by OM in soil N bio-availability, especially under farming systems not employing mineral fertilizers. Also in this case, the significance of differences between the two vineyards was affected by a high variability within vineyard.

Soil C / N ratio was quite low across the whole area, tending to be smaller in the new vineyard. Similar C / N values are reported by other authors for tilled vineyards on sloping land, under different soil and climate conditions (Stevanato et al., 2014). Commonly, in the topsoil of arable land, soil C / N ratio ranges from 10 to 12 and is always lower in the subsoil. Conventional tillage-based managements that limit the input of fresh organic residues and enhance mineralization of existing soil OM cause the C / N ratio to progressively decrease with time (Osman, 2013). It is interesting to note that C / N was in absolute rather low also in the old vineyard, despite having it been treated organically and partly left grass-covered for many years.

The three variables considered together (TOC, TN, C / N) seem to suggest that the organic management carried out in the farm produces only a slight improvement in soil biochemical fertility.

A further difference between the two vineyards was marked by the soil soluble salt concentration, with the new vineyard averaging lower EC for the whole duration of the trial, though with poor statistical significance. This was an additional consequence of the mixing action of pre-planting earthworks on soil horizons, given the non-saline nature and relatively lower weathering status of the soil parent material that was incorporated into the topsoil.

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4.2 Soil microbial activity and diversity

The assessment of the structure of soil bacterial communities by DGGE revealed significant differences between the new and the old vineyard. Interestingly, these differences changed with time; the similarity between the two vineyards, in particular, increased by 10.3 % over the considered period (from 78 % in 2010 to 86 % in 2014). However, as observed for all other soil properties, microbial diversity showed a high within-vineyard variability, which in the old vineyard was probably enhanced by the alternated grass-covered/tilled inter-row management. Soil variability was well evidenced by microbial respiration (Fig. 6) and PCA analysis (Fig. 9) for each sampling year, especially after 2010.

Interestingly, at the beginning of the trial (2010) both H′ and Band Number values appeared to be poorly correlated to other soil properties, and in particular TOC and TN, (Fig. 8) likely due to the short time elapsed from the earthwork treatment. After 2010, microbial diversity was higher in the old vineyard and positively related to TOC, microbial respiration, clay content and other biological indicators. The Simpson index values in the new vineyard indicated the dominance of few species. The diversity indices appeared to be related to climate factors, in particular to the seasonal temperature (Fig. 10), soil $CaCO_3$ content was strictly related to low levels of microbial diversity and activity, inducing the selection of few dominant species (higher Simpson values). The better homeostatic conditions of the old vineyard soil explain its higher values in terms of microbial diversity and function as compared to the new vineyard, according to the chemical parameters. This confirms the potential role of microbial diversity as indicator of recovery processes, as also suggested by previous authors (Bezdicek et al., 1996; Seybold et al., 1999). In contrast, microbial respiration, one of the most common and sensitive biological indicators for soil quality, appeared to be more affected by other parameters such as soil organic carbon quantity or temperature.

As soil resilience can be quantified experimentally by measuring the rate of recovery of the original pre-disturbance conditions, we calculated the resilience rate based

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on similarity values. The results indicated a slow but constant increase of similarity between the bacterial communities of the two vineyards, with a recovery rate of about 2.5 % year^{-1} in terms of structural diversity. According to this trend, at least further three years would be needed for the new vineyard to recover a bacterial diversity similar as that of the old vineyard.

4.3 Soil mesobiology and QBS-ar index

Among soil organisms that can be affected by the application of different cultivation techniques and crop managements, Annelida and microarthropods are the organisms most representative of mesofauna. In this study, microarthropod density can be considered as a mirror of the aging of the situation tested. It's likely that the densities registered reflected the management adopted and, consequently, their movements into the micro-scale compartment.

The microarthropod abundance differed considerably between the new and the old vineyard. The new vineyard, after a starting period of very scarce arthropod presence (abundance < 5/soil core), immediately following the pre-planting earthworks, showed only moderate signs of recovery, leading to an abundance relatively stable over time (around 62/soil core).

The old vineyard, instead, since the beginning of the trial revealed a larger arthropod richness than the new vineyard, with abundance values increasing over time (on average, by a 77 % per year). As a result, at the end of the trial, the microarthropod abundance in the old vineyard was 2.8 times higher than in the new vineyard. Taking into account climate variables, the microarthropod abundance in the old vineyard appeared closely related to the annual precipitation and, in particular, to the amount of rainfall occurred during the winter-spring period (from January to April, Pearson $R = 0.980$, Fig. 7a). Our results are in agreement with findings by other Authors, demonstrating a positive correlation between microarthropod density (mites and springtails) and soil moisture content (Hassall et al., 1986; Chikoski et al., 2006).

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It is noteworthy that, despite the same climate influence, this relation was not observed in the new vineyard. This was possibly due to a contrasting effect of tillage-induced soil conditions on the development of microarthropod population; in particular, lower organic matter content, which is a primary source of nutrients for detritivore arthropods, and overall worse soil physical environment, impacted by pre-planting earthworks and annual tillage practices, created a less suitable habitat for arthropod survival.

Mites and springtails vary their abundance in a similar way (Narula et al., 1996). For both arthropods vertical migrations have been found to occur as a response to changes in soil moisture in grassland soils (Hassal et al., 1986). The highest abundance of springtails in the last sampling is typically a response to higher soil moisture. Actually, the reproduction rate of springtails is highly dependent on the optimal habitat, therefore high densities of their populations arise following rainfall (Schaefer, 1995; Badejo et al., 1998). At the same time, springtail fecundity and longevity are optimal with appropriate N and C availability (Johnston, 2000), however, these concentrations wer not much increased in the last year.

Mite abundance seems to be more associated to changes in soil characteristics than springtails, possibly due to narrower micro-habitat requirements and longer cycles of development. Nevertheless, in the last year of the study, springtails were more represented and a significant change occurred in the communities structure.

4.4 Interactions between state factors and soil biology

The outcomes of the PCA clearly evidenced the differences between the old and the new vineyards. As the average variance on PC1 is around 43.6 % (about double of the PC2) for the observed period, most of the differences between the two vineyards are related to PC1 (Fig. 8). PC1 can be interpreted as the factor that contrasts the components of soil biology from the physical and chemical soil properties. Apart from the Simpson index and Band Number, which vary between years, all the other variables related to soil biology, biodiversity and biological quality, namely TOC, total N, C / N

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(except for 2011), Taxa, QBS, Class, H′ (apart from 2014), and respiration show a significant communality over the years and are associated with PC1.

It is worthy to observe that also clay content and electrical conductivity are associate with PC1. In the case of clay, the direct correlation between clay and organisms has been found also by other authors (England et al., 1993; Sorensen, 1983), while EC, although rather low in both vineyard soils, points to a relatively more advanced weathering of the parent material in the soil of the older vineyard. Figure 9 shows that all these variables are well represented in the cases belonging to the old vineyard. On the other hand, total and active lime content, as well as sand and silt content, and pH, show a significant and stable communality over the years that contrast with the former variables. The cases plot shows that these variables are mostly related to the new vineyard (Fig. 9).

It is to emphasize that PCA showed consistent results concerning biological variables, which appeared to be strongly related to each other. In particular microbial diversity (H′, Band Number) were always positively related to QBS, nitrogen availability and clay content, whereas they were negatively related to $CaCO_3$ and sand content (Fig. 8). Interestingly, biological diversity appeared to be poorly affected by climatic parameters, such as rainfall (which was then excluded from the PC, Fig. 10). In contrast, both microbial and arthropod diversity were positively related to temperature, but microbial respiration did not. This could be due to the fact that microbial mineralization is more stably related to C and N availability, rather than to climatic factors.

As previously observed, PC2 plays a minor role in the model, however, it tends to differentiate biochemical variables (TOC, Total N, Respiration, together with clay and EC) from those which are related to biodiversity and biological quality (QBS, Class, H′, Taxa, Bands). This would indicate the presence of two different processes: the first one driven by TOC accumulation, which increments biological fertility, and the other one characterised by the increase of biodiversity and biological organization, consequence of the progressive adjusting of micro and mesobiology to the new soil conditions.

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In 2010, the initial internal heterogeneity of the new vineyard was quite higher compared to the old one, but since 2011 an increase of internal variability within the old vineyard samples occurred over time. Ultimately, the plot of cases on the principal components (Fig. 9) reveals that after five year from the earthworks and three years from vine plantation the two vineyards are still well separated and there is not any apparent resilience over time.

5 Conclusions

At the best of our knowledge, this work is the first attempt to set up an integrated monitoring activity of the development of soil physical, chemical, micro and meso-biological soil functions in a new vineyard, planted after earthworks which deeply influenced soil features and, in particular, biological fertility. The comparison with a neighbouring old vineyard, planted on the same soil type, evidenced that after four years from planting most soil properties are still significantly different, and only biodiversity tends to converge. It is expected that biodiversity in the two soil will be similar in about three years, that is, after eight years from the earthworks and six years from vine plantation. For the other soil functions it is difficult to foresee the resilience time, also because the soil under the relatively older vineyard has not reached yet, after 14 years from vine plantation, a steady state for many chemical properties.

The partial permanent grass cover of the old vineyard did not result to improve significantly soil biology, and also the organic farming cultivation system did not speed up markedly the recovery process, probably because of the limited amount of the distributed compost. It seems to be plausible, instead, that the different soil organic matter content and biology between the new and old vineyard are mainly related to vine development and slow accumulation of plant residues.

In conclusion, the whole results of this work showed that in these specific conditions, which are however representative of many premium viticultural farms, soils with very poor biological fertility, like the one which result from the earthworks made before vine

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plantation, need a rather long time to restore soil functions, probably longer than that needed to obtain an economic grape production.

The perspectives of the research work foresee to continue the annual soil sampling and multidisciplinary analysis and, at the same time, to start monitoring vine and grass biomass, at least until grape yield of the new and old vineyard will be similar.

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Table 1. Soil properties of the selected sampling sites in the first sudy year (2010).

Vineyard	Clay (%)	Sand (%)	USDA texture class	Field Capacity (% w/w)	Wilting Point (% w/w)	TOC (%)	TN (%)	C / N (%)	Total $CaCO_3$ (%)	Active $CaCO_3$ (%)	pH	EC (µS)
P1 new	20.8	32.5	Loam	24.3	10.3	0.45	0.08	5.9	34.7	8.0	8.2	206.9
P2 new	18.9	33.1	Loam	22.9	9.8	0.43	0.08	5.6	37.6	8.8	8.3	166.0
P3 new	18.1	34.4	Loam	22.2	9.5	0.39	0.07	5.7	39.5	9.0	8.2	167.0
P4 new	20.7	35.1	Loam	22.3	9.6	0.47	0.06	7.6	40.9	7.3	8.2	171.8
P5 old-G	25.1	31.7	Loam	24.8	12.3	0.68	0.10	6.8	27.8	6.1	8.2	211.3
P6 old-T	28.6	31.4	Clay Loam	25.4	12.9	0.81	0.11	7.6	27.4	5.0	8.2	245.9
P7 old-G	26.4	31.9	Loam	24.7	13.3	0.65	0.10	6.8	21.7	4.3	8.2	186.0
P8 old-T	25.6	32.5	Loam	22.2	11.4	0.46	0.08	5.6	36.3	6.1	8.2	273.5

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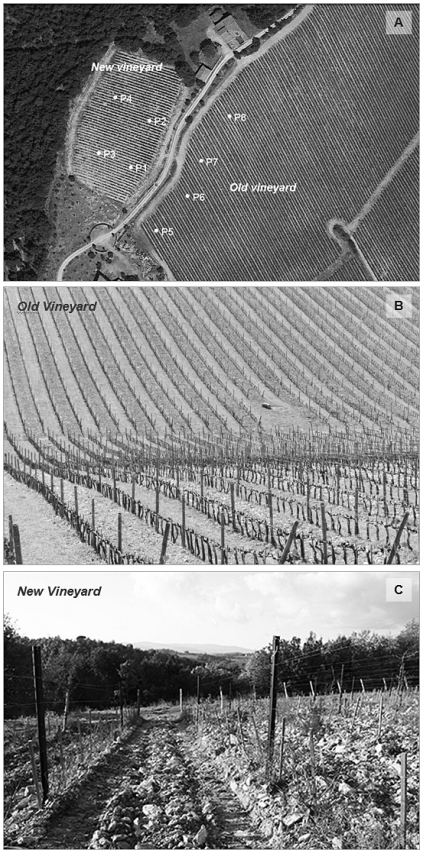

Figure 1. The new and the old vineyards with the respective monitoring sites.

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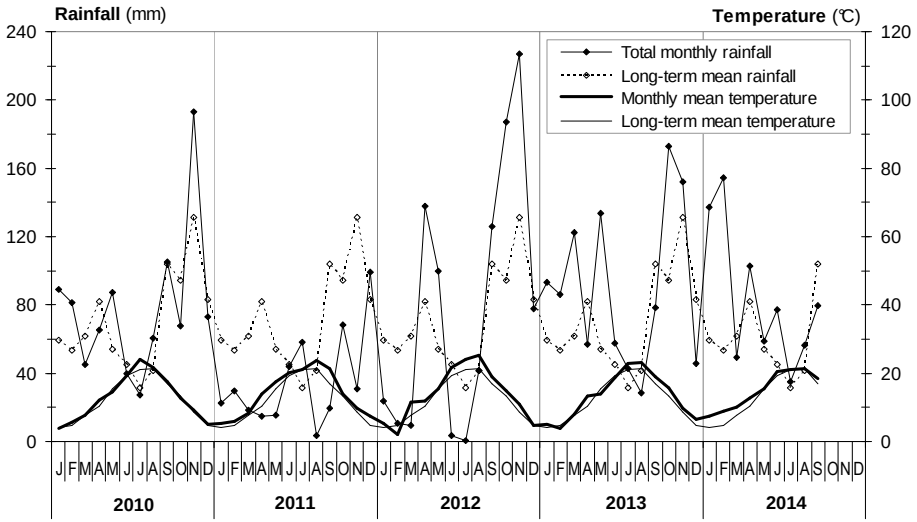

Figure 2. Trends of rainfall and temperature during the experimental period with the respective long-term average trends (1990–2010).

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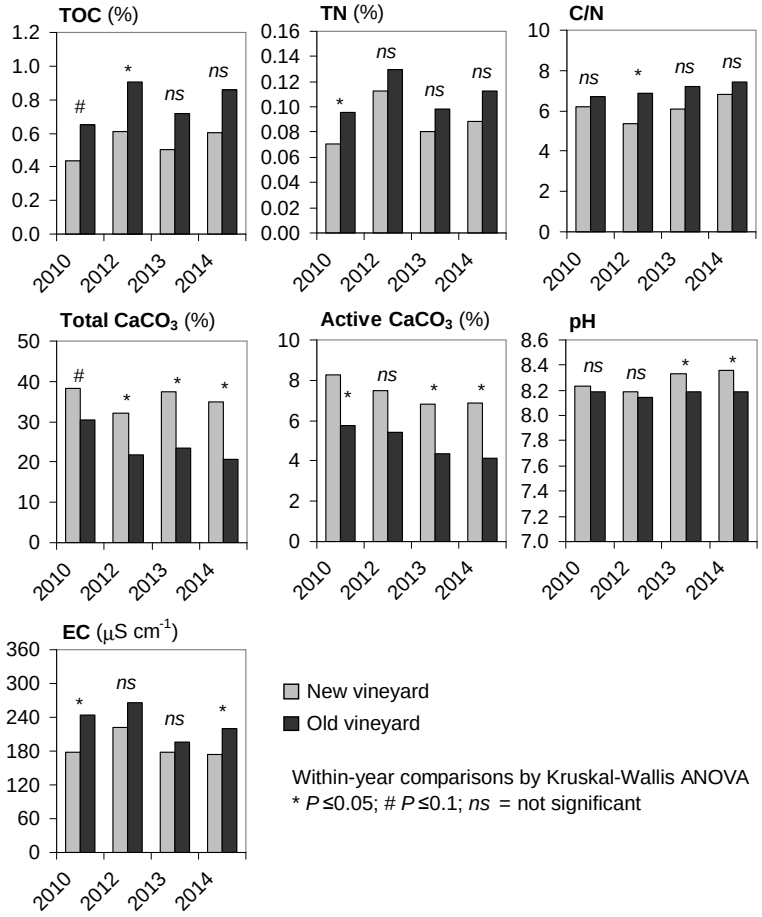

Figure 3. Soil chemical properties in the new and the old vineyard during the experimental period.

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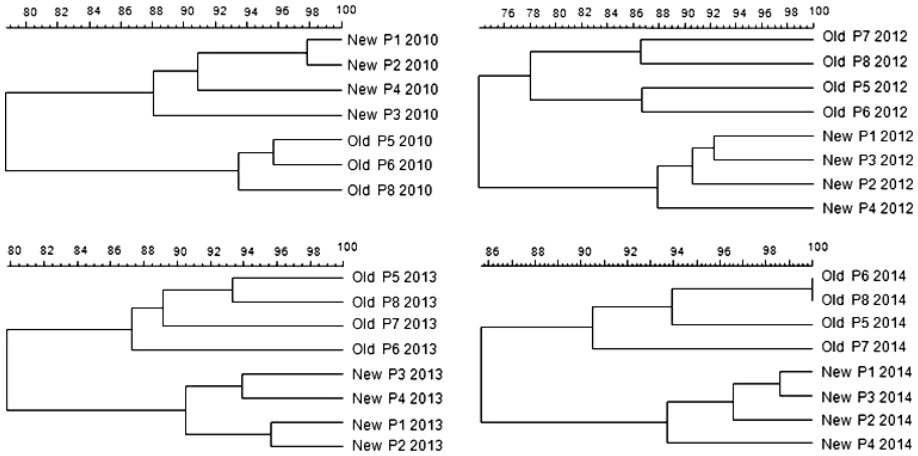

Figure 4. Dendrograms of hierarchical cluster analysis based on UPGMA and Dice's coefficient of DGGE banding patterns of the 16S rDNA generated by Gel Compare II software.

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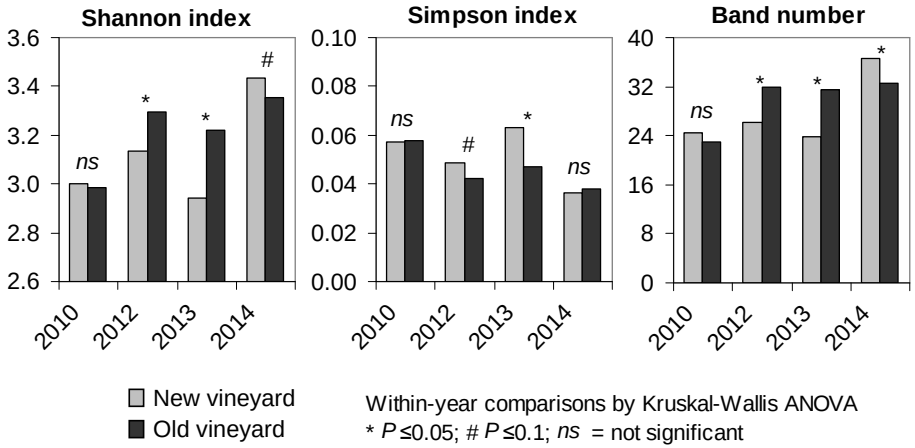

Figure 5. Diversity indices and band number of the DGGE banding patterns generated by Gel Compare II software.

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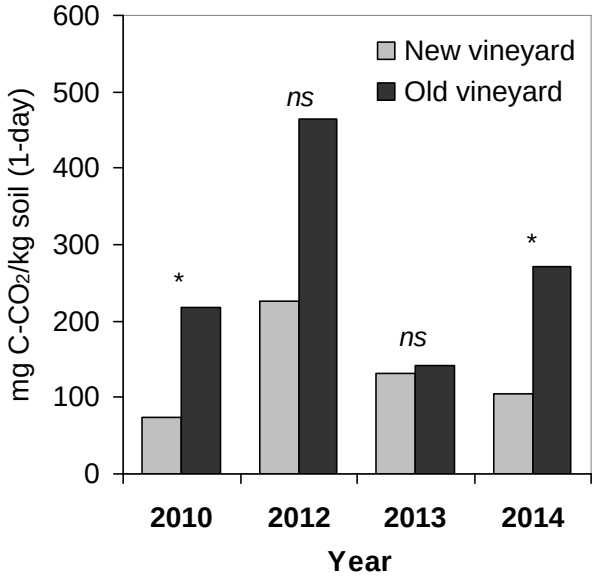

Within-year comparisons by Kruskal-Wallis ANOVA
* $P \leq 0.05$; *ns* = not significant

Figure 6. Microbial respiration in the two vineyards during the experimental period.

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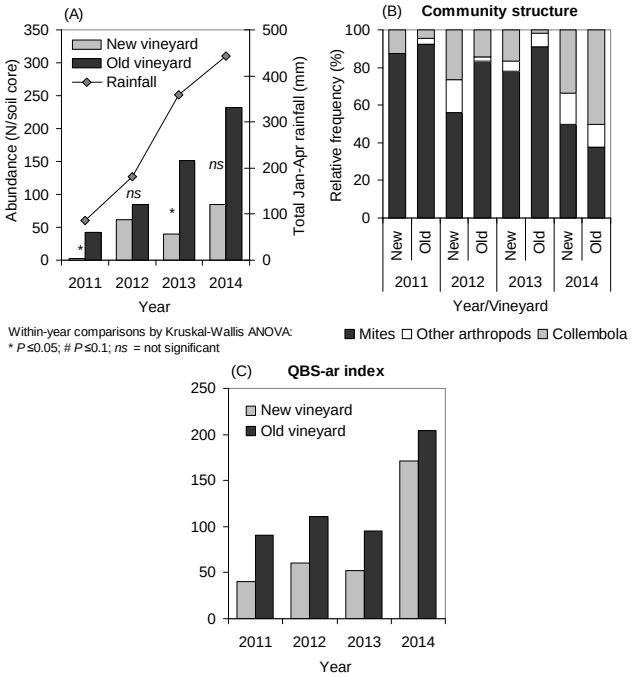

Figure 7. Soil microarthropod community, biological quality index (QBS-ar) and cumulated rainfall in the months before sampling (January to April).

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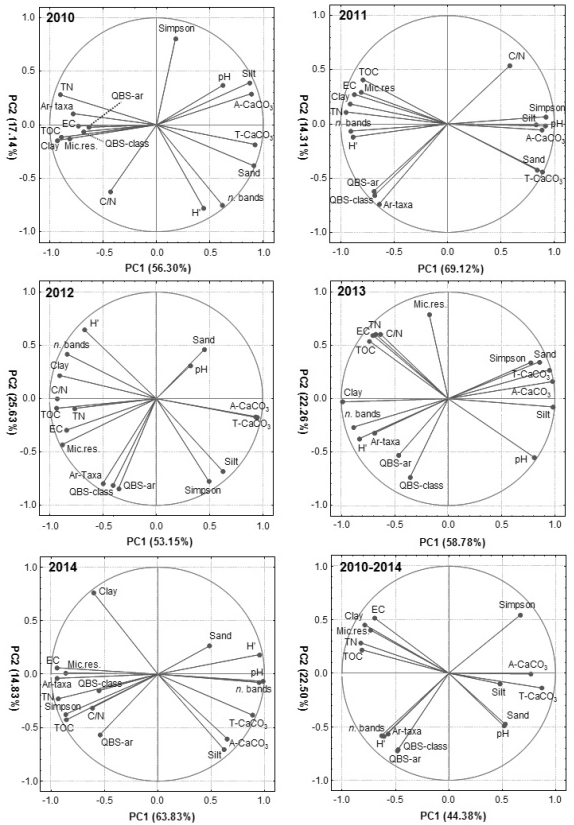

TOC = soil total OC; TN = soil total N; C/N = soil TOC to TN ratio; T-CaCO3 = soil total CaCO3; A-CaCO3 = soil active CaCO3; EC = soil electrical conductivity; Mic.res. = microbial respiration; H' = Shannon index; Simpson = Simpson index; *n.* bands = number of DGGE bands; Ar-taxa = number of soil microarthropod taxa; QBS-ar = soil biological quality index; QBS-class = soil biological quality class.

Figure 8. PCA biplots for each year and for the whole experimental period (not including climate parameters).

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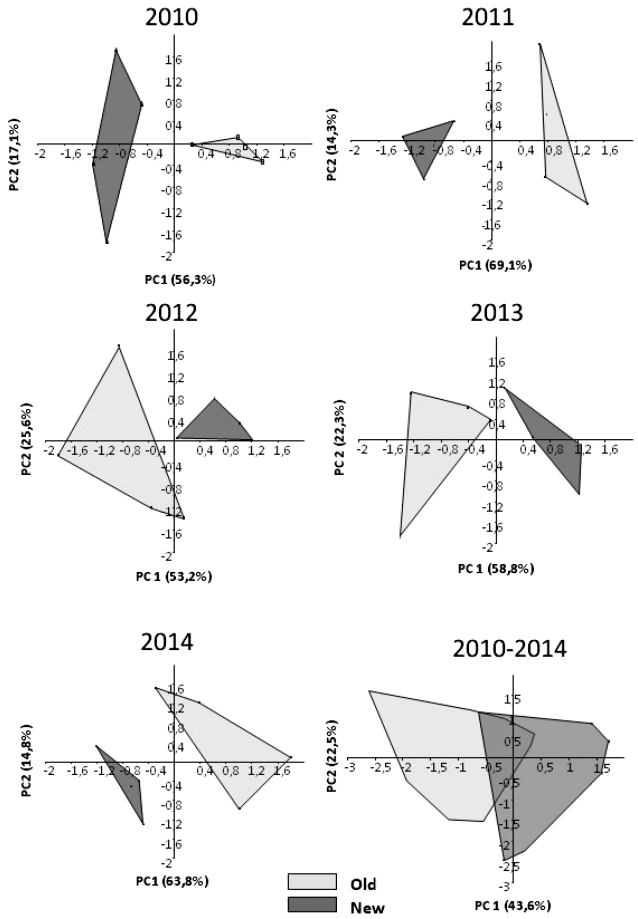

Figure 9. PCA plots of cases, for each year and for the whole experimental period (not including climatic parameters).

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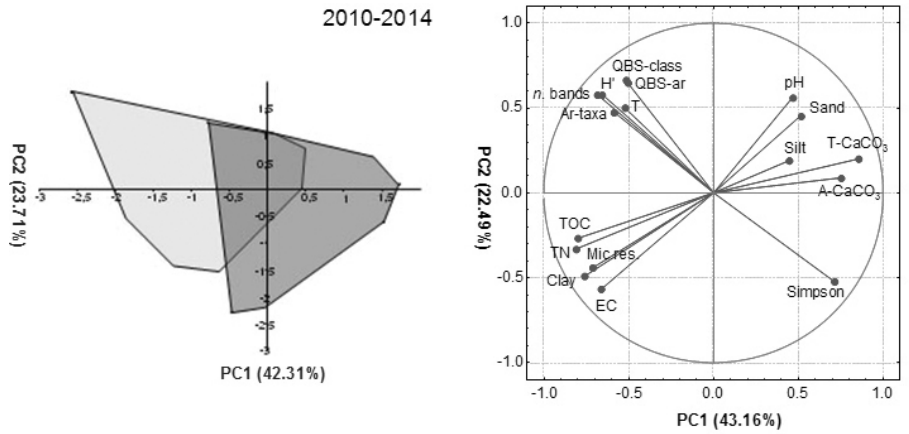

TOC = soil total OC; TN = soil total N; C/N = soil TOC to TN ratio; T-CaCO3 = soil total CaCO3; A-CaCO3 = soil active CaCO3; EC = soil electrical conductivity; Mic.res. = microbial respiration; H' = Shannon index; Simpson = Simpson index; *n.* bands = number of DGGE bands; Ar-taxa = number of soil microarthropod taxa; QBS-ar = soil biological quality index; QBS-class = soil biological quality class; T = temperature.

Figure 10. PCA plots for the whole 2010–2014 period (including climate parameters).

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