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 a 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 with compost only every Autumn (1,000 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 fixed locations in each vineyard, every Spring from 2010 to 2014. The old vineyard was sampled in both tilled and grass-covered inter-rows.

According to the results from physical and chemical analyses, the new vineyard, during the whole 2010-2014 period, showed lower total organic carbon, total nitrogen, carbon to nitrogen ratio and electrical conductivity, 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. Rainfall appeared to have enhancing effect on microarthropod abundance, but only in the old vineyard, where the biota was more structured than

in new one. The euedaphic forms, well adapted to soil life, were always rare. Microbiological analysis revealed a different structure of eubacterial communities between the old and the new vineyard in the whole period. However, the DGGE similarity values of these communities increased by 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 the time needed to obtain an economic grape production.

Key words: vine, biology, resilience, terroir, Italy

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 ensures 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 the 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 underlying 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, where only a marginal proportion of the new vineyards is planted on non-agricultural lands. In this context, whenever a new vineyard is established on the same place of the old one, the time needed to reach a new equilibrium should be assessed with reference to the previous conditions.

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 to soil biological activity. Nevertheless, organic viticulture may have limitations in the recovery of some soil functions, in particular, nitrogen nutrition of vines in very poor soils, like those interested by bulldozing and scalping (Costantini et a., 2013).

Monitoring the degree of soil degradation and resilience over time requires the use of suitable soil quality indicators. These are commonly based on a variety of soil chemical, physical and biological properties that have a direct link to soil ecosystem functions and are highly responsive to soil perturbation, such as soil organic matter, aggregate stability, microbial respiration, biological activity and diversity.

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).

More recently, new bio-indicators involving the characterization of soil arthropod communities have been proposed for soil quality assessment. Microarthropods, in particular, are a major component of soil biota and are known to be important contributors to soil formation, organic matter transformation, nutrient cycling, C accumulation and plant and microbial diversity. Furthermore, they respond significantly to changes in land management, thus gaining increasing interest as effective indicators of soil quality (Brussaard et al., 1997; Culliney, 2013; Parisi, 2001; Parisi et al., 2005).

The abundance and diversity of soil fauna integrate soil physical, chemical and microbiological properties and reflect general ecological changes, becoming an important asset in the landscape ecology and conservation tool box (Menta et al., 2008; Yan et al., 2012; Wardle, 2002). The spatial distribution of soil microarthropods and their functional groups' abundance are influenced by human induced disturbance related to farming activities, such as soil cultivation (Paoletti and Bressan, 1995).

Our research was based on the monitoring of soil quality 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 disturbance by pre-planting earthworks. In this paper, the results from the first five years of study are presented.

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 top of a small hill, with gentle slope (near 5 %), at about 400 m a.s.l. altitude. The area is 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). Based on the long-term average data (1990–2010 period), mean annual temperature is 12.3 °C and precipitation 800 mm, mostly concentrated in Autumn and Spring. The potential evapotranspiration (ET_0) from April to September is 850 mm (Hargreaves and Samani, 1982), and the Winkler index is 1.856 degree days. Climate data were collected from a weather station located close to the site.

The experimental area (figures 1.A, B, C) extends to approximately 40 ha and consists of two zones: one with South-West facing aspect, 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 one, with South-West aspect, covered by a new vineyard established in 2011 after equivalent earthworks, carried out in the summer of 2009.

According to the ordinary management, the vineyards are periodically uprooted and re-planted, with a rest period between one vineyard and the following one. In the present case, before the new vineyard establishment the soil had been covered by an older vineyard until 1990, followed by a set aside period up to 2009. During this period, the soil was kept abandoned, allowing the development of shrubs, weeds and wild vine plant vegetation.

Over the whole duration of the experiment, the new vineyard was entirely cultivated by periodic tillage, according to the farm strategy to maintain the soil surface free from weeds until the start of a commercial level of grape production.

The old vineyard was managed with alternating tilled (T) and grass-covered (G) inter-rows; the latter were kept under natural weed development, which was periodically mowed (two or three times per year), shredded together with plant residues and spread on the soil surface. Once a year, the grass-covered soil was scarified to 40-50 cm depth without soil inversion, to allow soil aeration and avoid soil compaction.

154 The vine disease control was based on copper treatments. This aspect was not studied, anyway,
155 no particular fungal or pest disease was recorded during the study period. Overall, in the new
156 vineyard there has been comparatively less machine traffic, because of a lower need for plant
157 management and protection treatments, due to the lower plant development and poor grape yields.
158 Despite that, possible traffic-related differences between the two vineyards are supposed to be
159 negligible, since soil mechanical stress in the old vineyard is reduced by the grass cover (this is one
160 of the main benefits at which the grass covering is aimed).

161 Both vineyards were managed organically, applying with 1.0 Mg ha^{-1} compost per year in
162 Autumn. The compost was a commercial pelletized product obtained by dry-composting of
163 livestock manure, with the following properties: total N = 3.6 %, organic N = 2,8 %, total OC =
164 33.4 %, C/N = 9.3, humic + fulvic acids = 15.2 %, total P (P_2O_5) = 3.3 %, total K = 0.28 % (s.s).

165 Four soil profiles per vineyard were dug close to the experimental plots, to describe, analyse,
166 and classify soil types. In the old vineyard, two of them were dug in the grass-covered inter-rows
167 and the other two in the tilled inter-rows. Not any soil profile study was performed at a detailed
168 scale prior to 2009 earthworks; however, an antecedent soil survey of the entire farm indicated that
169 the soil type across the selected vineyards was uniform. Table 1 shows the main features of the
170 representative soil type of the experimental area, under ordinary viticultural management and grape
171 production.

172 The monitoring of soil chemical, physical, and biological properties over time was carried out
173 by means of representative samples, collected annually from each vineyard in four selected 10 m^2
174 georeferenced plots (referred to as P1–P4 in the new vineyard and P5–P8 in the old vineyard (figure
175 1.A). Each plot was sampled during Spring in four separate points, using different sampling
176 procedures depending on the specific analyses to be performed (details are provided in the
177 following paragraphs). The sampling locations were the same for the whole duration of the
178 experimentation. The old vineyard was sampled in both grass-covered (P5 and P7 plots) and tilled
179 inter-rows (P6 and P8 plots). In this regards it must be pointed out that, during the study period, no

significant differences for selected soil properties were observed between the two inter-row managements ($P < 0.05$). This was determined by the fact that extensive weed development promoted by the Autumn–Spring rainfall often occurred also in cultivated spaces, and that soil sampling was always performed before the first grass mowing. For this reason, the grass-cover and tillage data were pooled together for all statistical evaluations.

Experimental data were not available for soil microarthropods in 2010 (both vineyards) and for soil properties in 2011 (old vineyard); therefore, for the mentioned years, not all selected variables could be considered..

Neither vine phenology nor production were recorded during the five years, since in the old vineyard, owing to the plant youth and delayed growth caused by poor soil conditions, no significant grape production was obtained until the end of the experimental period, except for a few small clusters in 2013 and 2014, which however were not suitable for harvest or grape yield monitoring.

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. 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

206 calculated from the difference between the total C measured before and after the HCl treatment
207 (Sequi and De Nobili, 2000).

208 Active lime was determined according to the Drouineau method; the procedure involved
209 reaction of the soil with 0.1 M ammonium oxalate for 2 h under agitation, followed by the
210 determination of unreacted oxalate by back-titration with 0.1 M $KMnO_4$ (Loeppert and Suarez,
211 1996). Soil pH was measured potentiometrically in a 1:2.5 soil-water suspension. Electrical
212 conductivity was measured in a 1:2 soil-water extract, after 2 hour shaking, overnight standing and
213 filtration. The main soil properties at the beginning of the study are reported in table 2.

214

215 **2.3 Soil microbiological analysis**

216 Soil microbiological communities were characterized using subsamples of the same soil
217 samples collected for soil physical and chemical analyses.

218 Estimation of soil organic OC mineralisation was performed by measuring the C-CO_2 developed
219 [mg (C-CO_2) kg soil^{-1} day^{-1}] from the soil in closed jars (Isermeyer 1952). A 25 g amount of oven-
220 dried soil was rewetted to a -33 kPa water tension and incubated at 30°C. The CO_2 evolution after
221 one day (representing the soil easily mineralisable C) was determined by back titration of the
222 NaOH-absorbed CO_2.

223 The structure of microbial communities was determined by means of denaturing gradient gel
224 electrophoresis (DGGE), a PCR-based molecular technique which has been widely used in
225 microbial ecology for the rapid evaluation of soil microbial community structure of multiple soil
226 samples (Muyzer and Smalla, 1998; Nannipieri et al., 2003). Soil DNA was extracted by the bead-
227 beating method using FastDNA SPIN Kit and the FastPrep instrument (Bio 101, USA). The
228 eubacterial community structure was determined by amplifying the 16S rRNA genes, using the
229 primer set GC-968f (5′-CGC CCG GGG CGC GCC CCG GGC GGG GCG GGG GCA CGG GGG
230 GAA CGC GAA GAA CCT TA-3′) and 1401r (5′-GCG TGT GTA CAA GAC CC-3′) designed by
231 Felske and Akkermans (1998). Soil template DNA was amplified with a mix containing 1U 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 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 DGGE patterns and band intensity were used to calculate the Shannon-Wiener index (H') and the Simpson index (D), which, along with the number of DGGE bands, were used to characterize soil microbial diversity:

$$H' = -\sum_{i=1}^{S} p_i \ln p_i;$$

$$D = -\sum_{i=1}^{S} p_i^2$$

where S is the total number of bands and p_i is the relative abundance of the i band calculated as the ratio between i band intensity and the sum of the intensities of all the bands.

Calculations were performed using the 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 has 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).

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 at 0-10 cm depth from 4 replicated zones within each vineyard. For the extraction of microarthropods, the soil samples were placed in Berlese-Tullgren funnels for 5 days. The soil was allowed to dry from the top down, by means of a heating light; the microarthropods moving through

282 the soil were collected into a preservative solution (80 % ethanol) and afterwards identified to the
283 order level using a stereo microscope.
284
285 **2.5 Statistical analysis**
286 Differences in soil properties between the new and the old vineyards were tested statistically by
287 the non-parametric Kruskal-Wallis test, to avoid inaccuracies due to variance heterogeneity and
288 non-normality patterns in data distribution (Statsoft STATISTICA v. 7; SPSS v. 15.0). Soil QBS-ar
289 data were analyzed using the Mann-Whitney rank test (SPSS v. 15.0; $P = 0.05$).
290 A principal component analysis (PCA) was performed for each experimental year, in order to
291 explore similarities and differences between the two vineyards and to understand the pattern of
292 interrelationships among selected soil parameters over time. A separate PCA was done for the
293 whole 2010-2014 dataset, with and without the inclusion of climate variables. The results are
294 displayed graphically as score and loading plots. As previously mentioned in the paragraph 2.1,
295 most of soil chemical and microbiological data were not available in 2011 for the old vineyard;
296 therefore, in order to perform the PCA, the old vineyard dataset was completed by replacing the
297 missing value of each variable with the average of that variable across all other trial years in the
298 same experimental condition. This procedure is justified by the fact that PCA was mainly aimed at
299 interpreting the phenomenon under study through new latent components resulting from the
300 correlation among variables, and not to classify the values itself of the variables (ISTAT, 2000).
301
302 **3. Results**
303 **3.1 Climatic conditions during the trial**
304 The trends of rainfall and temperature recorded during the monitoring period are shown in
305 Figure 2 with the respective long-term average trends.
306 In 2010, the temperature and rainfall values were close to the long-term means. Starting from
307 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 from Winter to 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 (Figure 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 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 (Figure 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 (figure 5). The latter had similar (2010 and 2014) or higher (2012 and 2013) Shannon index than the former. The Simpson index showed no significant differences at the beginning and at the end of the experimental period, while during 2012 and 2013 it averaged higher values in the new vineyard (statistical significance levels $P = 0.1$ and $P = 0.05$, respectively). Furthermore, 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 (Figure 5).

Microbial respiration (Figure 6) was significantly higher in the old vineyard in 2010 and 2014 (by 61 % and 66 %, respectively). A large difference also occurred in 2012 (51 %), which was, however, not statistically significant due to a high within-vineyard variability. In 2013, the two vineyards had comparable respiration values.

3.4 Soil mesobiological quality

As concerns microarthropod communities, more than three thousand organisms were extracted from the soil samples over the entire experimental period (Table 3). On the whole, arthropod abundance was relatively low in both vineyards, however it averaged higher values in the old vineyard (the difference was not statistically significant only in 2012), following an increasing trend with time until the end of the trial (Figure 7.A).

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 (figure 7.B). 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) (figure 7.C). 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). In all samplings, collembolans always included eudaphic forms (e.g. Onychiuridae; EMI=20). 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).

4. Discussion

4.1 Soil physical and chemical properties

Earthwork operations carried out before planting in the new vineyard caused the upsetting of the soil layers and a surface enrichment of the silt-sized mineral particles originating from the

385 mechanical grinding of the sedimentary marly rock of substratum. The overturning action of tillage
386 caused a relatively higher $CaCO_3$ level in the surface layer, which combined with a lowered soil
387 buffering capacity due to the organic matter depletion, may account for the tendency, even if slight,
388 of soil pH in the new vineyard to increase with time.

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

401 As to soil OC status, this depends upon the balance between degrading and restorative
402 processes, which are strongly affected by the management system employed. In our case, both
403 vineyards had a poor soil OM level, like most vineyards in the area under the same management
404 (Costantini et al., 2013). The level was lower in the new vineyard, as a result of tillage-based
405 management of the soil surface, which limited the potential for OM accumulation. To this respect, it
406 must be considered that plant residues are here the main source of soil OM, and that the whole
407 residue biomass provided by the young vines in the new vineyard is lower, due to the reduced plant
408 development.

409 Soil TN followed similar trend as TOC (TN vs TOC: $R^2 = 0.800^{**}$), averaging lower contents
410 in the new vineyard. The outcomes confirm the crucial role played by OM in soil N bio-availability,

411 especially under farming systems not employing mineral fertilizers. Also in this case, the
412 significance of differences between the two vineyards was affected by a high variability within
413 vineyard.

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

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

425 A further difference between the two vineyards was marked by the soil soluble salt
426 concentration, which in the new vineyard averaged lower levels for the whole duration of the trial,
427 though with not statistically significant differences in 2012 and 2013 ($P > 0.1$). This was an
428 additional consequence of the mixing action of pre-planting earthworks on soil horizons, given the
429 non-saline nature and relatively lower weathering status of the soil parent material that was
430 incorporated into the topsoil.

431

432 **4.2 Soil microbial activity and diversity**

433 The assessment of the structure of soil bacterial communities by DGGE revealed significant
434 differences between the new and the old vineyard. Interestingly, these differences changed with
435 time; the similarity between the two vineyards, in particular, increased by 10.3 % over the
436 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 alternate grass-covered/tilled inter-row management. Soil variability was well evidenced by microbial respiration (figure 6) and PCA analysis (Fig. 9) for each sampling year, especially after 2010.

At the beginning of the trial (2010), both H' and DGGE band number were poorly correlated to other soil properties, and, in particular, TOC and TN (Figure 8), likely due to the short time elapsed from the earthwork treatment. From 2010 to 2013, microbial diversity was higher in the old vineyard and positively related to TOC, clay content, microbial respiration and other biological indicators. The diversity indices H' and *n*. bands appeared, moreover, related to the seasonal temperature (Figure 10), while the close relation between soil $CaCO_3$ and the Simpson index indicates a lower microbial diversity in the presence of higher $CaCO_3$ amounts.

The better homeostatic conditions of the old vineyard soil explain its higher values in terms of microbial diversity and functions 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 of soil quality, appeared to be 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 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

463 Among soil organisms that can be affected by the application of different cultivation techniques
464 and crop managements, Annelida and microarthropods are the organisms most representative of
465 mesofauna. In this study, microarthropod density can be considered as a mirror of the aging of the
466 situation tested. It's likely that the densities registered reflected the management adopted and,
467 consequently, their movements into the micro-scale compartment.
468 The microarthropod abundance differed considerably between the new and the old vineyard.
469 The new vineyard, after a starting period of very scarce arthropod presence (abundance < 5/soil
470 core) following the pre-planting earthworks, showed only moderate signs of recovery, with a
471 relatively stable abundance over time (around 62/soil core).
472 The old vineyard, instead, since the beginning of the trial revealed a larger arthropod richness
473 than the new vineyard, with abundance values increasing over time (on average, by a 77 % per
474 year). As a result, at the end of the trial, the microarthropod abundance in the old vineyard was 2.8
475 times higher than in the new vineyard. Taking into account climate variables, the microarthropod
476 abundance in the old vineyard appeared closely related to the annual precipitation and, in particular,
477 to the amount of rainfall occurred during the Winter–Spring period (from January to April;
478 Spearman $\rho = 1.000$, $P = 0.01$). Our results are in agreement with findings by other authors,
479 demonstrating a positive correlation between microarthropod density (mites and springtails) and
480 soil moisture content (Hassall et al., 1986; Chikoski et al., 2006).
481 It is noteworthy that, despite the same climate influence, this relation was not observed in the
482 new vineyard. This was possibly due to a contrasting effect of tillage-induced soil conditions on the
483 development of microarthropod population. In particular, a lower organic matter content, which is a
484 primary source of nutrients for detritivore arthropods, and overall worse soil physical environment,
485 impacted by pre-planting earthworks and annual tillage practices, created a less suitable habitat for
486 arthropod survival (Kautz et al., 2006; Parisi et al., 2005).
487 Mites and springtails vary their abundance in a similar way (Narula et al., 1996). For both
488 arthropods vertical migrations have been observed in response to changes in soil moisture in

489 grassland soils (Hassal et al., 1986). However, their abundance may follow different patterns over
490 time, depending on the lifecycle length and reproductive strategy, as well as on their individual
491 tolerance to temperature and moisture in the soil.

492 It is known that the rate of increase of springtail population is highly dependent on optimal
493 habitat with adequate N and C supply (Johnston, 2000) and is enhanced by rainfall (Schaefer, 1995;
494 Badejo et al., 1998). In the present study, there was no significant evidence of a relationship
495 between the total microarthropod dynamics and soil OC and N changes over time. In the last year,
496 the rise in the springtail population was presumably due to the high rainfall and was particularly
497 emphasized in the old vineyard, as a result of a larger availability at the soil surface of
498 microenvironments colonized by emi- and epiedaphic forms**.**

499

500 **4.4 Interactions between state factors and soil biology**

501 The outcomes of the PCA showed a clear separation between the old and the new vineyard
502 along the PC1 (Figure 8), which explained 53 % to 69 % of variance over the years (43.6 % for the
503 overall 2010–2014 period). The results, moreover, indicated a contrasting contribution of soil
504 biological properties (negative loadings) and most of soil physical-chemical properties (positive
505 loadings) (Figure 9). PC1 can be interpreted as the factor that contrasts the components of soil
506 biology from the physical and chemical soil properties. Apart from the Simpson index and band
507 number, which varied among years, all the other variables related to soil biology, biodiversity and
508 biological quality, namely TOC, total N, C/N (except for 2011), *n*. microarthropod taxa, QBS-ar,
509 QBS-ar class, H' (except for 2014) and microbial respiration showed a significant communality
510 over the years and were associated with PC1.

511 It is worthy to observe that also clay content and electrical conductivity were associated with
512 PC1. The direct correlation between clay and organisms has been found also by other authors
513 (England et al., 1993; Sorensen, 1983), while EC, although rather low in both vineyard soils, points
514 to a relatively more advanced weathering of the parent material in the soil of the older vineyard.

Figure 9 shows that all these variables were well represented in the cases belonging to the old vineyard. On the other hand, total and active lime, as well as sand, silt and pH, showed a significant and stable communality over the years that contrasted with the former variables. The case plot shows that these variables were mostly related to the new vineyard (figure 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-ar, nitrogen availability and clay content, whereas they were negatively related to $CaCO_3$ and sand contents (figure 8). In regards to climate effect, biological diversity was positively related to the temperature, but was not related to the rainfall (which was then excluded from the PCA; Figure10). Differently, microbial respiration appeared to be more affected by TOC and TN contents rather than by climatic factors.

As previously observed, PC2 played a minor role in the model, however, it tended to differentiate physicochemical and biochemical variables (TOC, total N, respiration, together with clay and EC) from those which are related to biodiversity and biological quality (QBS-ar, QBS-ar class, H', *n.* microarthropod taxa, *n.* DGGE 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, as a consequence of the progressive adjusting of micro- and mesobiology to the new soil conditions.

In 2010, the new vineyard had a higher spatial heterogeneity compared to the old vineyard; however, since 2011, the latter showed an increasing variability over time. Ultimately, the plot of cases on the principal components (figure 9) reveals that, after five years from the earthworks and three years from vine plantation, the two vineyards were still well separated and there was no apparent resilience over time.

5. Conclusions

To the best of our knowledge, this work is the first attempt to set up an integrated monitoring activity of soil physical, chemical, micro- and meso-biological functions over time in a new vineyard, with the aim to understand their changes in response to pre-planting earthworks and assess a possible recovery to their original or a new equilibrium status. The results demonstrate that earthworks caused strong modifications in the topsoil physical and chemical properties and negatively impacted soil biological communities, at both the microbial and the mesofauna level.

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 soils 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 several chemical properties.

The partial permanent grass cover in the old vineyard did not result to improve significantly soil biology, and the organic farming itself appeared to be scarcely effective in enhancing the recovery process, probably because of the small amount of supplied compost. It seems to be plausible, instead, that the different soil organic matter content and biology between the new and old vineyard were mainly related to the different vine development and plant residue availability.

In conclusion, from the overall results of this work it can be stated that, in these specific soil and environmental conditions, which are however representative of many premium viticultural farms, soils with very poor biological fertility, like those upset by pre-planting earthworks, need a rather long time to restore their functions, probably longer than the time needed to obtain a commercially acceptable grape production.

The perspective of our research is to continue the annual soil monitoring and multidisciplinary analysis and, at the same time, to start monitoring vine plants and grass biomass, at least until the grape yield of the new and old vineyard will be similar.

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References

Andrenelli, M.C., Fiori, V. and Pellegrini, S.: Soil particle-size analysis up to 250 μm by X-ray granulometer: device set-up and regressions for data conversion into pipette-equivalent values, Geoderma, 192, 380-393, 2013.

Badejo, M.A., Nathaniol, T.I. and Tian, G.: Abundance of springtails (Collembola) under four agroforestry tree species with contrasting litter quality. Biological Fertility of Soils, 27, 15-20, 1998.

Bazzoffi, P., Abbattista, F., Vanino, S. and Pellegrini S.: Impact of land levelling for vineyard plantation on soil degradation in Italy, Bollettino della Societa Geologica Italiana, 6, 191-199, 2006.

Bazzoffi, P. and Tesi, P.C.: Effectiveness of the GAEC standard of cross compliance Prohibition of performing unauthorized land levelling on soil erosion control, Italian Journal of Agronomy, 6 (1), 25-34, 2011.

Bezdicek, D., Papendick, R.I. and Lal, R.: Importance of soil quality to health and sustainable land management, in: Methods for assessing soil quality, edited by: Doran, J.W. and Jones, A.J., SSSA Spec. Publ. 49, Soil Science Society of America, Madison, WI, 1–18, 1996.

Blanco-Canqui, H.and Lal, R.: Principles of Soil Conservation and Management. New York, Springer, 2008.

Bloem, J., Benedetti, A. and Hopkins, D.W.: Microbiological methods for assessing soil quality, Wallingford, UK, Cabi Publishing, ISBN 0-85199-098-3, 2006.

Brussaard, L., Behan-Pelletier, V.M., Bignell, D., Brown, V.K., Didden, W., Folgarait, P., Fragoso, C., Wall Freckman, D., Gupta, V.V.S.R., Hattori, T., Hawksworth, D.L., Klopatek, C., Lavelle P., Malloch, D.W., Rusek, J., Söderström, B., Tiedje, J.M., Ross, A.V. Biodiversity and ecosystem functionning in soil. Ambio, 26 , 563–570, 1997.

595 Chikoski, J.M., Ferguson, S.H. and Meyer, L.: Effects of water addition on soil arthropods and
596 soil characteristics in a precipitation-limited environment. Acta Oecologica, 30, 203–211,
597 2006.
598 Costantini, E.A.C., Agnelli, A., Bucelli, P., Ciambotti, A., Dell'Oro, V., Natarelli, L., Pellegrini,
599 S., Perria, R., Priori, S., Storchi, P., Tsolakis. C. and Vignozzi, N.: Unexpected relationships
600 between δ13c and wine grape performance in organic farming, J. Int. Sci. Vigne Vin, 47
601 (4), 269-285, 2013.
602 Costantini, E.A.C. and Barbetti R.: Environmental and visual impact analysis of viticulture and
603 olive tree cultivation in the province of Siena (Italy), European Journal of Agronomy, 28
604 (3), 412-426, 2008.
605 Costantini, E.A.C., Bucelli, P. and Priori, S.: Quaternary landscape history determines the soil
606 functional characters of terroir, Quaternary International, 265, 63-73, 2012.
607 Costantini, E.A.C., Fantappié, M. and L'Abate, G.: Climate and pedoclimate of Italy. In:
608 Costantini, E.A.C. and Dazzi, C. (Eds.), The Soils of Italy. World Soils Book Series,
609 Springer, DOI 10.1007/978-94-007-5642-7, 2013.
610 Culliney, T.W.: Role of Arthropods in Maintaining Soil Fertility, Agriculture, 3, 629–659, DOI
611 10.3390/agriculture3040629, 2013.
612 D'Avino, L.: Esposizione del metodo di Vittorio Parisi per la valutazione della Qualità
613 Biologica del Suolo (QBS) e proposta di standardizzazione delle procedure. CD ROM.
614 Museo di Storia Naturale di Parma, Italy, 2002.
615 Decaëns, T., Jiménez, J. J., Gioia, C., Measey, G. J., and Lavelle, P.: The values of soil animals
616 for conservation biology, Eur. J. Soil Biol., 42, 23–38, 2006.
617 Egli, M. and Fitze, P.: Quantitative aspects of carbonate leaching of soils with differing ages
618 and climates. Catena, 46, 35-62, 2001.

619 Eilers, K.G., Debenport, S., Anderson, S. and Fierer, N.: Digging deeper to find unique
620 microbial communities: The strong effect of depth on the structure of bacterial and archaeal
621 communities in soil. Soil Biol. Biochem., 50, 58-65, 2012.
622 England, L.S., Lee, H. and Trevors, J. T.: Bacterial survival in soil: effect of clays and protozoa.
623 Soil Biology and Biochemistry, 25(5), 525-531,1993.
624 Fabiani, A.. Gamalero, E., Castaldini, M., Cossa, G.P., Musso, C., Pagliai, M. and Berta, G.:
625 Microbiological polyphasic approach for soil health evaluation in an Italian pollutted site,
626 Science of the Total Environment, 407, 4954-4964, 2009.
627 Felske, A. and Akkermans, A.D.L.: Spatial homogeneity of abundant bacterial 16S rRNA
628 molecules in grassland soils, Microbial Ecology, 36 (1), 31-36, 1998.
629 Fierer, N., Schimel, J. P. and Holden, P. A.: Variations in microbial community composition
630 through two soil depth profiles. Soil Biol. Biochem., 35, 167–176, 2003.
631 Garcia-Ruiz, J.M.: The effects of land uses on soil erosion in Spain: A review, Catena, 81 (1), 1-
632 11, 2010.
633 Hargreaves, G.H. and Samani, Z.A.: Estimating potential evapotranspiration. J. Irrig. Drain.
634 Div., 108 (3), 225-230, 1982.
635 Hassall, M., Visser, S. and Parkinson, D.: Vertical migration of Onychiurus subtenuis
636 (Collembola) in relation to rainfall and microbial activity. Pedobiologia, 29, 175-82, 1986.
637 Huber, L., Eisenbeis, G., Porten, M. and Ruhl, E.H.: The influence of organically managed
638 vineyard-soils on the phylloxera-populations and the vigour of grapevines, Acta
639 Horticulturae, 617, 55-59, 2003.
640 Isermeyer, H.: Eine einfache Methode zur Bestimmung der Bodenatmung und der Karbonate
641 im Boden, Z. Pflanzenernaehr Bodenkd, 56, 26–38, 1952.
642 ISTAT: Statistical data editing. Essays n. 6, Rome, Itituto Nazionale di Statistica, ISBN: 88-
643 458-0284-12000, 2000.

644 IUSS Working Group WRB: World Reference Base for Soil Resources. World Soil Resources
645 Reports No.106, FAO, Rome, 2014.
646 Johnston, J.M: The contribution of microarthropods to aboveground food webs: a review and
647 model of belowground transfer in a coniferous forest. American Midland Naturalist, 143,
648 226-238, 2000.
649 Kautz, T., López-Fando, C. and Ellmer, F.: Abundance and biodiversity of soil microarthropods
650 as influenced by different types of organic manure in a long-term field experiment in Central
651 Spain, Appl. Soil Ecol., 33, 278-285, 2006.
652 Lal, R.: Degradation and resilience of soils. Phil. Trans. R. Soc. Lond. B, 352, 997-1010, 1997.
653 Lamb, R. O.: Geotechnical aspects of leaching of carbonates from loessial soils. In: Hoddinott,
654 K.B. and Lamb, R.O. (Eds.), Physico-chemical Aspects of Soil and Related Materials,
655 ASTM STP 1095, American society for testing and materials Physic, Philadelphia, 29-43,
656 1990.
657 Le Bissonnais, Y., Blavet, D., De Noni, G., Laurent, J.Y., Asseline, J. and Chenu, C.:
658 Erodibility of Mediterranean vineyard soils: relevant aggregate stability methods and
659 significant soil variables, European Journal of Soil Science, 58, 188–195, 2007
660 Le Bissonnais, Y., Montier, C., Jamagne, M., Daroussin, J. and King, D.: Mapping erosion risk
661 for cultivated soil in France, Catena, 46 (2-3), 207-220, 2002.
662 Loeppert, R.H. and Suarez, D.L.: Carbonate and gypsum, in: D.L. Sparks (Ed.) Methods of soil
663 analysis, Part 3: Chemical methods. SSSA and ASA, Madison, WI, 437-474, 1996
664 Martínez-Casasnovas, J.A. and Concepción Ramos, M.: Soil alteration due to erosion,
665 ploughing and levelling of vineyards in north east Spain. Soil Use and Management, 25(2),
666 183-192, 2009.
667 Menta, C., Leoni, A., Bardini, M., Gardi, C., and Gatti, F.: Nematode and microarthropod com-
668 munities: comparative use of soil quality bioindicators in covered dump and natural soils,
669 Environ. Bioind., 3, 35–46, 2008.

Muyzer, G. and Smalla, K.: Application of denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE) in microbial ecology. Antonie van Leeuwenhoek, 73(1), 127-141, 1998.

Nannipieri, P., Ascher, J., Ceccherini, M., Landi, L., Pietramellara, G. and Renella, G.: Microbial diversity and soil functions, European Journal of Soil Science, 54(4), 655-670, 2003.

Narula, A., Vats, L.K. and Handa, S.: Collembolas and mites of deciduous forest stand. Indian Journal of forestry, 21 (2), 147-149, 1998.

Osman, K.T.: Soils: Principles, properties and management. Dordrecht, Springer, 2013.

Paoletti, M.G. and Bressan, M.: Soil invertebrates as bioindicators of human disturbance. Crit. Rev. Plant Sci., 15 (1), 21-26, 1995.

Parisi, V.: La qualità biologica del suolo. Un metodo basato sui microartropodi. Acta Naturalia de l'Ateneo Parmense, 37, 97–106, 2001.

Parisi, V., Menta, C., Gardi, C., Jacomini, C. and Mozzanica, E.: Microarthropod communities as a tool to assess soil quality and biodiversity: a new approach in Italy. Agric., Ecosys. Env., 105, 323-333, 2005.

Probst, B., Schuler, C. and Joergensen, R.G. : Vineyard soils under organic and conventional management - Microbial biomass and activity indices and their relation to soil chemical properties. Biology and Fertility of Soils, 44 (3), 443-450, 2008.

Ramos, M.C. and Martinez-Casasnovas, J.A.: Nutrient losses by runoff in vineyards of the Mediterranean Alt Penedès region (NE Spain) Agriculture, Ecosystems and Environment, 113, 1-4, 356-363, 2006.

Ramos, M.C. and Martínez-Casasnovas, J.A.: Soil loss and soil water content affected by land levelling in Penedès vineyards, NE Spain Catena, 71 (2), 210-217, 2007.

Rawnsley, B.: Assessment of soil health in vineyards Acta Horticulturae, Volume 1018, 417-424, 2014.

696 Reinecke, A.J., Albertus, R.M.C., Reinecke, S.A. and Larink, O.: The effects of organic and
697 conventional management practices on feeding activity of soil organisms in vineyards.
698 African Zoology, 43 (1) , 66-74, 2008.
699 Seddaiu, G., Porcu, G., Ledda, L., Roggero, P.P., Agnelli, A. and Corti, G.: Soil organic matter
700 content and composition as influenced by soil management in a semi-arid Mediterranean
701 agro-silvo-pastoral system, Agriculture Ecosystems and Environment, 167, 1-11, 2013.
702 Sequi, P. and De Nobili, M.: Carbonio organico. In: Angeli, F. (Ed.), Metodi di Analisi Chimica
703 del Suolo. Ministero per le Politiche Agricole e Forestali, Osservatorio Nazionale
704 Pedologico e per la Qualità del Suolo, VII.1, 1–13, 2000.
705 Seybold, C. A., Herrick, J. E. and Brejda, J. J.: Soil resilience: a fundamental component of soil
706 quality. Soil Science, 164(4), 224-234, 1999.
707 Schaefer, M.: Interspecific interactions in the soil community. Acta Zoologica Fennica, 196,
708 101-106, 1995.
709 Sharp-Heward, S., Almond, P. and Robinson, B.: Soil disturbance and salinisation on a vineyard
710 affected by landscape recontouring in marlborough, new zealand, Catena, 122, 170-179,
711 2014.
712 Sorensen, L.H.: The infuence of stress treatments on the microbial biomass and the rate of
713 decomposition of humified matter in soils containing different amounts of clay. Plant Soil,
714 75, 107-119, 1983.
715 Stevanato, P., Concheri, G., Squartini, A., Saccomani, M., Piffanelli, P., Fricano, A., Angelini,
716 E. and Fornasier, F.: Soil biological and Biochemical traits linked to nutritional status in
717 grapevine, Journal of Soil Science and Plant Nutrition,14 (2), 421-432, 2014.
718 Van Leeuwen, C., Friant, P., Choné, X., Tregoat, O., Koundouras, S. and Dubourdieu, D.:
719 Influence of Climate, Soil, and Cultivar on Terroir. Am. J. Enol., 55(3), 207-217, 2004.
720 Van Leeuwen, C. and Seguin, G.: The Concept of Terroir in Viticulture, Journal of Wine
721 Research, 17, 1-10, 2006.

722 Vaudour, E.: The quality of grapes and wine in relation to geography: notions of terroir at
723 various scales, J. Wine Res., 13 (2), 117-141, 2002.
724 White, R.E.: Soils for fine wines, Oxford University Press, New York, 2003.

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Table 1. Main soil properties of the experimental area under ordinary vineyard management and grape production.

Profile horizon	Depth cm	Sand %	Silt %	Clay %	pH	$CaCO_3$ %	TOC %	TN g kg^{-1}
Ap	0-28	26	35	39	8.3	25.3	0.81	0.83
Bw	28-100	30	28	42	8.3	27.5	0.61	0.65
BC	100-120	28	29	43	7.9	27.5		

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Table Table 2. Soil properties of the selected monitoring plots within each vineyard in the first year of study (soil depth = 0-15 cm).

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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Table 3. Abundance of soil microarthropods, number of taxa and QBS-ar index (as resulting from the sum of the EMI scores) in the old (OV) and in the new (NV) vineyard (2011–2014).

	Microarthropod Abundance									EMI (Eco-Morphological Index)							
	2011		2012		2013		2014			2011		2012		2013		2014	
	OV	NV	OV	NV	OV	NV	OV	NV		OV	NV	OV	NV	OV	NV	OV	NV
Acari	7	224	138	353	123	643	168	352		20	20	20	20	20	20	20	20
Collembola	1	11	65	60	26	14	114	466		20	20	20	20	20	20	20	20
Araneida						1	1	1							1	5	5
Chilopoda				1		2	3	17					10		10	10	10
Coleoptera				1			1	1					15		1	11	20
Holometabolous insects larvae			41	3		14	4	8				10	10			10	10
Diplura		2			1		4	2			20				20	20	20
Diptera larvae		1	3	1	1	11	5	40			10	10	10	10	10	10	10
Hymenoptera				2		2	2	26					5	1	5	5	5
Homoptera		1		2							1		1		5		
Protura				1			4	1					20			20	20
Symphyla		3					2	2			20					20	20
Tisanoptera						1		1							1		1
Diptera					7	9		1						1	1		1
Rincota						1		4							1		1
Pauropoda							30	4								20	20
Psocoptera								1									1
Diplopoda								2									10
Isopoda								1									10
Total arthropods	8	242	247	424	158	698	338	930	QBS-ar	40	91	60	111	52	95	171	204
									n. taxa	2	6	4	9	5	12	12	18

730

Figure captions

Figure 1. . The new and the old vineyards with their respective monitoring plots (P1–P5 in the new vineyard, P6–P8 in the old vineyard).

Figure 2. Rainfall and temperature during the experimental period with their respective long-term average trends (1990-2010).

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

Figure 4. Dendrograms of hierarchical cluster analysis based on UPGMA and Dice's coefficient of DGGE banding patterns of the 16S rDNA.

Figure 5. Diversity indices and number of bands of the DGGE banding patterns.

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

Figure 7. Abundance and community structure of soil microarthropods and soil biological quality index (QBS-ar) in the new and old vineyard over the experimental period. The annual abundance is shown together with the cumulative rainfall from January to April (before sampling).

Figure 8. PCA score plots for each year and for the whole study period (not including climate).

Figure 9. PCA loading plots for each year and for the whole study period (not including climate).

Figure 10. PCA score and loading plots for the whole 2010-2014 period (including climate).

747 Figure 1. The new and the old vineyards with their respective monitoring plots (P1–P5 in the new

748 vineyard, P6–P8 in the old vineyard).

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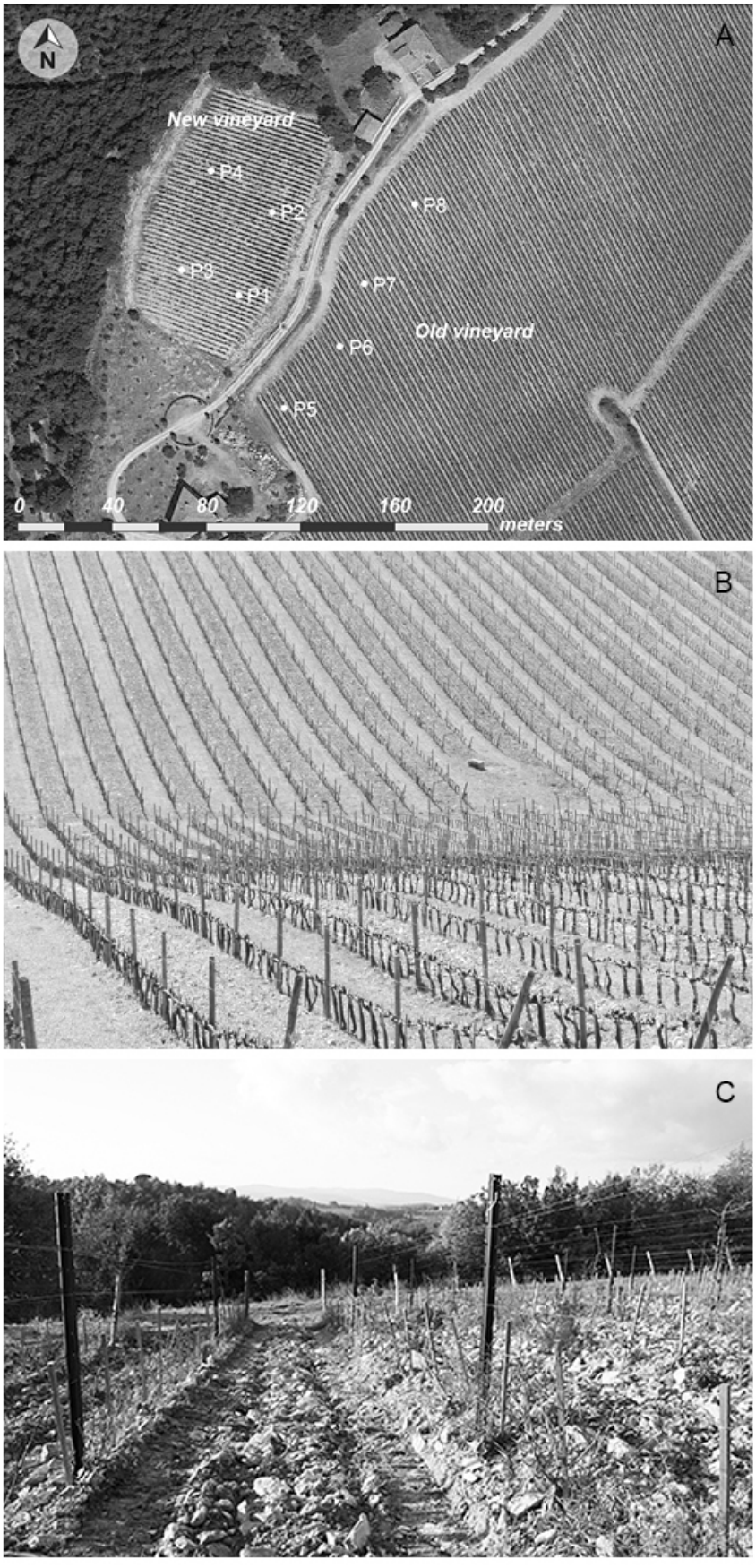


Figure 2. Rainfall and temperature during the experimental period with their respective long-term average trends (1990-2010).

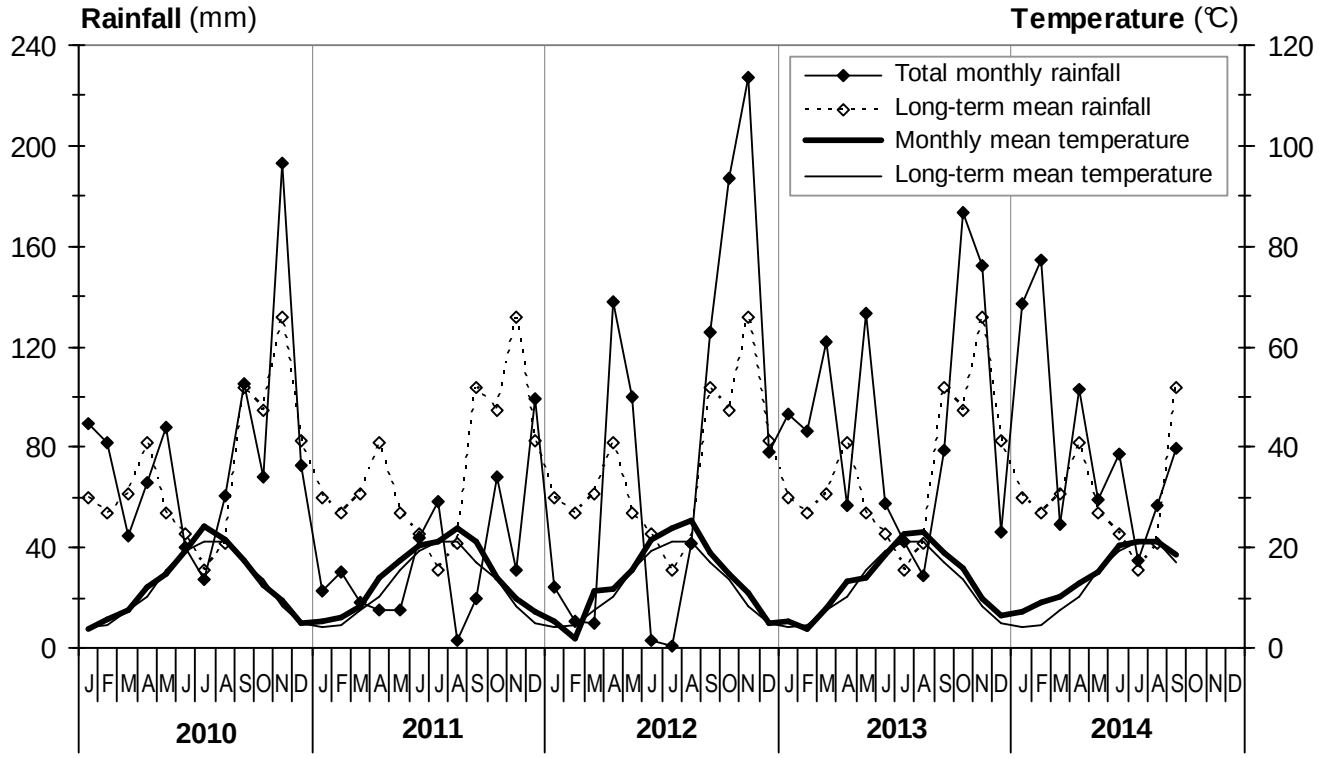


Figure 3. Soil chemical properties in the new and the old vineyard during the experimental period.

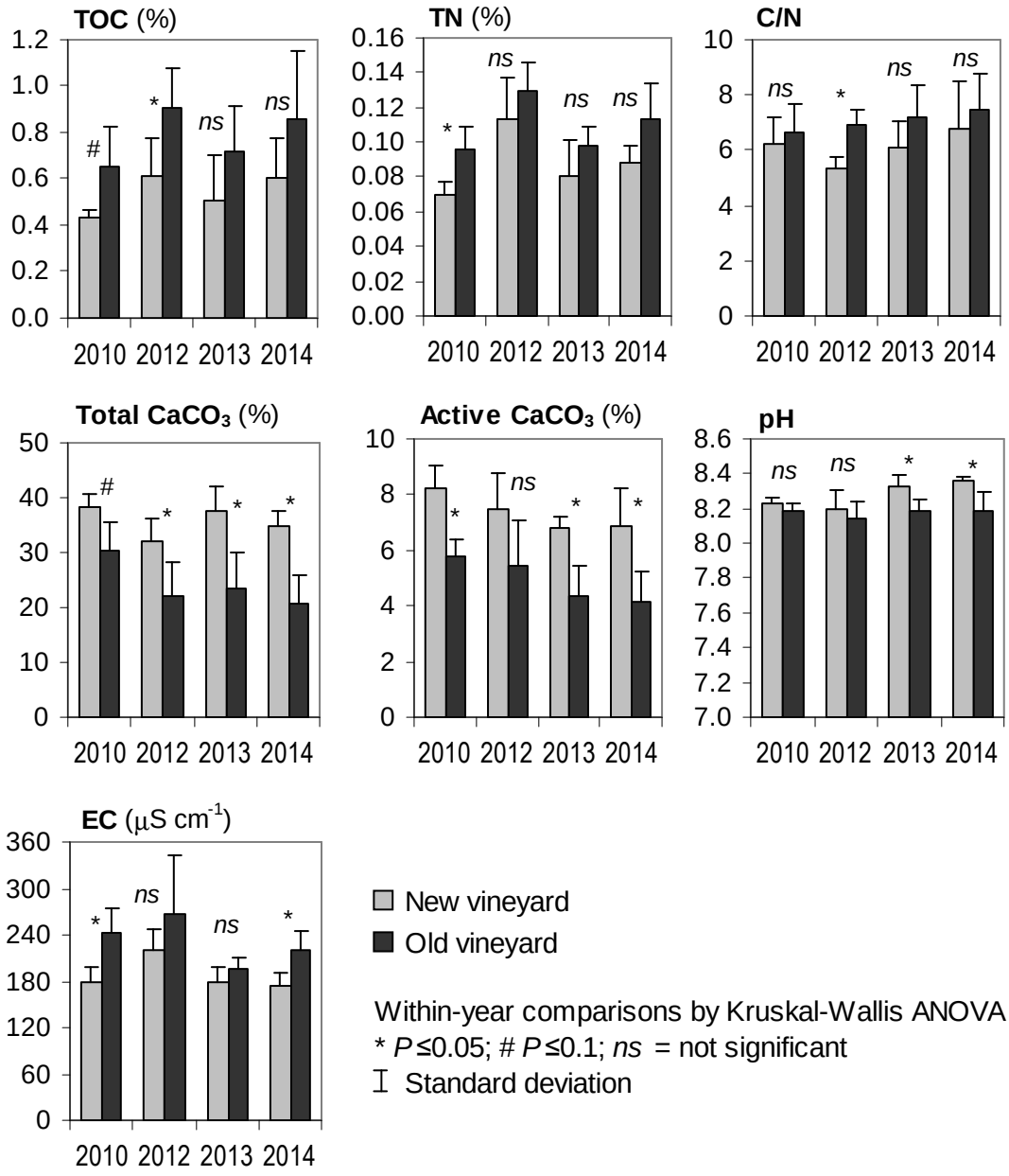
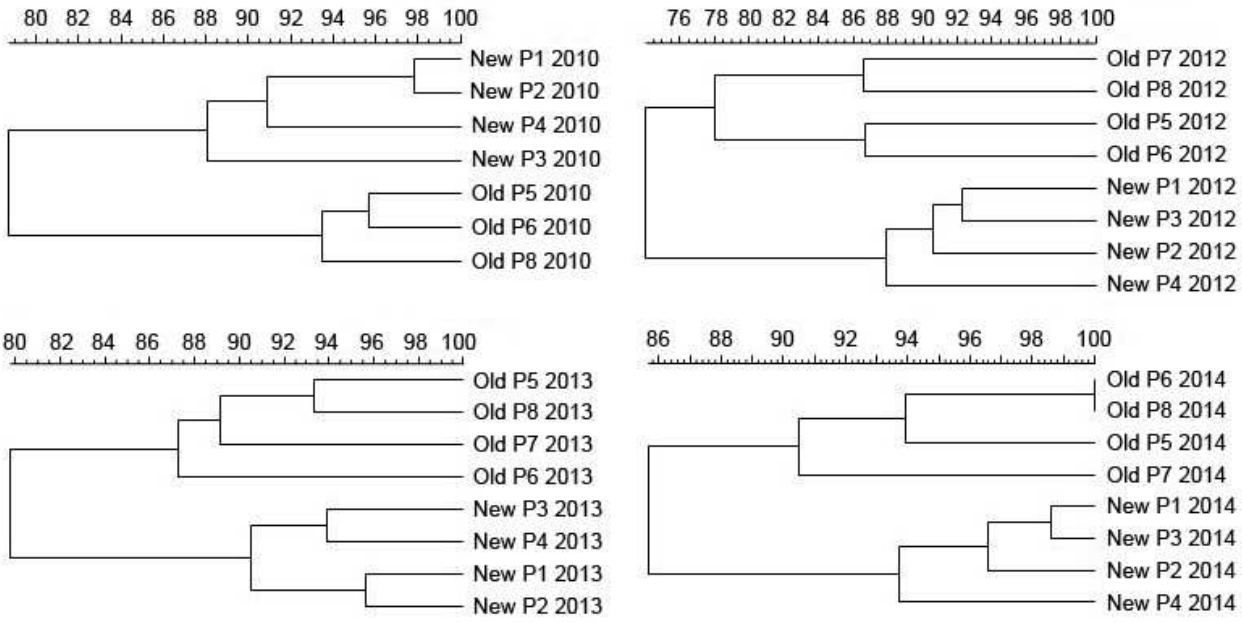


Figure 4. Dendrograms of hierarchical cluster analysis based on UPGMA and Dice's coefficient of DGGE banding patterns of the 16S rDNA.



799 Figure 5. Diversity indices and number of bands of the DGGE banding patterns.

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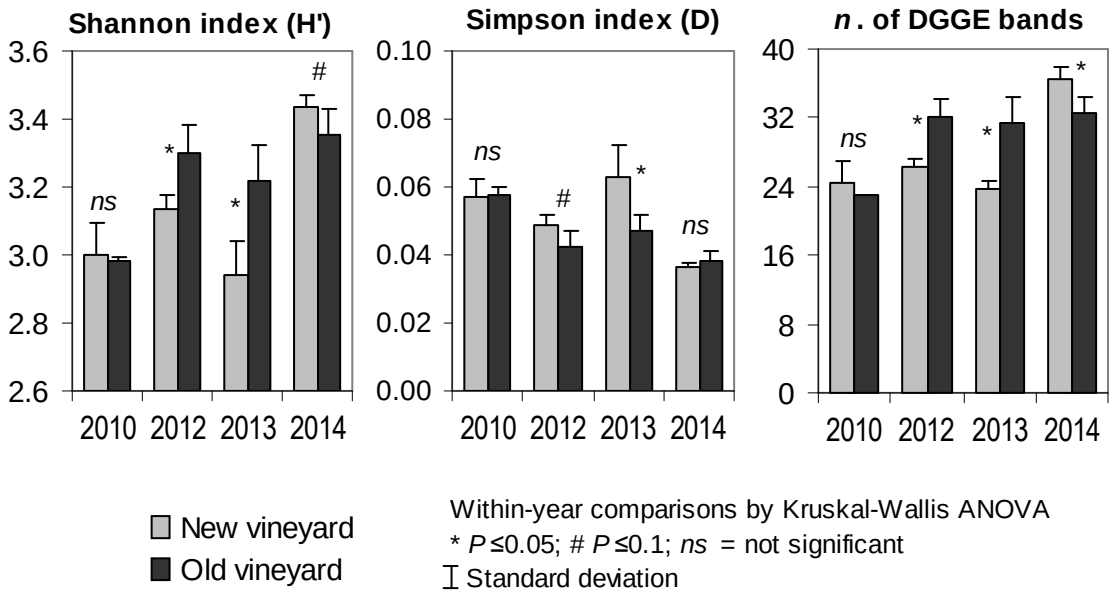
801 

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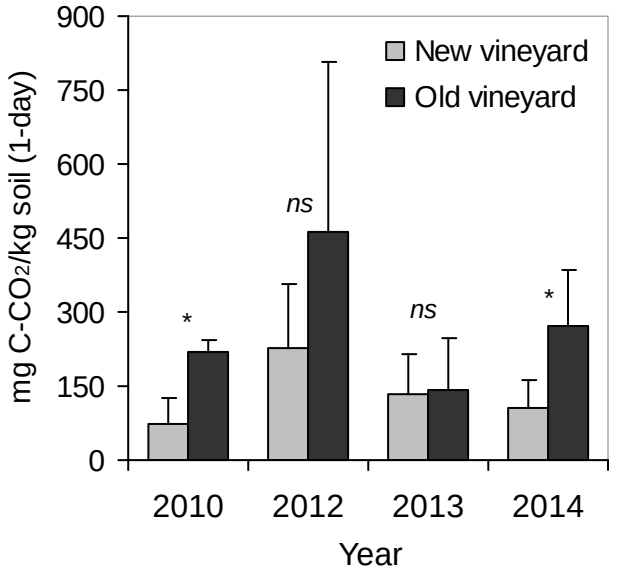
Figure 6. Microbial respiration in the two vineyards during the experimental period.



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

Figure 7. Abundance and community structure of soil microarthropods and soil biological quality index (QBS-ar) in the new and old vineyard over the experimental period. The annual abundance is shown together with the cumulative rainfall from January to April (before sampling).

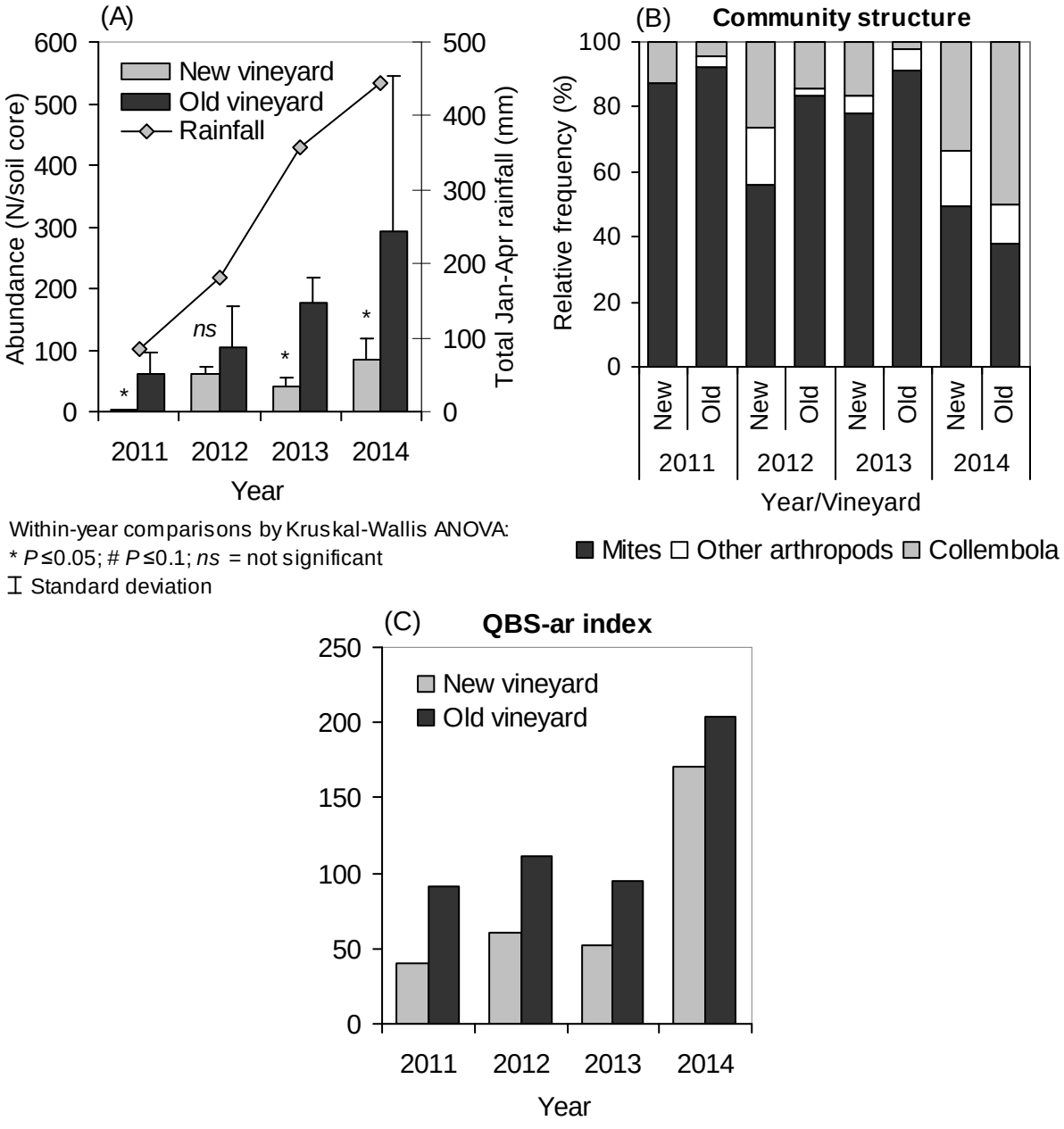


Figure 8. PCA score plots for each year and for the whole study period (not including climate).

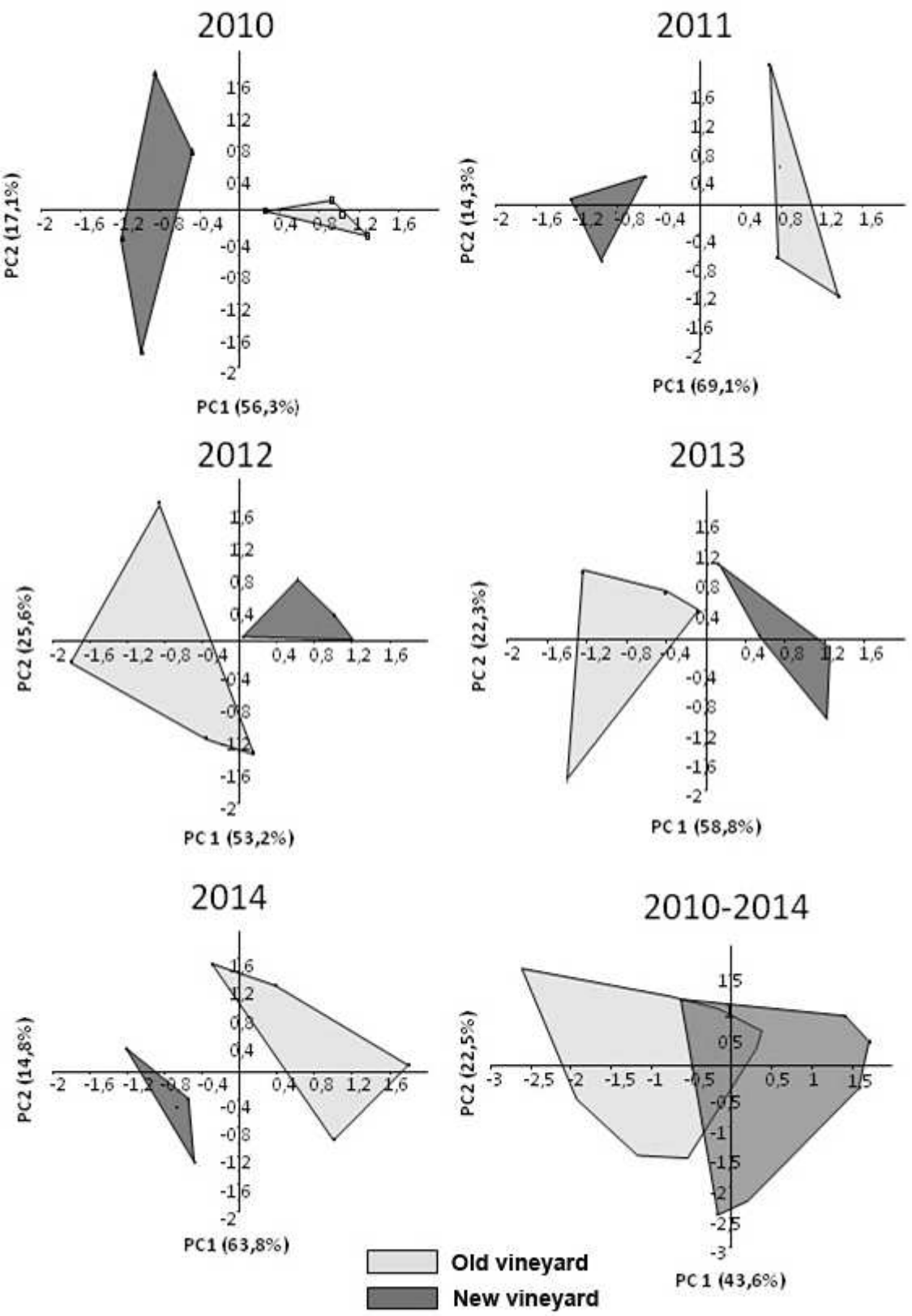
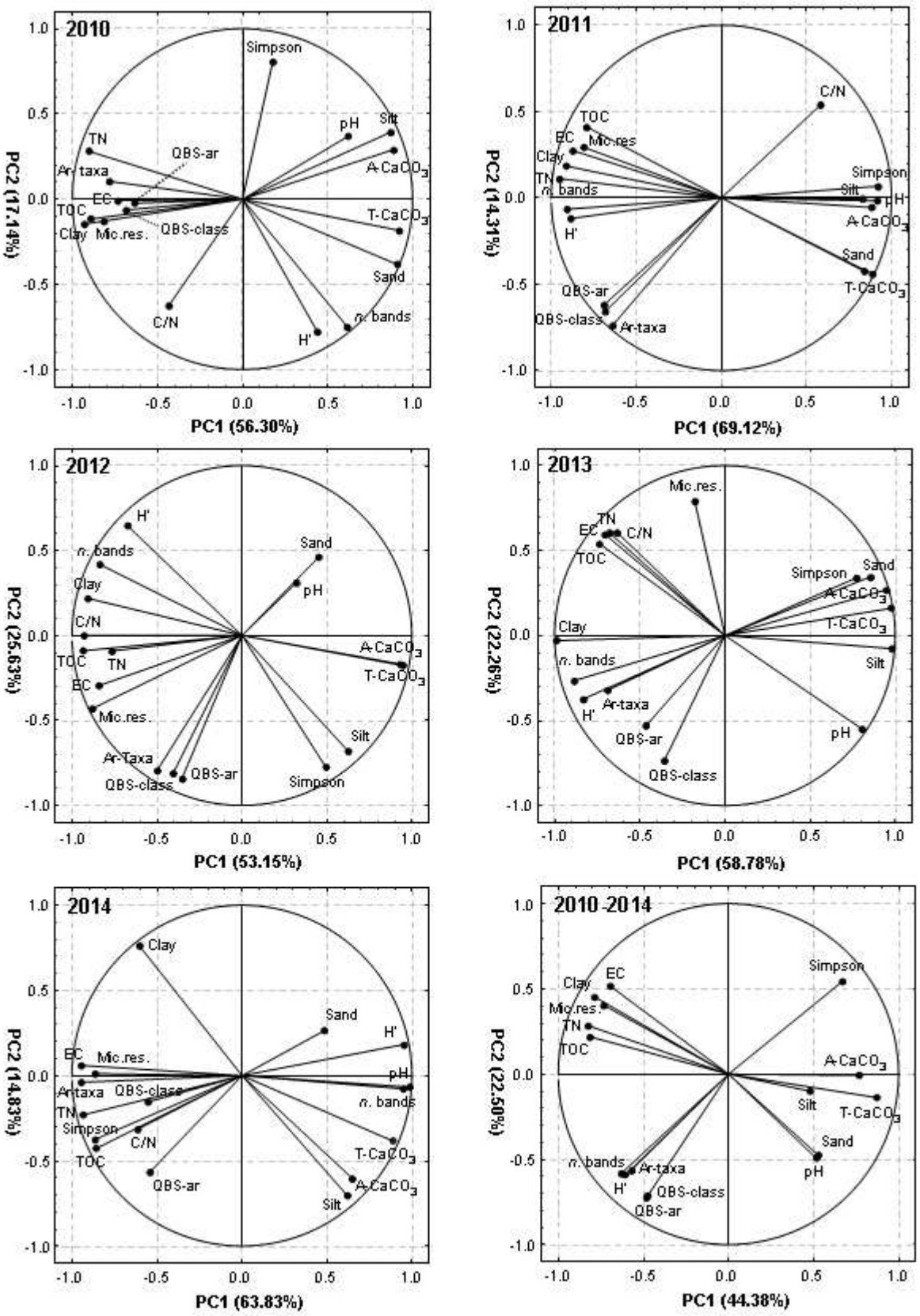
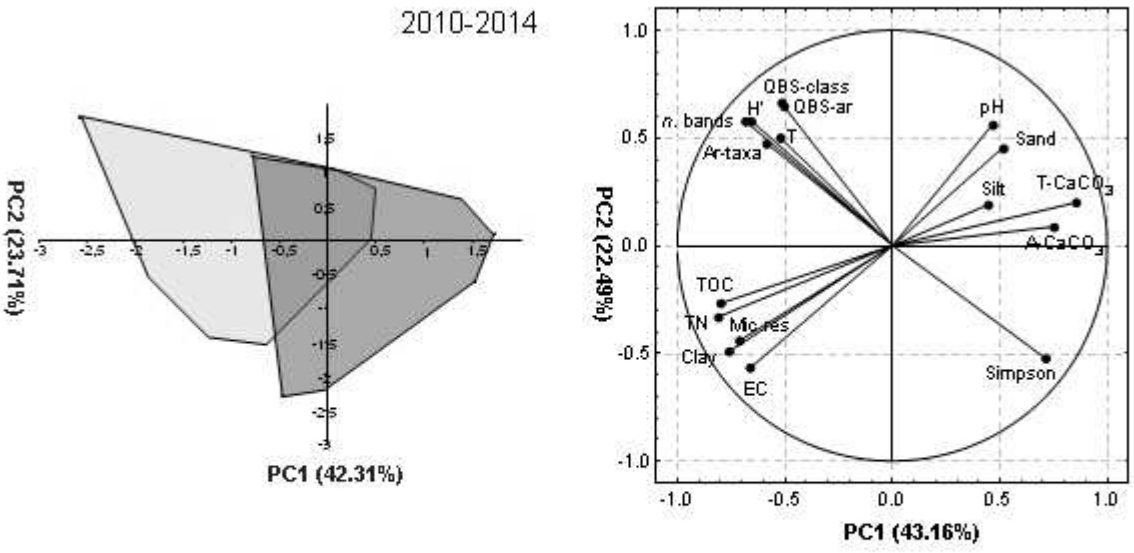


Figure 9. PCA loading plots for each year and for the whole study period (not including climate).



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 10. PCA score and loading plots for the whole 2010-2014 period (including climate).



TOC = soil total OC; TN = soil total N; C/N = soil TOC to TN ratio; T-$CaCO_3$ = soil total $CaCO_3$; A-$CaCO_3$ = soil active $CaCO_3$; 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.