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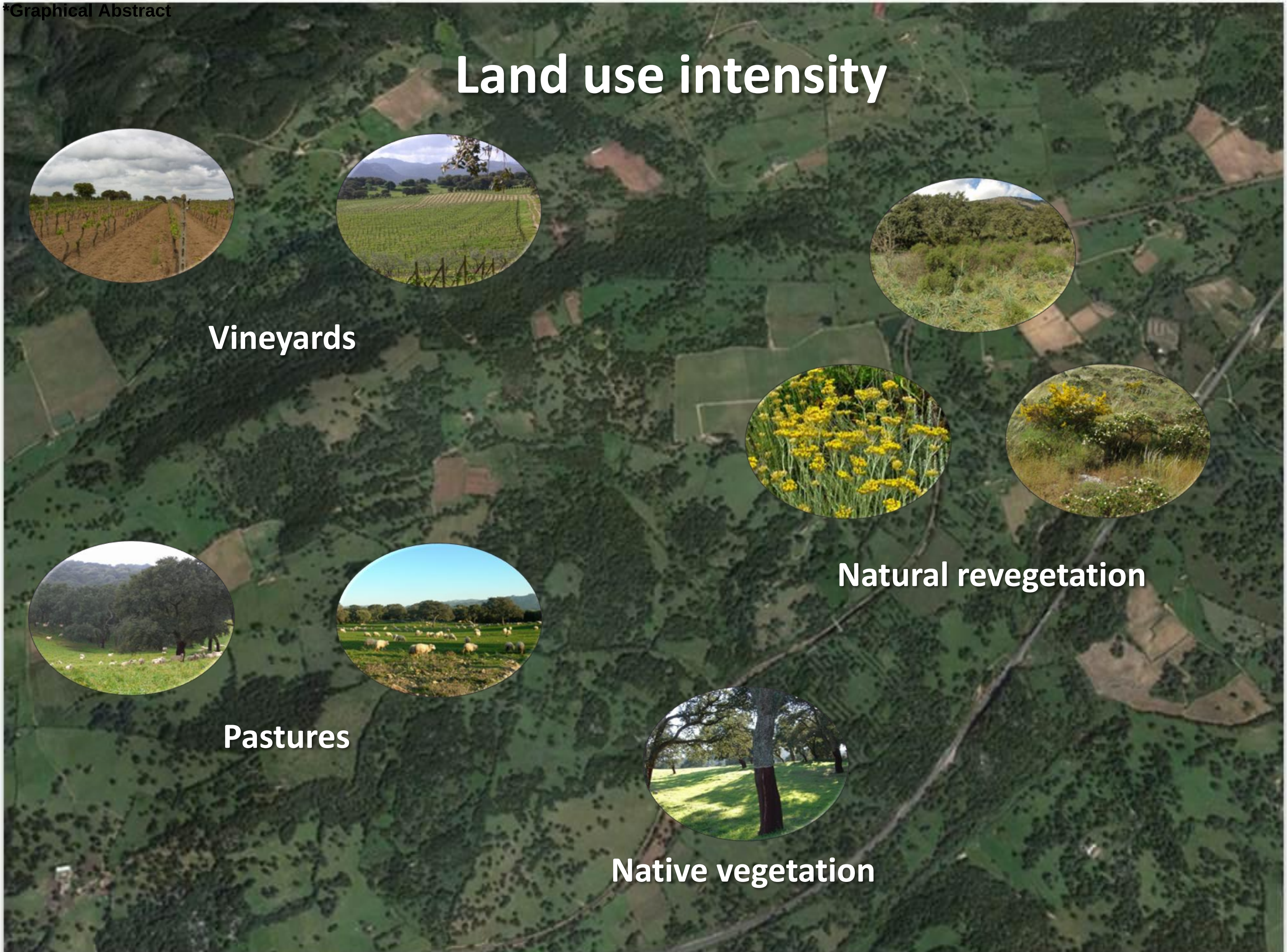
*Graphical Abstract



Organic carbon pools and soil biological fertility are affected by land use intensity in Mediterranean ecosystems of Sardinia, Italy

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Abstract

Soil quality is mainly studied from the chemical and physical point of view, whereas soil biochemical and microbiological parameters are relatively more scarcely explored to assess the effect of management practices. This study aimed to evaluate soil organic carbon (SOC) and its pools; soil microbial activity parameters; and the Biological Fertility Index (BFI), in six land uses characteristics of the Mediterranean basin in north-eastern Sardinia. These land uses differed in management intensity and consisted of: tilled vineyard (TV), no tilled grassed vineyard (GV), former vineyards (FV), hay crop and pasture (HC and PA), cork oak forest (CO).

Significant differences among ecosystems were found in most cases in (SOC), the related pools (total extractable carbon, humic and fulvic acids, not humified, not extractable), humification parameters (degree, rate and index of humification), and soil microbial activity (microbial carbon, respiration, metabolic quotient, and mineralization quotient). Pasture and cork oak forest showed in average a better soil quality for most biochemical and microbial parameters in comparison with the other ecosystems. The index of soil biological fertility (BFI) was higher under cork oak forest which is supposed to be the most sustainable ecosystem in the long term in this environment, able to maintain soil biological fertility and microbial diversity.

Keywords: Land use intensity; Soil organic carbon pools; Biological fertility index; Mediterranean ecosystems

1. Introduction

Soil quality studies have focused mainly on chemical (Bone et al., 2014) such as soil organic carbon (SOC) and pH (Zornoza et al., 2015), and physical soil quality parameters such as aggregate stability, bulk density and soil porosity (Pulido-Moncada et al., 2015; Zornoza et al., 2015). Some authors have suggested minimum datasets of soil parameters to assess soil quality deriving from: i) a combination of physical and biological parameters, e.g. soil texture, bulk density, water retention, organic carbon, soil biomass and biomass activity (Doran and Parkin, 1994), or ii) physical, chemical and biological characteristics, e.g. total C and N, anaerobically mineralizes N, pH, phosphate, bulk density and macroporosity (Sparling et al., 2003; Hedo et al., 2015; Muñoz-Rojas et al., 2016).

Land use, sensitivity to change in soil management, ease of measure in routine laboratory analyses for soil monitoring, and the relations with soil functions have been included among the attributes for the selections of indicators (Verhoef, 2004). Biogeochemical cycles and biological indicators need to be addressed as a tool to assess different ecosystem services that are supported by soil properties and functions (Smith et al., 2011; Costantini et al., 2016), and in this context microbial biomass and enzyme activities are the most studied indicators (Abbott and Murphy 2007; Zornoza et al., 2015). In addition, most used indicators include microbial respiration and enzyme activities, and related indices (Kieft et al., 1998; Bastida et al., 2006; Muscolo et al., 2014; Muñoz-Rojas et al., 2016). For example, the Biologic Index of Fertility (BIF) includes respiration and enzymatic activities (Stefanic et al., 1984). Kang et al. (2005) proposed the Microbial index of soil (Mi) based on microbial biomass Carbon (C) and Nitrogen (N), potentially mineralizable N, soil respiration, bacterial population, mycorrhizal infection, and dehydrogenase and phosphatase activities, combined with a crop index and a nutrient index. The Enzyme Activity Number (EAN) includes the activity of five enzymes, namely dehydrogenase, catalase, alkaline phosphatase, amylase and protease (Beck, 1994). But specific indexes combining a limited number of easy measurable biochemical and microbial parameters are less studied, and a general agreement on the selection of proper indicators to assess soil biological fertility is still missing (Beed et al., 2011).

When considering the link between soil quality and agricultural management, less disturbed land uses may have a high soil quality and SOC content (Parras-Alcántara et al., 2015), and soil aggregation (Grandy and Robertson, 2007), which in turn may influence soil microbial community activity (García-Orenes et al., 2010; Camilli et al., 2016).

Ecosystems in the Mediterranean Basin are considered important for their plant diversity, but are more prone to land degradation due to soil degradation and SOC depletion, often coupled with erosive processes (Muñoz-Rojas et al., 2015) due to extensive land use changes in the last decades (Anaya-Romero et al., 2011). Studies dealing with the effects of different land use intensities on the biochemical and microbiological characteristics in these ecosystems are rather limited (Caravaca et al., 2002; Riffaldi et al., 2002; García-Orenes et al., 2010; Marzaioli et al., 2010, Lagomarsino et al., 2011; Novara et al., 2012; Bevivino et al., 2014; Laudicina et al., 2015), and available studies have not yet been fully addressed to the application of comprehensive indicators in complex Mediterranean mosaic landscapes, where different ecosystems typical of the heterogeneous agricultural land uses adopted in the study area spatially coexist.

An indicator system called soil Biological Fertility Index (BFI) was proposed for soil monitoring in Italy (Pompili et al., 2008; Renzi et al., 2017). In detail, the index is based on soil organic matter (SOM=SOC×1.724), basal respiration at the last day of incubation (Cbas), cumulated respiration during the incubation period (Ccum), microbial biomass carbon (Cmic), and metabolic quotient (qCO_2). The indicator has proved to be more sensitive than microbial activity or microbial biomass alone to detect differences in soil quality, and to discriminate soil biological fertility status under different treatments (Pompili et al., 2008; Renzi and Benedetti, 2015; Renzi et al., 2017).

The aims of this study were: i) to evaluate a set of soil chemical, biochemical and microbial parameters under different land use intensities frequently adopted in the agro-ecosystems of the

Mediterranean area, ii) to test the Biological Fertility Index in these specific conditions, and iii) to derive information about the ecosystem sustainability in terms of soil biological fertility.

2. Materials and methods

2.1. The study site

The study site is located in the Berchidda district (40° 46' N, 9° 10' E), in the Gallura region of north-eastern Sardinia (Italy). In the study area (Fig. 1), long-term human activities have created a spatial mosaic landscape of ecosystems (Eldon and Gershenson, 2015), where patches of native vegetation (cork-oak forest) are intermingled with more intensive cropped ecosystems (vineyards), and pastures at different level of intensification (Bevivino et al., 2014). In addition, natural revegetated areas (scrublands, maquis and Helichrysum meadows) are present.

Climate is warm temperate with dry and hot summers, and mean annual rainfall and temperature are 623 mm and 15.0°C respectively. Long term climate data (1985-2006) were obtained from Servizio Agrometeorologico Regionale (SAR) of Sardinia Region. The area is hilly (mean altitude 285 m a.s.l.), soils have sandy-loam and loamy-sand textures, derive from granitic rocks, and are classified as Dystric and Eutric Cambisols (IUSS Working Group WRB, 2015).

Six land uses intensities as described in Francaviglia et al. (2014) were compared in monitoring plots (Table 1): Tilled vineyard (TV), 0.99 ha; No-tilled grassed vineyard (GV), 1.94 ha; Former vineyard (FV), 1.15 ha, presently naturally revegetated after the abandonment of vineyards; Hay crop (HC), 3.31 ha, with 5 years of cereals or legumes grown for hay, followed by spontaneous herbaceous vegetation in the sixth year; Pasture (PA), 3.17 ha, with 5 years of spontaneous herbaceous vegetation, and one year of hay crop; Cork oak forest (CO), 1.22 ha. The total surface of the study area is 1472 ha, where TV represent 5% of the total area (73.2 ha), GV 3.8% (55.2 ha), FV 3.7% (54.7 ha), HC 7.9% (115.7 ha), PA 34.3% (505 ha), and CO 26.8% (395 ha). Other land uses (e.g. broad-leafed and coniferous woodlands) were not included in this study (218.8 ha, 14.9%), while urban areas occupy 55.1 ha (3.7% of the total area).

Scattered cork-oak trees are included in PA and HC, and grazing activity by sheep is present during some months of the year. As a fact, these ecosystems are derived from the former cork forests, and are considered part of the silvo-pastoral ecosystem known as Mediterranean Dehesa landscape (Francaviglia et al., 2012; Lozano-García et al., 2016).

2.2. Monitoring scheme and soil characterization

The monitoring scheme was setup to highlight possible differences due to land use and management intensity within similar climatic and pedologic conditions. A random sampling scheme was adopted (Fig. 1), pits were digged with a mini excavator, and samples for a general characterization of entire soil profiles were taken along the different soil horizons using a hand trowel. Main soil characteristics are reported in Table 2, and data presented were normalized at a fixed depth (20 cm) to enable the comparison among the different soil profiles.

Soil samples for the biochemical and biological fertility determinations were collected from the topsoil layer (20 cm) in February, May and November 2007, with three random replicates in each land use to consider local differences due to soil slope and/or vegetation heterogeneity. Each replicate was composed of three subsamples combined into one composite sample, and the total number of samples analyzed for each land use during the study was nine (3 replicates × 3 periods). Samplings were limited to 20 cm of topsoil excluding the subsoil, since the main changes in soil microbial parameters due to conversion among land uses can be expected in the upper centimeters (Conant et al. 2001).

2.3. Laboratory analyses

Samples were air-dried at ambient temperature (less than one week), and the analyses were made on the < 2 mm dried soil fraction after sieving.

Soil reaction (pH) was determined in 1:2.5 soil:water suspension by potentiometric method using an Orion Ionalyzer Model 901 pH meter, particle-size analysis with the wet sieving and sedimentation procedure after pre-treatment with hydrogen peroxide to remove organic matter and cementing substances, soil texture according to the USDA classification (Soil Survey Staff, 2011): clay (< 0.002 mm), silt (< 0.05 mm), sand (< 2 mm), total N with the Kjeldahl method (Bremner and Sparks, 1996), available P with a spectrophotometer using the Olsen method, cation exchange capacity as sum of exchangeable cations (CEC), and exchangeable K with the barium chloride-triethanolamine method buffered at pH 8.2 with an atomic absorption spectrophotometer.

Soil organic carbon (SOC) was determined with the Springer-Klee method (1954), and soil organic matter was determined by SOM=SOC×1.724 applying the Van Bemmelen coefficient (Nelson and Sommers, 1982). Total extractable carbon (TEC), and humic and fulvic acid carbon (HA+FA) were determined by the dichromate oxidation method (Nelson and Sommers, 1982). Not humified and more labile C fraction (NHC) was calculated by the difference [TEC–(HA+FA)], and not extractable organic carbon (NEC), conventionally defined as humin (a pool of organic carbon recalcitrant to microbial degradation), by the difference (SOC–TEC). Humification parameters DH (degree of humification), HR (humification rate), and HI (humification index) were determined according to Sequi et al. (1986) and Ciavatta et al. (1990). DH is given by (HA+FA×100)/TEC, HR by (HA+FA×100)/SOC, and HI (dimensionless) by (TEC–HA+FA)/HA+FA.

Microbial biomass carbon (Cmic), expressed in mg C kg^{-1} soil, was determined with the chloroform fumigation-extraction method (Vance et al., 1987), on air-dried soils, pre-conditioned by a 10-d incubation in open glass jars at field capacity and 30°C.

To measure soil microbial respiration, 25 g of sample were placed in closed glass jars, and incubated in the dark at field capacity and 30°C. The CO_2 evolved was trapped by 0.5 N NaOH after 1, 2, 4, 7, 10, 14, 17, 21, 25 days of incubation, and determined by titration of the excess NaOH with 0.5 N HCl (Isermeyer, 1952). Basal respiration (Cbas) is the respiration rate at the last day of incubation in mg CO_2–C kg^{-1} soil d^{-1}, Ccum the cumulated respiration during the incubation period in mg CO_2–C kg^{-1} soil. The metabolic quotient (qCO_2) is the hourly CO_2 evolved per unit of microbial biomass. It expresses the relation between the activity (basal respiration) and the carbon content of the microbial biomass, i.e. the amount of CO_2–C produced per unit microbial biomass carbon, and allows evaluating the effects of external disturbances. The unit is mg CO_2–C 10^{-2} h^{-1} mg $Cmic^{-1}$ (Anderson and Domsch, 1990; 1993). The mineralisation quotient (qM) is the ratio between the cumulated respiration and the SOC, and is expressed in %. The quotient indicates the efficiency of micro-flora in metabolising SOC (Dommergues, 1960).

2.4. *Soil biological fertility index*

The Biological Fertility Index (BFI) is an indicator system considering SOM, Cbas, Ccum, Cmic, qCO_2 and qM. Five intervals of values were set for each parameter, and scores increasing from 1 to 5 were assigned to each interval (Table 3) based on evidences deduced from earlier studies available in the literature (Brookes, 1995; Vance et al., 1987; Oberholzer and Höper, 2000; Bloem et al., 2006). The algebraic sum of the scores for each parameter providing the proposed classes of biological fertility is shown in Table 4.

Previous validations of the indicator were carried out in Italy: i) in two land uses (grassland and maize crop) with different fertilization treatments (no fertilization, manure+mineral, and sludge), where results indicated a lower soil biological fertility in the sludge treatment (Pompili et al., 2008), and ii) in different sites with pollution from heavy metals or organic compounds, where the indicator allowed to discriminate the soil biological fertility status in comparison with not polluted soils (Renzi and Benedetti, 2015). A recent validation using different combinations of the 6 variables was performed over 1079 soil samples collected in Italy, confirming its appropriateness as a multi-domain indicator to discriminate soil biological fertility (Renzi et al., 2017).

Increasing scores were assigned to SOM, Cbas, Ccum, Cmic and qM when the value of the parameter is increasing, i.e. “the more is better”; conversely qCO_2 has increasing scores when the value of the parameter decreases, i.e. "the less is better" and the ecosystem is more stable (Insam and Haseiwandter, 1989; Anderson, 1994; Andrews et al., 2004).

2.5. Statistical analyses

Statistical analyses were performed using the *Statistica 8.0* software package (Statsoft, Tulsa, USA). Significant differences among means ($p < 0.05$) were evaluated through the Fisher's protected least significant difference test (LSD post hoc test).

3. Results and discussion

3.1. Soil organic carbon and pools

Pasture (PA) and cork oak forest (CO) showed significantly higher SOC and TEC contents ($p<0.05$) across ecosystems (Table 5). In particular, SOC and TEC were 2.08% and 1.60% respectively in PA, and 2.10% and 1.66% in CO. In the other land uses, SOC was lower and ranged from 1.32 (FV) to 1.54% (TV, GV), TEC from 0.95 (FV) to 1.15% (HC). The ranking was CO>PA>TV+GV>HC>FV for SOC content, CO>PA>HC>TV+GV>FV for TEC.

No significant differences in SOC were found in the two vineyards ecosystems (TV and GV), and SOC was 1.54% in both. The most likely explanations are: i) the yearly addition to TV of organic carbon as organic fertilizer (200 kg C ha^{-1} $year^{-1}$), and ii) the supplementary irrigation provided in GV in case of water stress. In Mediterranean conditions this might promote microbial activity and enhance SOC mineralization (Nuñes et al., 2007; Butenschoen et al., 2011; Arroita et al., 2013), thus offsetting the positive effect of the grass cover (Lagomarsino et al., 2011; Muñoz-Rojas et al., 2015). PA and HC, even if both grazed by sheep for some months during the year (Table 1), showed significantly different SOC and TEC contents ($p<0.05$), due to the differences of cropping intensity and the lower soil disturbance by tillage in PA (Francaviglia et al., 2014; Muñoz-Rojas et al., 2015). In particular, SOC content of these ecosystems is in agreement with the findings obtained in Sardinia (Italy) by Salis et al. (2015), reporting 2.11% of SOC for a natural pasture grazed by sheep, and 1.56% for a pasture annually ploughed and sowed for forage production.

Humic and fulvic acids carbon (HA+FA) did not show significant differences among the ecosystems, even if the contents of PA and CO (0.86-0.89%) were slightly higher in comparison with GV, TV, FV, and HC (0.73-0.79%), consistently with SOC and TEC values. (HA+FA) showed a ranking similar to SOC and TEC, i.e. CO>PA>GV>TV>FV>HC. The higher SOC and TEC amounts under PA and CO ecosystems can be due to different reasons: i) the higher organic inputs returned to soil by the plant and root biomass, in contrast with the ecosystems where crop harvesting or removal of pruning residues reduces plant inputs; ii) the organic input from sheep grazing in PA; or iii) the higher microbial decomposition in the more intensive or disturbed ecosystems such as the vineyards and the hay crop (Gregory et al., 2016). Results are in agreement with other findings (Bevivino et al., 2014), reporting a relevant effect of land use intensification on SOC and TEC; a lower agricultural management intensity was related to a higher and more stable chemical and biochemical soil composition.

In addition, we might speculate that CO is in a steady-state condition due to the absence of any soil disturbance, while PA is undoubtedly less disturbed than HC. Furthermore, there is an inherent but not quantifiable source of variation in the data, due the different time periods after the conversion from CO: PA and HC were established in the 70s, and TV and GV in the 90s. FV represent a revegetation of former vineyards, established in the 50s and abandoned in the 70s.

Not humified carbon (NHC) ranged from 0.19 to 0.77% in FV and CO respectively (Table 5), with significantly higher contents under pasture (PA) and cork oak forest (CO), indicating a higher labile C fraction with a rapid turnover in comparison with the other ecosystems (Jenkinson and Ladd, 1981; McGill et al., 1986; Jenkinson and Parry, 1989; von Lützow et al., 2007). The ranking was CO>PA>HC>GV+TV>FV, in agreement with SOC content. Pastures have been shown to transfer a large amount of organic matter to soil (Fischer et al., 1994) mainly through the root systems, as well woody plants are reported to be potential sources of recalcitrant organic materials in soils (Lorenz et al. 2007).

Humin, i.e. not extractable organic carbon (NEC), did not show significant differences among the ecosystems, and ranged from 0.28 to 0.48% in HC and PA respectively. The ranking was PA>CO>GV+TV>FV>HC. The highest contents were found in PA (0.48%) and CO (0.44%), indicating the presence of a C pool more stable and less affected by mineralization processes, and preferentially stabilized in more stable forms chemically or physically protected (Camilli et al., 2016).

The average ratio TEC/SOC was 0.75 (ranging from 0.71 to 0.81), not significantly different among ecosystems. The quite high ratio indicates that most of SOC is not represented by humin (NEC), which is conversely chemically protected by the processes of stabilization mediated by the silt+clay fraction, and less physically protected due to the lowest formation of soil aggregates in sandy soils (Six et al., 2002). Results are in agreement with other findings for sandy soils in Mediterranean environments under pastures, forests and Mediterranean maquis (Trinchera et al., 2015), where a TEC/SOC ratio of 0.79 was found.

Humification degree (DH), humification rate (HR) and humification index (HI) in the different ecosystems are reported in Table 5. Humification parameters (DH, HR, and HI) are commonly considered as an index of soil humification activity as well as of availability of non humified labile fractions (Ciavatta et al., 1990; Vittori Antisari et al., 2010). DH ranged from 81.6 to 56.5% respectively in FV and PA, was significantly different in PA and CO in comparison with GV, TV and GV, while HC showed an intermediate value (63.5%) not significantly different from the other ecosystems. Soil organic matter is well humified, as indicated by the DH values (56-82%), higher in the two vineyards (71-72%) and in the former vineyards (82%); the higher is DH the higher is the soil ability to humify the organic materials available, meaning a higher chemical and biological stability of organic matter in the soil (Allison, 1973). Differences among land uses might be ascribed to the effect of a reduced soil disturbance in PA and CO, as well as to an increase of C inputs in these ecosystems. DH values are consistent with the reference value of 70-80% which is commonly measured in the Italian soils (Benedetti et al., 2006). HR, indicating the fraction of humified carbon in comparison with SOC, was higher in the two vineyards (about 52%) and in the former vineyards (58%), in agreement with DH and with significant differences in comparison to PA and CO ecosystems. HI was lower and significantly different in the two vineyards (0.41-0.44) and in the former vineyards (0.25). The higher and significantly different values in PA and CO (0.91-0.93) might indicate that mineralization prevails on humification, in agreement with DH and HR values.

3.2. Soil biological fertility parameters

Microbial biomass carbon (Cmic) showed the highest and significantly different value (193.8 mg C kg^{-1}) in the CO ecosystem (Table 6), followed by GV (156.3 mg C kg^{-1}), but in the latter case Cmic value was not significantly different in comparison with PA and TV land uses (114.9 and 119.9 mg C kg^{-1} respectively). The lowest and significantly different values were found in HC and FV (78.7 and 58.5 mg C kg^{-1} respectively). The ranking for Cmic was CO>GV>TV>PA>HC>FV. Similarly to SOC pools, microbial biomass carbon (Cmic) increased in the less disturbed ecosystems, reflecting mainly ranking already observed in SOC values. Cmic and its ratio to SOC (q*mic*) can indicate both a larger substrate availability to the soil microorganisms (Anderson and Domsch, 1989), and an increasing trend to stock organic C in the long term in the natural or less disturbed systems, i.e. CO, and GV in this study.

Cbas (mg CO_2–C kg^{-1} d^{-1}) and the cumulated respiration after 25 days of incubation (Ccum in mg CO_2–C kg^{-1}) were significantly higher in CO (Cbas 11.7, Ccum 486.3) and to a lesser extent in GV (Cbas 4.8, Ccum 234.1). The rankings were CO>GV>TV>FV>PA>HC for Cbas, CO>GV>FV>TV>PA>HC for Ccum. We found the highest values of Cbas and Ccum in CO, and might suppose that in this land use the higher C inputs from vegetation to soil were counterbalanced by an increase in microbial respiration.

The metabolic quotient (qCO_2), showed values always high (Table 6), independently from the land use intensity. qCO_2 was significantly different in CO and FV compared with the other land uses. The ranking was CO>FV>TV>GV>PA>HC. It must be considered that for qCO_2 the less is better (Insam and Haseiwandter, 1989; Anderson, 1994; Andrews et al., 2004), i.e. more favorable conditions for microbial survival are present, while high values are indicative of an increase of CO_2 related to a microbial stress (Jenkinson and Ladd, 1981; Andersen, 2003). High qCO_2 values can be ascribed also to changes in the bacterial-to-fungal ratio (Sakamoto and Oba, 1994; Landi et al., 2000; Nannipieri et al., 2003).

The mineralization quotient (qM) was higher and significantly different in CO (2.36%), FV (1.66%) and GV (1.54%), indicating a better efficiency of micro-organisms in metabolizing the organic matter (Mocali et al., 2008). The ranking was CO>FV>GV>TV>HC>PA.

Overall, results indicated that the land uses with none or lower disturbance (cork-oak forest and pasture) showed as average a more stable chemical, biochemical and microbiological condition in comparison with the others. Similarly, Moscatelli et al. (2007) and van Eekeren et al. (2008) found that SOM content in permanent or grazed grasslands was higher than in permanent arable lands more disturbed by tillage. Pastures have a great potential to stock SOC (Franzluebbers et al., 2000), and given the organic input by grazing, PA ecosystem might improve its soil biological fertility in the long term, and potentially store as much SOC as the native forest.

3.3. Soil biological fertility index

To calculate the biological fertility index, soil organic matter (SOM) and microbial activity parameters determined in the different ecosystems were used to evaluate their sustainability (Table 7). Data showed that the average scores of the parameters and BFI, and the lower and upper values based on the standard deviation of the measured parameters. Cork oak forest ecosystem (CO) showed the highest BFI (18 as average, range 16-20), and ranked in the medium-good class of soil biological fertility commonly observed in in Italy (Renzi et al., 2017). Tilled vineyard (TV), no-tilled grassed vineyard (GV) and pasture (PA) had lower BFIs (12 as average, range 11-15), ranking in the pre stress-medium BFI class. The other land uses (FV and HC) had the lowest BFIs (10-11 as average, range 9-12), and the corresponding soil biological fertility class was always typical of a pre-stress (alarm) situation. This was particularly evident in hay crop (HC), the more disturbed ecosystem due to the frequent tillage operations, which had the lowest BFI (10 as average, range 9-12) denoting a low condition of biological fertility which highlights the need for a sustainable management of pasturelands to avoid soil degradation if improperly managed (Papini et al., 2011).

Results confirmed the relevance of land use changes in maintaining soil biological fertility, as well as the need to adopt sustainable practices at lower intensification management to avoid soil degradation. In particular, pastures should be managed with the minimum soil disturbance by tillage, allowing a higher resilience both of SOM and microbial activity parameters. Moreover, supplementary irrigation in vineyards enhances SOC mineralization offsetting the positive effect of the grass cover, and should be avoided.

4. Conclusions

The study of total SOC and its pools, and microbial activity parameters, allowed deriving relevant conclusions about the effect of increasing levels of land use intensification in the studied ecosystems. Pasture and cork oak forest showed the highest contents of SOC in the different pools (total, extractable, humic and fulvic, not humified, and not extractable), while microbial biomass carbon and respiration activity were higher in cork oak forest and grassed vineyard. Given the organic input by roots, plant material and grazing, and the lower soil disturbance by tillage, pasture might potentially increase the storage of soil organic carbon.

The BFI index indicated a higher soil biological fertility under cork oak forest, and we can reasonably suppose that the maintenance of this ecosystem can increase globally the soil biological fertility level and the microbial diversity on the area. Pasture might improve its soil biological fertility in the long-term, as well as the grassed vineyards provided that supplementary irrigation is avoided.

The methodology presented in this research might be easily applied to other ecosystems with available information on soil and land use management, possibly coupled with more detailed and complex microbiological studies about functional and genetic soil biodiversity.

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Organic carbon pools and soil biological fertility are affected by land use intensity in Mediterranean ecosystems of Sardinia, Italy

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Abstract

Soil quality is mainly studied from the chemical and physical point of view, whereas soil biochemical and microbiological parameters are relatively more scarcely explored to assess the effect of management practices. This study aimed to evaluate soil organic carbon (SOC) and its pools; soil microbial activity parameters; and the Biological Fertility Index (BFI), in six land uses characteristics of the Mediterranean basin in north-eastern Sardinia. These land uses differed in management intensity and consisted of: tilled vineyard (TV), no tilled grassed vineyard (GV), former vineyards (FV), hay crop and pasture (HC and PA), cork oak forest (CO).

Significant differences among ecosystems were found in most cases in (SOC), the related pools (total extractable carbon, humic and fulvic acids, not humified, not extractable), humification parameters (degree, rate and index of humification), and soil microbial activity (microbial carbon, respiration, metabolic quotient, and mineralization quotient). Pasture and cork oak forest showed in average a better soil quality for most biochemical and microbial parameters in comparison with the other ecosystems. The index of soil biological fertility (BFI) was higher under cork oak forest which is supposed to be the most sustainable ecosystem in the long term in this environment, able to maintain soil biological fertility and microbial diversity.

Keywords: Land use intensity; Soil organic carbon pools; Biological fertility index; Mediterranean ecosystems

1. Introduction

Soil quality studies have focused mainly on chemical (Bone et al., 2014) such as soil organic carbon (SOC) and pH (Zornoza et al., 2015), and physical soil quality parameters such as aggregate stability, bulk density and soil porosity (Pulido-Moncada et al., 2015; Zornoza et al., 2015). Some authors have suggested minimum datasets of soil parameters to assess soil quality deriving from: i) a combination of physical and biological parameters, e.g. soil texture, bulk density, water retention, organic carbon, soil biomass and biomass activity (Doran and Parkin, 1994), or ii) physical, chemical and biological characteristics, e.g. total C and N, anaerobically mineralizes N, pH, phosphate, bulk density and macroporosity (Sparling et al., 2003; Hedo et al., 2015; Muñoz-Rojas et al., 2016).

Land use, sensitivity to change in soil management, ease of measure in routine laboratory analyses for soil monitoring, and the relations with soil functions have been included among the attributes for the selections of indicators (Verhoef, 2004). Biogeochemical cycles and biological indicators need to be addressed as a tool to assess different ecosystem services that are supported by soil properties and functions (Smith et al., 2011; Costantini et al., 2016), and in this context microbial biomass and enzyme activities are the most studied indicators (Abbott and Murphy 2007; Zornoza et al., 2015). In addition, most used indicators include microbial respiration and enzyme activities, and related indices (Kieft et al., 1998; Bastida et al., 2006; Muscolo et al., 2014; Muñoz-Rojas et al., 2016). For example, the Biologic Index of Fertility (BIF) includes respiration and enzymatic activities (Stefanic et al., 1984). Kang et al. (2005) proposed the Microbial index of soil (Mi) based on microbial biomass Carbon (C) and Nitrogen (N), potentially mineralizable N, soil respiration, bacterial population, mycorrhizal infection, and dehydrogenase and phosphatase activities, combined with a crop index and a nutrient index. The Enzyme Activity Number (EAN) includes the activity of five enzymes, namely dehydrogenase, catalase, alkaline phosphatase, amylase and protease (Beck, 1994). But specific indexes combining a limited number of easy measurable biochemical and microbial parameters are less studied, and a general agreement on the selection of proper indicators to assess soil biological fertility is still missing (Beed et al., 2011).

When considering the link between soil quality and agricultural management, less disturbed land uses may have a high soil quality and SOC content (Parras-Alcántara et al., 2015), and soil aggregation (Grandy and Robertson, 2007), which in turn may influence soil microbial community activity (García-Orenes et al., 2010; Camilli et al., 2016).

Ecosystems in the Mediterranean Basin are considered important for their plant diversity, but are more prone to land degradation due to soil degradation and SOC depletion, often coupled with erosive processes (Muñoz-Rojas et al., 2015) due to extensive land use changes in the last decades (Anaya-Romero et al., 2011). Studies dealing with the effects of different land use intensities on the biochemical and microbiological characteristics in these ecosystems are rather limited (Caravaca et al., 2002; Riffaldi et al., 2002; García-Orenes et al., 2010; Marzaioli et al., 2010, Lagomarsino et al., 2011; Novara et al., 2012; Bevivino et al., 2014; Laudicina et al., 2015), and available studies have not yet been fully addressed to the application of comprehensive indicators in complex Mediterranean mosaic landscapes, where different ecosystems typical of the heterogeneous agricultural land uses adopted in the study area spatially coexist.

An indicator system called soil Biological Fertility Index (BFI) was proposed for soil monitoring in Italy (Pompili et al., 2008; Renzi et al., 2017). In detail, the index is based on soil organic matter (SOM=SOC×1.724), basal respiration at the last day of incubation (Cbas), cumulated respiration during the incubation period (Ccum), microbial biomass carbon (Cmic), and metabolic quotient (qCO_2). The indicator has proved to be more sensitive than microbial activity or microbial biomass alone to detect differences in soil quality, and to discriminate soil biological fertility status under different treatments (Pompili et al., 2008; Renzi and Benedetti, 2015; Renzi et al., 2017).

The aims of this study were: i) to evaluate a set of soil chemical, biochemical and microbial parameters under different land use intensities frequently adopted in the agro-ecosystems of the

Mediterranean area, ii) to test the Biological Fertility Index in these specific conditions, and iii) to derive information about the ecosystem sustainability in terms of soil biological fertility.

2. Materials and methods

2.1. The study site

The study site is located in the Berchidda district (40° 46’ N, 9° 10’ E), in the Gallura region of north-eastern Sardinia (Italy). In the study area (Fig. 1), long-term human activities have created a spatial mosaic landscape of ecosystems (Eldon and Gershenson, 2015), where patches of native vegetation (cork-oak forest) are intermingled with more intensive cropped ecosystems (vineyards), and pastures at different level of intensification (Bevivino et al., 2014). In addition, natural revegetated areas (scrublands, maquis and Helichrysum meadows) are present.

Climate is warm temperate with dry and hot summers, and mean annual rainfall and temperature are 623 mm and 15.0°C respectively. Long term climate data (1985-2006) were obtained from Servizio Agrometeorologico Regionale (SAR) of Sardinia Region. The area is hilly (mean altitude 285 m a.s.l.), soils have sandy-loam and loamy-sand textures, derive from granitic rocks, and are classified as Dystric and Eutric Cambisols (IUSS Working Group WRB, 2015).

Six land uses intensities as described in Francaviglia et al. (2014) were compared in monitoring plots (Table 1): Tilled vineyard (TV), 0.99 ha; No-tilled grassed vineyard (GV), 1.94 ha; Former vineyard (FV), 1.15 ha, presently naturally revegetated after the abandonment of vineyards; Hay crop (HC), 3.31 ha, with 5 years of cereals or legumes grown for hay, followed by spontaneous herbaceous vegetation in the sixth year; Pasture (PA), 3.17 ha, with 5 years of spontaneous herbaceous vegetation, and one year of hay crop; Cork oak forest (CO), 1.22 ha. The total surface of the study area is 1472 ha, where TV represent 5% of the total area (73.2 ha), GV 3.8% (55.2 ha), FV 3.7% (54.7 ha), HC 7.9% (115.7 ha), PA 34.3% (505 ha), and CO 26.8% (395 ha). Other land uses (e.g. broad-leafed and coniferous woodlands) were not included in this study (218.8 ha, 14.9%), while urban areas occupy 55.1 ha (3.7% of the total area).

Scattered cork-oak trees are included in PA and HC, and grazing activity by sheep is present during some months of the year. As a fact, these ecosystems are derived from the former cork forests, and are considered part of the silvo-pastoral ecosystem known as Mediterranean Dehesa landscape (Francaviglia et al., 2012; Lozano-García et al., 2016).

2.2. Monitoring scheme and soil characterization

The monitoring scheme was setup to highlight possible differences due to land use and management intensity within similar climatic and pedologic conditions. A random sampling scheme was adopted (Fig. 1), pits were digged with a mini excavator, and samples for a general characterization of entire soil profiles were taken along the different soil horizons using a hand trowel. Main soil characteristics are reported in Table 2, and data presented were normalized at a fixed depth (20 cm) to enable the comparison among the different soil profiles.

Soil samples for the biochemical and biological fertility determinations were collected from the topsoil layer (20 cm) in February, May and November 2007, with three random replicates in each land use to consider local differences due to soil slope and/or vegetation heterogeneity. Each replicate was composed of three subsamples combined into one composite sample, and the total number of samples analyzed for each land use during the study was nine (3 replicates × 3 periods). Samplings were limited to 20 cm of topsoil excluding the subsoil, since the main changes in soil microbial parameters due to conversion among land uses can be expected in the upper centimeters (Conant et al. 2001).

2.3. Laboratory analyses

Samples were air-dried at ambient temperature (less than one week), and the analyses were made on the < 2 mm dried soil fraction after sieving.

Soil reaction (pH) was determined in 1:2.5 soil:water suspension by potentiometric method using an Orion Ionalyzer Model 901 pH meter, particle-size analysis with the wet sieving and sedimentation procedure after pre-treatment with hydrogen peroxide to remove organic matter and cementing substances, soil texture according to the USDA classification (Soil Survey Staff, 2011): clay (< 0.002 mm), silt (< 0.05 mm), sand (< 2 mm), total N with the Kjeldahl method (Bremner and Sparks, 1996), available P with a spectrophotometer using the Olsen method, cation exchange capacity as sum of exchangeable cations (CEC), and exchangeable K with the barium chloride-triethanolamine method buffered at pH 8.2 with an atomic absorption spectrophotometer.

Soil organic carbon (SOC) was determined with the Springer-Klee method (1954), and soil organic matter was determined by SOM=SOC×1.724 applying the Van Bemmelen coefficient (Nelson and Sommers, 1982). Total extractable carbon (TEC), and humic and fulvic acid carbon (HA+FA) were determined by the dichromate oxidation method (Nelson and Sommers, 1982). Not humified and more labile C fraction (NHC) was calculated by the difference [TEC–(HA+FA)], and not extractable organic carbon (NEC), conventionally defined as humin (a pool of organic carbon recalcitrant to microbial degradation), by the difference (SOC–TEC). Humification parameters DH (degree of humification), HR (humification rate), and HI (humification index) were determined according to Sequi et al. (1986) and Ciavatta et al. (1990). DH is given by (HA+FA×100)/TEC, HR by (HA+FA×100)/SOC, and HI (dimensionless) by (TEC–HA+FA)/HA+FA.

Microbial biomass carbon (Cmic), expressed in mg C kg^{-1} soil, was determined with the chloroform fumigation-extraction method (Vance et al., 1987), on air-dried soils, pre-conditioned by a 10-d incubation in open glass jars at field capacity and 30°C.

To measure soil microbial respiration, 25 g of sample were placed in closed glass jars, and incubated in the dark at field capacity and 30°C. The CO_2 evolved was trapped by 0.5 N NaOH after 1, 2, 4, 7, 10, 14, 17, 21, 25 days of incubation, and determined by titration of the excess NaOH with 0.5 N HCl (Isermeyer, 1952). Basal respiration (Cbas) is the respiration rate at the last day of incubation in mg CO_2–C kg^{-1} soil d^{-1}, Ccum the cumulated respiration during the incubation period in mg CO_2–C kg^{-1} soil. The metabolic quotient (qCO_2) is the hourly CO_2 evolved per unit of microbial biomass. It expresses the relation between the activity (basal respiration) and the carbon content of the microbial biomass, i.e. the amount of CO_2–C produced per unit microbial biomass carbon, and allows evaluating the effects of external disturbances. The unit is mg CO_2–C 10^{-2} h^{-1} mg $Cmic^{-1}$ (Anderson and Domsch, 1990; 1993). The mineralisation quotient (qM) is the ratio between the cumulated respiration and the SOC, and is expressed in %. The quotient indicates the efficiency of micro-flora in metabolising SOC (Dommergues, 1960).

2.4. Soil biological fertility index

The Biological Fertility Index (BFI) is an indicator system considering SOM, Cbas, Ccum, Cmic, qCO_2 and qM. Five intervals of values were set for each parameter, and scores increasing from 1 to 5 were assigned to each interval (Table 3) based on evidences deduced from earlier studies available in the literature (Brookes, 1995; Vance et al., 1987; Oberholzer and Höper, 2000; Bloem et al., 2006). The algebraic sum of the scores for each parameter providing the proposed classes of biological fertility is shown in Table 4.

Previous validations of the indicator were carried out in Italy: i) in two land uses (grassland and maize crop) with different fertilization treatments (no fertilization, manure+mineral, and sludge), where results indicated a lower soil biological fertility in the sludge treatment (Pompili et al., 2008), and ii) in different sites with pollution from heavy metals or organic compounds, where the indicator allowed to discriminate the soil biological fertility status in comparison with not polluted soils (Renzi and Benedetti, 2015). A recent validation using different combinations of the 6 variables was performed over 1079 soil samples collected in Italy, confirming its appropriateness as a multi-domain indicator to discriminate soil biological fertility (Renzi et al., 2017).

Increasing scores were assigned to SOM, Cbas, Ccum, Cmic and qM when the value of the parameter is increasing, i.e. "the more is better"; conversely qCO_2 has increasing scores when the value of the parameter decreases, i.e. “the less is better” and the ecosystem is more stable (Insam and Haseiwandter, 1989; Anderson, 1994; Andrews et al., 2004).

2.5. Statistical analyses

Statistical analyses were performed using the *Statistica 8.0* software package (Statsoft, Tulsa, USA). Significant differences among means ($p < 0.05$) were evaluated through the Fisher’s protected least significant difference test (LSD post hoc test).

3. Results and discussion

3.1. Soil organic carbon and pools

Pasture (PA) and cork oak forest (CO) showed significantly higher SOC and TEC contents (p<0.05) across ecosystems (Table 5). In particular, SOC and TEC were 2.08% and 1.60% respectively in PA, and 2.10% and 1.66% in CO. In the other land uses, SOC was lower and ranged from 1.32 (FV) to 1.54% (TV, GV), TEC from 0.95 (FV) to 1.15% (HC). The ranking was CO>PA>TV+GV>HC>FV for SOC content, CO>PA>HC>TV+GV>FV for TEC.

No significant differences in SOC were found in the two vineyards ecosystems (TV and GV), and SOC was 1.54% in both. The most likely explanations are: i) the yearly addition to TV of organic carbon as organic fertilizer (200 kg C ha^{-1} $year^{-1}$), and ii) the supplementary irrigation provided in GV in case of water stress. In Mediterranean conditions this might promote microbial activity and enhance SOC mineralization (Nuñes et al., 2007; Butenschoen et al., 2011; Arroita et al., 2013), thus offsetting the positive effect of the grass cover (Lagomarsino et al., 2011; Muñoz-Rojas et al., 2015). PA and HC, even if both grazed by sheep for some months during the year (Table 1), showed significantly different SOC and TEC contents (p<0.05), due to the differences of cropping intensity and the lower soil disturbance by tillage in PA (Francaviglia et al., 2014; Muñoz-Rojas et al., 2015). In particular, SOC content of these ecosystems is in agreement with the findings obtained in Sardinia (Italy) by Salis et al. (2015), reporting 2.11% of SOC for a natural pasture grazed by sheep, and 1.56% for a pasture annually ploughed and sowed for forage production.

Humic and fulvic acids carbon (HA+FA) did not show significant differences among the ecosystems, even if the contents of PA and CO (0.86-0.89%) were slightly higher in comparison with GV, TV, FV, and HC (0.73-0.79%), consistently with SOC and TEC values. (HA+FA) showed a ranking similar to SOC and TEC, i.e. CO>PA>GV>TV>FV>HC. The higher SOC and TEC amounts under PA and CO ecosystems can be due to different reasons: i) the higher organic inputs returned to soil by the plant and root biomass, in contrast with the ecosystems where crop harvesting or removal of pruning residues reduces plant inputs; ii) the organic input from sheep grazing in PA; or iii) the higher microbial decomposition in the more intensive or disturbed ecosystems such as the vineyards and the hay crop (Gregory et al., 2016). Results are in agreement with other findings (Bevivino et al., 2014), reporting a relevant effect of land use intensification on SOC and TEC; a lower agricultural management intensity was related to a higher and more stable chemical and biochemical soil composition.

In addition, we might speculate that CO is in a steady-state condition due to the absence of any soil disturbance, while PA is undoubtedly less disturbed than HC. Furthermore, there is an inherent but not quantifiable source of variation in the data, due the different time periods after the conversion from CO: PA and HC were established in the 70s, and TV and GV in the 90s. FV represent a revegetation of former vineyards, established in the 50s and abandoned in the 70s.

Not humified carbon (NHC) ranged from 0.19 to 0.77% in FV and CO respectively (Table 5), with significantly higher contents under pasture (PA) and cork oak forest (CO), indicating a higher labile C fraction with a rapid turnover in comparison with the other ecosystems (Jenkinson and Ladd, 1981; McGill et al., 1986; Jenkinson and Parry, 1989; von Lützow et al., 2007). The ranking was CO>PA>HC>GV+TV>FV, in agreement with SOC content. Pastures have been shown to transfer a large amount of organic matter to soil (Fischer et al., 1994) mainly through the root systems, as well woody plants are reported to be potential sources of recalcitrant organic materials in soils (Lorenz et al. 2007).

Humin, i.e. not extractable organic carbon (NEC), did not show significant differences among the ecosystems, and ranged from 0.28 to 0.48% in HC and PA respectively. The ranking was PA>CO>GV+TV>FV>HC. The highest contents were found in PA (0.48%) and CO (0.44%), indicating the presence of a C pool more stable and less affected by mineralization processes, and preferentially stabilized in more stable forms chemically or physically protected (Camilli et al., 2016).

The average ratio TEC/SOC was 0.75 (ranging from 0.71 to 0.81), not significantly different among ecosystems. The quite high ratio indicates that most of SOC is not represented by humin (NEC), which is conversely chemically protected by the processes of stabilization mediated by the silt+clay fraction, and less physically protected due to the lowest formation of soil aggregates in sandy soils (Six et al., 2002). Results are in agreement with other findings for sandy soils in Mediterranean environments under pastures, forests and Mediterranean maquis (Trinchera et al., 2015), where a TEC/SOC ratio of 0.79 was found.

Humification degree (DH), humification rate (HR) and humification index (HI) in the different ecosystems are reported in Table 5. Humification parameters (DH, HR, and HI) are commonly considered as an index of soil humification activity as well as of availability of non humified labile fractions (Ciavatta et al., 1990; Vittori Antisari et al., 2010). DH ranged from 81.6 to 56.5% respectively in FV and PA, was significantly different in PA and CO in comparison with GV, TV and GV, while HC showed an intermediate value (63.5%) not significantly different from the other ecosystems. Soil organic matter is well humified, as indicated by the DH values (56-82%), higher in the two vineyards (71-72%) and in the former vineyards (82%); the higher is DH the higher is the soil ability to humify the organic materials available, meaning a higher chemical and biological stability of organic matter in the soil (Allison, 1973). Differences among land uses might be ascribed to the effect of a reduced soil disturbance in PA and CO, as well as to an increase of C inputs in these ecosystems. DH values are consistent with the reference value of 70-80% which is commonly measured in the Italian soils (Benedetti et al., 2006). HR, indicating the fraction of humified carbon in comparison with SOC, was higher in the two vineyards (about 52%) and in the former vineyards (58%), in agreement with DH and with significant differences in comparison to PA and CO ecosystems. HI was lower and significantly different in the two vineyards (0.41-0.44) and in the former vineyards (0.25). The higher and significantly different values in PA and CO (0.91-0.93) might indicate that mineralization prevails on humification, in agreement with DH and HR values.

3.2. Soil biological fertility parameters

Microbial biomass carbon (Cmic) showed the highest and significantly different value (193.8 mg C kg^{-1}) in the CO ecosystem (Table 6), followed by GV (156.3 mg C kg^{-1}), but in the latter case Cmic value was not significantly different in comparison with PA and TV land uses (114.9 and 119.9 mg C kg^{-1} respectively). The lowest and significantly different values were found in HC and FV (78.7 and 58.5 mg C kg^{-1} respectively). The ranking for Cmic was CO>GV>TV>PA>HC>FV. Similarly to SOC pools, microbial biomass carbon (Cmic) increased in the less disturbed ecosystems, reflecting mainly ranking already observed in SOC values. Cmic and its ratio to SOC (q*mic*) can indicate both a larger substrate availability to the soil microorganisms (Anderson and Domsch, 1989), and an increasing trend to stock organic C in the long term in the natural or less disturbed systems, i.e. CO, and GV in this study.

Cbas (mg CO_2–C kg^{-1} d^{-1}) and the cumulated respiration after 25 days of incubation (Ccum in mg CO_2–C kg^{-1}) were significantly higher in CO (Cbas 11.7, Ccum 486.3) and to a lesser extent in GV (Cbas 4.8, Ccum 234.1). The rankings were CO>GV>TV>FV>PA>HC for Cbas, CO>GV>FV>TV>PA>HC for Ccum. We found the highest values of Cbas and Ccum in CO, and might suppose that in this land use the higher C inputs from vegetation to soil were counterbalanced by an increase in microbial respiration.

The metabolic quotient (qCO_2), showed values always high (Table 6), independently from the land use intensity. qCO_2 was significantly different in CO and FV compared with the other land uses. The ranking was CO>FV>TV>GV>PA>HC. It must be considered that for qCO_2 the less is better (Insam and Haseiwandter, 1989; Anderson, 1994; Andrews et al., 2004), i.e. more favorable conditions for microbial survival are present, while high values are indicative of an increase of CO_2 related to a microbial stress (Jenkinson and Ladd, 1981; Andersen, 2003). High qCO_2 values can be ascribed also to changes in the bacterial-to-fungal ratio (Sakamoto and Oba, 1994; Landi et al., 2000; Nannipieri et al., 2003).

The mineralization quotient (qM) was higher and significantly different in CO (2.36%), FV (1.66%) and GV (1.54%), indicating a better efficiency of micro-organisms in metabolizing the organic matter (Mocali et al., 2008). The ranking was CO>FV>GV>TV>HC>PA.

Overall, results indicated that the land uses with none or lower disturbance (cork-oak forest and pasture) showed as average a more stable chemical, biochemical and microbiological condition in comparison with the others. Similarly, Moscatelli et al. (2007) and van Eekeren et al. (2008) found that SOM content in permanent or grazed grasslands was higher than in permanent arable lands more disturbed by tillage. Pastures have a great potential to stock SOC (Franzluebbers et al., 2000), and given the organic input by grazing, PA ecosystem might improve its soil biological fertility in the long term, and potentially store as much SOC as the native forest.

3.3. Soil biological fertility index

To calculate the biological fertility index, soil organic matter (SOM) and microbial activity parameters determined in the different ecosystems were used to evaluate their sustainability (Table 7). Data showed that the average scores of the parameters and BFI, and the lower and upper values based on the standard deviation of the measured parameters. Cork oak forest ecosystem (CO) showed the highest BFI (18 as average, range 16-20), and ranked in the medium-good class of soil biological fertility commonly observed in in Italy (Renzi et al., 2017). Tilled vineyard (TV), no-tilled grassed vineyard (GV) and pasture (PA) had lower BFIs (12 as average, range 11-15), ranking in the pre stress-medium BFI class. The other land uses (FV and HC) had the lowest BFIs (10-11 as average, range 9-12), and the corresponding soil biological fertility class was always typical of a pre-stress (alarm) situation. This was particularly evident in hay crop (HC), the more disturbed ecosystem due to the frequent tillage operations, which had the lowest BFI (10 as average, range 9-12) denoting a low condition of biological fertility which highlights the need for a sustainable management of pasturelands to avoid soil degradation if improperly managed (Papini et al., 2011).

Results confirmed the relevance of land use changes in maintaining soil biological fertility, as well as the need to adopt sustainable practices at lower intensification management to avoid soil degradation. In particular, pastures should be managed with the minimum soil disturbance by tillage, allowing a higher resilience both of SOM and microbial activity parameters. Moreover, supplementary irrigation in vineyards enhances SOC mineralization offsetting the positive effect of the grass cover, and should be avoided.

4. Conclusions

The study of total SOC and its pools, and microbial activity parameters, allowed deriving relevant conclusions about the effect of increasing levels of land use intensification in the studied ecosystems. Pasture and cork oak forest showed the highest contents of SOC in the different pools (total, extractable, humic and fulvic, not humified, and not extractable), while microbial biomass carbon and respiration activity were higher in cork oak forest and grassed vineyard. Given the organic input by roots, plant material and grazing, and the lower soil disturbance by tillage, pasture might potentially increase the storage of soil organic carbon.

The BFI index indicated a higher soil biological fertility under cork oak forest, and we can reasonably suppose that the maintenance of this ecosystem can increase globally the soil biological fertility level and the microbial diversity on the area. Pasture might improve its soil biological fertility in the long-term, as well as the grassed vineyards provided that supplementary irrigation is avoided.

The methodology presented in this research might be easily applied to other ecosystems with available information on soil and land use management, possibly coupled with more detailed and complex microbiological studies about functional and genetic soil biodiversity.

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Table 1

Land uses description and management.

Land use	Establishment	Management	Fertilization (kg ha^{-1})	Irrigation
Tilled vineyard, TV*	1994	Soil tillage with rotovator (Feb., May, Aug.). Pruning in Jan-Feb. Residues removed from the field	200 organic carbon, 62.5-11-42 (N-P-K)	None
No tilled grassed vineyard, GV	1990	No tillage operations. Pruning in May-Jun. Residues left in the field	40-22-42(N-P-K)	Drip irrigation only in case of water stress
Former vineyard, FV	1951 (not cultivated since 1975-76)	-	-	-
Hay crop, HC	1970	Soil tillage (40 cm) and harrowing before sowing 5 years out of 6. Grazed (Jan-Mar) with 3-4 sheep ha^{-1}	50-39 (N-P) before sowing	None
Pasture, PA	1970	Soil tillage (40 cm) and harrowing before sowing 1 year out of 6. Grazed (Dec-Jun) with 6 sheep ha^{-1}	50-39 (N-P) before sowing	None
Cork oak forest, CO	Native vegetation	Used for cork production and cattle grazing	-	-

*TV is under organic farming, and a fertilizer for organic agriculture is supplied

Table 2

Main soil parameters in the 0-20 cm soil layer (mean ± SD).

Land use	Sand ($g\ kg^{-1}$)	Clay ($g\ kg^{-1}$)	Silt ($g\ kg^{-1}$)	Texture	pH	Total N ($g\ kg^{-1}$)	Available P ($mg\ kg^{-1}$)	CEC ($meq\ 100g^{-1}$)	Exchangeable K ($meq\ 100g^{-1}$)
TV (n=4)	830±34	120±14	50±44	Loamy Sand	5.1±0.1	0.99±0.07	34±4	12.67±2.16	0.22±0.08
GV (n=3)	800±6	115±13	85±14	Sandy Loam	6.2±0.3	0.93±0.05	30±14	15.03±1.97	0.75±0.18
FV (n=7)	791±36	101±31	108±37	Sandy Loam	6.0±0.3	1.07±0.05	6±2	18.08±5.50	0.24±0.10
HC (n=5)	733±5	131±9	136±5	Sandy Loam	5.6±0.4	1.73±0.45	35±22	16.94±4.55	0.51±0.24
PA (n=4)	732±12	135±8	133±5	Sandy Loam	5.5±0.4	2.08±0.15	26±8	17.17±3.73	0.44±0.08
CO (n=2)	761±29	116±3	123±26	Sandy Loam	5.7±0.2	1.69±0.22	5±3	17.83±2.29	0.36±0.18

SD standard deviation, TV tilled vineyard, GV no-tilled grassed vineyard, FV former vineyard, HC hay crop, PA pasture, CO cork oak forest, n number of soil profiles studied, CEC cation exchange capacity

Table 3

Scores of the intervals of values for the different parameters.

Parameter	Scores				
	1	2	3	4	5
Soil organic matter SOM (%)	< 1.0	≥ 1.0 ≤ 1.5	> 1.5 ≤ 2.0	> 2.0 ≤ 3.0	> 3.0
Basal respiration Cbas (mg CO_2–C kg^{-1} soil d^{-1})	< 5	≥ 5 ≤ 10	> 10 ≤ 15	> 15 ≤ 20	> 20
Cumulative respiration Ccum (mg CO_2–C kg^{-1} soil)	< 100	≥ 100 ≤ 250	> 250 ≤ 400	> 400 ≤ 600	> 600
Microbial biomass carbon Cmic (mg C kg^{-1} soil)	< 100	≥ 100 ≤ 200	> 200 ≤ 300	> 300 ≤ 400	> 400
Metabolic quotient (qCO_2) (mg CO_2–C 10^{-2} h^{-1} mg $Cmic^{-1}$)	≥ 0.4	< 0.4 ≥ 0.3	< 0.3 ≥ 0.2	< 0.2 ≥ 0.1	< 0.1
Mineralisation quotient qM (%)	< 1.0	≥ 1 ≤ 2	> 2 ≤ 3	> 3 ≤ 4	> 4

Table 4

Classes of the Biological Fertility Index (BFI).

Fertility class	I	II	III	IV	V
	Stress	Pre-stress (alarm)	Medium	Good	High
BFI scores sum	6	7-12	13-18	19-24	25-30

Table 5

Total soil organic carbon, organic carbon pools, and humification parameters in land uses (means ± SD). n=9 (3 replicates × 3 periods).

Land use	SOC (%)	TEC (%)	HA+HF (%)	NHC (%)	NEC (%)	TEC/SOC	DH (%)	HR (%)	HI
TV	1.54±0.26a	1.11±0.13a	0.78±0.12ns	0.32±0.15a	0.43±0.18ns	0.73±0.10ns	71.0±12.1ab	51.3±9.6bc	0.44±0.24a
GV	1.54±0.25a	1.11±0.13a	0.79±0.12ns	0.32±0.12a	0.43±0.16ns	0.73±0.08ns	71.9±8.4b	51.8±4.0bc	0.41±0.16a
FV	1.32±0.25a	0.95±0.23a	0.76±0.13ns	0.19±0.18a	0.38±0.02ns	0.71±0.04ns	81.6±15.9b	57.8±9.7c	0.25±0.22a
HC	1.43±0.28a	1.15±0.22a	0.73±0.16ns	0.42±0.18a	0.28±0.13ns	0.81±0.08ns	63.5±14.1ab	50.3±7.2abc	0.65±0.43ab
PA	2.08±0.14b	1.60±0.31b	0.86±0.05ns	0.74±0.41b	0.48±0.27ns	0.77±0.13ns	56.5±16.3a	41.6±6.2a	0.91±0.59b
CO	2.10±0.20b	1.66±0.14b	0.89±0.23ns	0.77±0.45b	0.44±0.14ns	0.79±0.06ns	56.6±19.3a	43.9±13.2ab	0.93±0.56b

SD standard deviation, TV tilled vineyard, GV no-tilled grassed vineyard, FV former vineyard, HC hay crop, PA pasture, CO cork oak forest, SOC total soil organic carbon, TEC total extractable carbon, HA+FA humic and fulvic acid carbon, NHC not humified carbon, NEC not extractable carbon, DH humification degree, HR humification rate, HI humification index. Different letters in each column indicate significant differences (p<0.05) among ecosystems (Fisher's LSD post hoc test), ns not significant

Table 6

Soil microbial activity parameters in land uses (means ± SD). n=9 (3 replicates × 3 periods).

Land use	Cmic (mg C kg^{-1})	Cbas (mg CO_2–C kg^{-1} d^{-1})	Ccum (mg CO_2–C kg^{-1})	qCO_2 (mg CO_2–C 10^{-2} h^{-1} mg $Cmic^{-1}$)	qM (%)
TV	119.9±26.9ab	4.3±0.9b	189.1±28.5ab	1.77±0.54a	1.26±0.26bc
GV	156.3±39.8b	4.8±0.8b	234.1±41.8b	1.54±0.37a	1.54±0.26c
FV	58.5±6.4a	3.7±0.5ab	215.5±0.93ab	2.63±0.23ab	1.66±0.29c
HC	78.7±24.1a	2.1±0.3a	132.4±31.6a	1.43±0.33a	0.96±0.22ab
PA	114.9±48.1ab	3.2±1.7ab	142.4±21.5a	1.53±0.87a	0.68±0.45a
CO	193.8±62.4b	11.7±4.4c	486.3±28.7c	3.28±1.28b	2.36±0.56d

SD standard deviation, TV tilled vineyard, GV no-tilled grassed vineyard, FV former vineyard, HC hay crop, PA pasture, CO cork oak forest, Cmic microbial biomass carbon, Cbas basal soil respiration, Ccum cumulated soil respiration, qCO_2 metabolic quotient, qM mineralisation quotient. Different letters in each column indicate significant differences ($p<0.05$) among ecosystems (Fisher's LSD post hoc test)

Table 7

Scores of the soil parameters, and Biological Fertility Index for the different land uses (lower and upper scores are in brackets). n=9 (3 replicates × 3 periods).

Land use	SOM	Cbas	Ccum	Cmic	qCO_2	qM	BFI score	BFI class
TV	4 (4-5)	1 (1-2)	2 (2-2)	2 (1-2)	1 (1-1)	2 (2-2)	12 (11-14)	II (II-III)
GV	4 (4-5)	1 (1-2)	2 (2-3)	2 (2-2)	1 (1-1)	2 (2-2)	12 (12-15)	II (II-III)
FV	4 (3-4)	1 (1-1)	2 (2-2)	1 (1-1)	1 (1-1)	2 (2-2)	11 (10-11)	II
HC	4 (4-3)	1 (1-1)	2 (2-2)	1 (1-2)	1 (1-1)	1 (1-2)	10 (9-12)	II
PA	5 (5-5)	1 (1-1)	2 (2-2)	2 (1-2)	1 (1-1)	1 (1-2)	12 (11-13)	II (II-III)
CO	5 (5-5)	3 (2-4)	4 (4-4)	2 (2-3)	1 (1-1)	3 (2-3)	18 (16-20)	III (III-IV)

TV tilled vineyard, GV no-tilled grassed vineyard, FV former vineyard, HC hay crop, PA pasture, CO cork oak forest, SOM soil organic matter (SOC×1.724), Cmic microbial biomass carbon, Cbas basal soil respiration, Ccum cumulated soil respiration, qCO_2 metabolic quotient, qM mineralisation quotient, BFI Biological Fertility Index, II pre-stress class, III medium class, IV good class.

Figure 1
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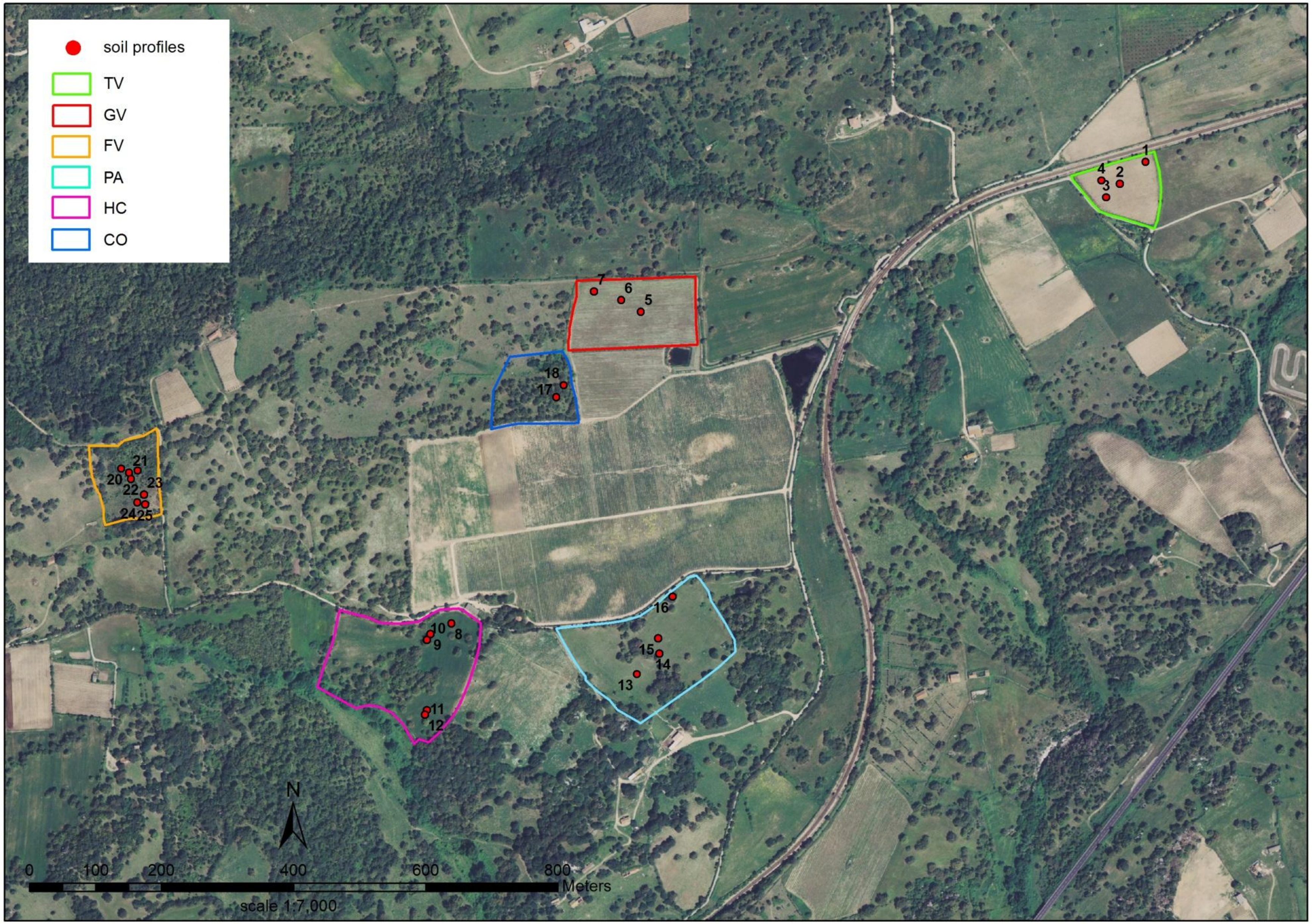

Fig. 1. Study area in northeast Sardinia (Italy).

TV tilled vineyard, GV no-tilled grassed vineyard, FV former vineyard, HC hay crop, PA pasture, CO cork oak forest

Figure 1 revised source

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