

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

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(Article begins on next page)

Land use intensity



Vineyards



Natural revegetation



Pastures



Native vegetation

13 **Abstract**

14

15 Soil quality is mainly studied from the chemical and physical point of view, **whereas** soil
16 biochemical and microbiological parameters are relatively more scarcely explored to assess the
17 effect of management practices. **This study aimed to evaluate soil organic carbon (SOC) and its**
18 **pools; soil microbial activity parameters; and the Biological Fertility Index (BFI), in six land uses**
19 **characteristics of the Mediterranean basin in north-eastern Sardinia. These land uses differed in**
20 **management intensity and consisted of: tilled vineyard (TV), no tilled grassed vineyard (GV),**
21 **former vineyards (FV), hay crop and pasture (HC and PA), cork oak forest (CO).**
22 **Significant** differences among **ecosystems** were found **in most cases** in **(SOC)**, the related pools
23 (total extractable carbon, humic and fulvic acids, not humified, not extractable), humification
24 parameters (degree, rate and index of humification), and soil microbial activity (microbial carbon,
25 respiration, metabolic quotient, and mineralization quotient). **Pasture** and **cork** oak forest showed **in**
26 average a better soil quality for **most biochemical and microbial** parameters in comparison with the
27 other ecosystems. The index of soil biological fertility (BFI) **was** higher under **cork** oak forest
28 which is **supposed to be** the most sustainable ecosystem in the long term in this environment, **able**
29 **to maintain soil biological fertility and microbial diversity.**

30

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

33

34

35 1. Introduction

36

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

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

61 biochemical and microbial parameters are less studied, and a general agreement on the selection of
62 proper indicators to assess soil biological fertility is still missing (Beed et al., 2011).

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

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

77 An indicator system called soil Biological Fertility Index (BFI) was proposed for soil monitoring in
78 Italy (Pompili et al., 2008; Renzi et al., 2017). In detail, the index is based on soil organic matter
79 ($SOM = SOC \times 1.724$), basal respiration at the last day of incubation (C_{bas}), cumulated respiration
80 during the incubation period (C_{cum}), microbial biomass carbon (C_{mic}), and metabolic quotient
81 (qCO_2). The indicator has proved to be more sensitive than microbial activity or microbial biomass
82 alone to detect differences in soil quality, and to discriminate soil biological fertility status under
83 different treatments (Pompili et al., 2008; Renzi and Benedetti, 2015; Renzi et al., 2017).

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

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

88

89 2. Materials and methods

90

91 2.1. The study site

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

98 Climate is warm temperate with dry and hot summers, and mean annual rainfall and temperature are
99 623 mm and 15.0°C respectively. Long term climate data (1985-2006) were obtained from Servizio
100 Agrometeorologico Regionale (SAR) of Sardinia Region. The area is hilly (mean altitude 285 m
101 a.s.l.), soils have sandy-loam and loamy-sand textures, derive from granitic rocks, and are classified
102 as Dystric and Eutric Cambisols (IUSS Working Group WRB, 2015).

103 Six land uses intensities as described in Francaviglia et al. (2014) were compared in monitoring
104 plots (Table 1): Tilled vineyard (TV), 0.99 ha; No-tilled grassed vineyard (GV), 1.94 ha; Former
105 vineyard (FV), 1.15 ha, presently naturally revegetated after the abandonment of vineyards; Hay
106 crop (HC), 3.31 ha, with 5 years of cereals or legumes grown for hay, followed by spontaneous
107 herbaceous vegetation in the sixth year; Pasture (PA), 3.17 ha, with 5 years of spontaneous
108 herbaceous vegetation, and one year of hay crop; Cork oak forest (CO), 1.22 ha. The total surface of
109 the study area is 1472 ha, where TV represent 5% of the total area (73.2 ha), GV 3.8% (55.2 ha),
110 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

111 uses (e.g. broad-leafed and coniferous woodlands) were not included in this study (218.8 ha,
112 14.9%), while urban areas occupy 55.1 ha (3.7% of the total area).

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

117

118 *2.2. Monitoring scheme and soil characterization*

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

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

133

134 *2.3. Laboratory analyses*

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

137 Soil reaction (pH) was determined in 1:2.5 soil:water suspension by potentiometric method using an
138 Orion Ionalyzer Model 901 pH meter, particle-size analysis with the wet sieving and sedimentation
139 procedure after pre-treatment with hydrogen peroxide to remove organic matter and cementing
140 substances, soil texture according to the USDA classification (Soil Survey Staff, 2011): clay (<
141 0.002 mm), silt (< 0.05 mm), sand (< 2 mm), total N with the Kjeldahl method (Bremner and
142 Sparks, 1996), available P with a spectrophotometer using the Olsen method, cation exchange
143 capacity as sum of exchangeable cations (CEC), and exchangeable K with the barium chloride-
144 triethanolamine method buffered at pH 8.2 with an atomic absorption spectrophotometer.

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

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

158 To measure soil microbial respiration, 25 g of sample were placed in closed glass jars, and
159 incubated in the dark at field capacity and 30°C. The CO_2 evolved was trapped by 0.5 N NaOH
160 after 1, 2, 4, 7, 10, 14, 17, 21, 25 days of incubation, and determined by titration of the excess
161 NaOH with 0.5 N HCl (Isermeyer, 1952). Basal respiration (C_{bas}) is the respiration rate at the last
162 day of incubation in $mg\ CO_2-C\ kg^{-1}\ soil\ d^{-1}$, C_{cum} the cumulated respiration during the incubation

163 period in $\text{mg CO}_2\text{-C kg}^{-1}$ soil. The metabolic quotient ($q\text{CO}_2$) is the hourly CO_2 evolved per unit of
164 microbial biomass. It expresses the relation between the activity (basal respiration) and the carbon
165 content of the microbial biomass, **i.e. the amount of $\text{CO}_2\text{-C}$ produced per unit microbial biomass**
166 **carbon**, and allows evaluating the effects of external disturbances. The unit is $\text{mg CO}_2\text{-C } 10^{-2} \text{ h}^{-1}$
167 mg Cmic^{-1} (Anderson and Domsch, 1990; 1993). The mineralisation quotient ($q\text{M}$) is the ratio
168 between the cumulated respiration and the **SOC**, and is expressed in %. The quotient indicates the
169 efficiency of micro-flora in metabolising **SOC** (Dommergues, 1960).

170

171 **2.4. Soil biological fertility index**

172 The Biological Fertility Index (BFI) is **an indicator system** considering SOM, C_{bas} , C_{cum} , C_{mic} ,
173 $q\text{CO}_2$ and $q\text{M}$. Five intervals of values **were** set for each parameter, and **scores** increasing from 1 to
174 5 **were** assigned to each interval (Table 3) **based on evidences deduced from earlier studies**
175 **available in the literature (Brookes, 1995; Vance et al., 1987; Oberholzer and Höper, 2000; Bloem**
176 **et al., 2006)**. The algebraic sum of the scores for each parameter **providing the proposed** classes of
177 biological fertility is shown in Table 4.

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

186 Increasing scores **were** assigned to SOM, C_{bas} , C_{cum} , C_{mic} and $q\text{M}$ when the value of the
187 parameter is increasing, i.e. “the more is better”; conversely $q\text{CO}_2$ has increasing scores when the

188 value of the parameter decreases, i.e. “the less is better” and the ecosystem is more stable (Insam
189 and Haseiwandter, 1989; Anderson, 1994; Andrews et al., 2004).

190

191 2.5. Statistical analyses

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

195

196 3. Results and discussion

197

198 3.1. Soil organic carbon and pools

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

204 No significant differences in SOC were found in the two vineyards ecosystems (TV and GV), and
205 SOC was 1.54% in both. The most likely explanations are: i) the yearly addition to TV of organic
206 carbon as organic fertilizer (200 kg C ha⁻¹ year⁻¹), and ii) the supplementary irrigation provided in
207 GV in case of water stress. In Mediterranean conditions this might promote microbial activity and
208 enhance SOC mineralization (Nuñez et al., 2007; Butenschoen et al., 2011; Arroita et al., 2013),
209 thus offsetting the positive effect of the grass cover (Lagomarsino et al., 2011; Muñoz-Rojas et al.,
210 2015). PA and HC, even if both grazed by sheep for some months during the year (Table 1),
211 showed significantly different SOC and TEC contents ($p < 0.05$), due to the differences of cropping
212 intensity and the lower soil disturbance by tillage in PA (Francaviglia et al., 2014; Muñoz-Rojas et
213 al., 2015). In particular, SOC content of these ecosystems is in agreement with the findings obtained

214 in Sardinia (Italy) by Salis et al. (2015), reporting 2.11% of SOC for a natural pasture grazed by
215 sheep, and 1.56% for a pasture annually ploughed and sowed for forage production.

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

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

233 Not humified carbon (NHC) ranged from 0.19 to 0.77% in FV and CO respectively (Table 5), with
234 significantly higher contents under pasture (PA) and cork oak forest (CO), indicating a higher labile
235 C fraction with a rapid turnover in comparison with the other ecosystems (Jenkinson and Ladd,
236 1981; McGill et al., 1986; Jenkinson and Parry, 1989; von Lützow et al., 2007). The ranking was
237 CO>PA>HC>GV+TV>FV, in agreement with SOC content. Pastures have been shown to transfer a
238 large amount of organic matter to soil (Fischer et al., 1994) mainly through the root systems, as well

239 woody plants are reported to be potential sources of recalcitrant organic materials in soils (Lorenz et
240 al. 2007).

241 Humin, i.e. not extractable organic carbon (NEC), did not show significant differences among the
242 ecosystems, and ranged from 0.28 to 0.48% in HC and PA respectively. The ranking was
243 PA>CO>GV+TV>FV>HC. The highest contents were found in PA (0.48%) and CO (0.44%),
244 indicating the presence of a C pool more stable and less affected by mineralization processes, and
245 preferentially stabilized in more stable forms chemically or physically protected (Camilli et al.,
246 2016).

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

254 Humification degree (DH), humification rate (HR) and humification index (HI) in the different
255 ecosystems are reported in Table 5. Humification parameters (DH, HR, and HI) are commonly
256 considered as an index of soil humification activity as well as of availability of non humified labile
257 fractions (Ciavatta et al., 1990; Vittori Antisari et al., 2010). DH ranged from 81.6 to 56.5%
258 respectively in FV and PA, was significantly different in PA and CO in comparison with GV, TV
259 and GV, while HC showed an intermediate value (63.5%) not significantly different from the other
260 ecosystems. Soil organic matter is well humified, as indicated by the DH values (56-82%), higher in
261 the two vineyards (71-72%) and in the former vineyards (82%); the higher is DH the higher is the
262 soil ability to humify the organic materials available, meaning a higher chemical and biological
263 stability of organic matter in the soil (Allison, 1973). Differences among land uses might be
264 ascribed to the effect of a reduced soil disturbance in PA and CO, as well as to an increase of C

265 **inputs in these ecosystems.** DH values are consistent with the reference value of 70-80% which is
266 commonly measured in the Italian soils (Benedetti et al., 2006). HR, indicating the fraction of
267 humified carbon in comparison with SOC, was higher in the two vineyards (about 52%) and in the
268 former vineyards (58%), in agreement with DH and with significant differences in comparison to
269 PA and CO ecosystems. HI was lower and significantly different in the two vineyards (0.41-0.44)
270 and in the former vineyards (0.25). The higher and significantly different values in PA and CO
271 (0.91-0.93) **might** indicate that mineralization prevails on humification, in agreement with DH and
272 HR values.

273

274 3.2. Soil biological fertility parameters

275 Microbial biomass carbon (Cmic) showed the highest and significantly different value (193.8 mg C
276 kg⁻¹) in the CO ecosystem (Table 6), followed by GV (156.3 mg C kg⁻¹), but in the latter case Cmic
277 value was not significantly different in comparison with PA and TV **land uses** (114.9 and 119.9 mg
278 C kg⁻¹ respectively). The lowest and significantly different values were found in HC and FV (78.7
279 and 58.5 mg C kg⁻¹ respectively). The ranking for Cmic was CO>GV>TV>PA>HC>FV. Similarly
280 to **SOC** pools, microbial biomass carbon (Cmic) increased in the less disturbed ecosystems,
281 reflecting mainly ranking already observed in SOC values. **Cmic and its ratio to SOC (qmic) can**
282 indicate both a larger substrate availability to the soil microorganisms (Anderson and Domsch,
283 1989), **and an increasing trend to stock organic C in the long term in the natural or less disturbed**
284 **systems, i.e. CO, and GV in this study.**

285 Cbas (mg CO₂-C kg⁻¹ d⁻¹) and the cumulated respiration after 25 days of incubation (Ccum in mg
286 CO₂-C kg⁻¹) were significantly higher in CO (Cbas 11.7, Ccum 486.3) and to a lesser extent in GV
287 (Cbas 4.8, Ccum 234.1). The rankings were CO>GV>TV>FV>PA>HC for Cbas,
288 CO>GV>FV>TV>PA>HC for Ccum. **We found the highest values of Cbas and Ccum in CO, and**
289 **might suppose that in this land use the higher C inputs from vegetation to soil were counterbalanced**
290 **by an increase in microbial respiration.**

291 The metabolic quotient (qCO_2), showed values always **high** (Table 6), independently from the land
292 use intensity. **qCO_2 was significantly different in CO and FV compared with the other land uses.**
293 **The ranking was CO>FV>TV>GV>PA>HC.** It must be considered that for qCO_2 the less is better
294 (Insam and Haseiwandter, 1989; Anderson, 1994; Andrews et al., 2004), i.e. more favorable
295 conditions for microbial survival are present, while high values are indicative of an increase of CO_2
296 related to a microbial stress (Jenkinson and Ladd, 1981; Andersen, 2003). **High qCO_2 values can be**
297 **ascribed also** to changes in the bacterial-to-fungal ratio (Sakamoto and Oba, 1994; Landi et al.,
298 2000; Nannipieri et al., 2003).

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

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

309

310 3.3. Soil biological fertility index

311 **To calculate the biological fertility index, soil organic matter (SOM) and microbial activity**
312 **parameters determined in the different ecosystems were used to evaluate their sustainability (Table**
313 **7).** Data **showed that** the average scores of the parameters and BFI, and the lower and upper values
314 based on the standard deviation of the measured parameters. **Cork** oak forest ecosystem (CO)
315 showed the highest BFI (18 as average, range 16-20), and ranked in the medium-good class of soil
316 biological fertility commonly observed in in Italy (Renzi et al., 2017). **Tilled** vineyard (TV), no-

317 tilled grassed vineyard (GV) and pasture (PA) had lower BFIs (12 as average, range 11-15), ranking
318 in the pre stress-medium BFI class. The other **land uses** (FV and HC) had the lowest BFIs (10-11 as
319 average, range 9-12), and the corresponding soil biological fertility class was always typical of a
320 pre-stress (alarm) situation. This was particularly evident in hay crop (HC), the more disturbed
321 ecosystem due to the frequent tillage operations, which had the lowest BFI (10 as average, range 9-
322 12) denoting a low condition of biological fertility which highlights the need for a sustainable
323 management of pasturelands to avoid soil degradation if improperly managed (Papini et al., 2011).

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

330

331 **4. Conclusions**

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

339 The BFI index indicated a higher soil biological fertility under cork oak forest, and we can
340 reasonably suppose that the maintenance of this ecosystem can increase globally the soil biological
341 fertility level and the microbial diversity on the area. **Pasture might improve its soil biological**

342 fertility in the long-term, as well as the grassed vineyards provided that supplementary irrigation is
343 avoided.

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

347

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349

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355

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13 **Abstract**

14

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

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

30

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

33

34

35 **1. Introduction**

36

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

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

61 biochemical and microbial parameters are less studied, and a general agreement on the selection of
62 proper indicators to assess soil biological fertility is still missing (Beed et al., 2011).

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

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

77 An indicator system called soil Biological Fertility Index (BFI) was proposed for soil monitoring in
78 Italy (Pompili et al., 2008; Renzi et al., 2017). In detail, the index is based on soil organic matter
79 ($SOM = SOC \times 1.724$), basal respiration at the last day of incubation (C_{bas}), cumulated respiration
80 during the incubation period (C_{cum}), microbial biomass carbon (C_{mic}), and metabolic quotient
81 (qCO_2). The indicator has proved to be more sensitive than microbial activity or microbial biomass
82 alone to detect differences in soil quality, and to discriminate soil biological fertility status under
83 different treatments (Pompili et al., 2008; Renzi and Benedetti, 2015; Renzi et al., 2017).

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

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

88

89 **2. Materials and methods**

90

91 *2.1. The study site*

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

98 Climate is warm temperate with dry and hot summers, and mean annual rainfall and temperature are
99 623 mm and 15.0°C respectively. Long term climate data (1985-2006) were obtained from Servizio
100 Agrometeorologico Regionale (SAR) of Sardinia Region. The area is hilly (mean altitude 285 m
101 a.s.l.), soils have sandy-loam and loamy-sand textures, derive from granitic rocks, and are classified
102 as Dystric and Eutric Cambisols (IUSS Working Group WRB, 2015).

103 Six land uses intensities as described in Francaviglia et al. (2014) were compared in monitoring
104 plots (Table 1): Tilled vineyard (TV), 0.99 ha; No-tilled grassed vineyard (GV), 1.94 ha; Former
105 vineyard (FV), 1.15 ha, presently naturally revegetated after the abandonment of vineyards; Hay
106 crop (HC), 3.31 ha, with 5 years of cereals or legumes grown for hay, followed by spontaneous
107 herbaceous vegetation in the sixth year; Pasture (PA), 3.17 ha, with 5 years of spontaneous
108 herbaceous vegetation, and one year of hay crop; Cork oak forest (CO), 1.22 ha. The total surface of
109 the study area is 1472 ha, where TV represent 5% of the total area (73.2 ha), GV 3.8% (55.2 ha),
110 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

111 uses (e.g. broad-leafed and coniferous woodlands) were not included in this study (218.8 ha,
112 14.9%), while urban areas occupy 55.1 ha (3.7% of the total area).

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

117

118 *2.2. Monitoring scheme and soil characterization*

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

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

133

134 *2.3. Laboratory analyses*

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

137 Soil reaction (pH) was determined in 1:2.5 soil:water suspension by potentiometric method using an
138 Orion Ionalyzer Model 901 pH meter, particle-size analysis with the wet sieving and sedimentation
139 procedure after pre-treatment with hydrogen peroxide to remove organic matter and cementing
140 substances, soil texture according to the USDA classification (Soil Survey Staff, 2011): clay (<
141 0.002 mm), silt (< 0.05 mm), sand (< 2 mm), total N with the Kjeldahl method (Bremner and
142 Sparks, 1996), available P with a spectrophotometer using the Olsen method, cation exchange
143 capacity as sum of exchangeable cations (CEC), and exchangeable K with the barium chloride-
144 triethanolamine method buffered at pH 8.2 with an atomic absorption spectrophotometer.

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

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

158 To measure soil microbial respiration, 25 g of sample were placed in closed glass jars, and
159 incubated in the dark at field capacity and 30°C. The CO_2 evolved was trapped by 0.5 N NaOH
160 after 1, 2, 4, 7, 10, 14, 17, 21, 25 days of incubation, and determined by titration of the excess
161 NaOH with 0.5 N HCl (Isermeyer, 1952). Basal respiration (C_{bas}) is the respiration rate at the last
162 day of incubation in $mg\ CO_2 - C\ kg^{-1}\ soil\ d^{-1}$, C_{cum} the cumulated respiration during the incubation

163 period in mg CO₂-C kg⁻¹ soil. The metabolic quotient (qCO₂) is the hourly CO₂ evolved per unit of
164 microbial biomass. It expresses the relation between the activity (basal respiration) and the carbon
165 content of the microbial biomass, i.e. the amount of CO₂-C produced per unit microbial biomass
166 carbon, and allows evaluating the effects of external disturbances. The unit is mg CO₂-C 10⁻² h⁻¹
167 mg Cmic⁻¹ (Anderson and Domsch, 1990; 1993). The mineralisation quotient (qM) is the ratio
168 between the cumulated respiration and the SOC, and is expressed in %. The quotient indicates the
169 efficiency of micro-flora in metabolising SOC (Dommergues, 1960).

170

171 *2.4. Soil biological fertility index*

172 The Biological Fertility Index (BFI) is an indicator system considering SOM, C_{bas}, C_{cum}, C_{mic},
173 qCO₂ and qM. Five intervals of values were set for each parameter, and scores increasing from 1 to
174 5 were assigned to each interval (Table 3) based on evidences deduced from earlier studies
175 available in the literature (Brookes, 1995; Vance et al., 1987; Oberholzer and Höper, 2000; Bloem
176 et al., 2006). The algebraic sum of the scores for each parameter providing the proposed classes of
177 biological fertility is shown in Table 4.

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

186 Increasing scores were assigned to SOM, C_{bas}, C_{cum}, C_{mic} and qM when the value of the
187 parameter is increasing, i.e. “the more is better”; conversely qCO₂ has increasing scores when the

188 value of the parameter decreases, i.e. “the less is better” and the ecosystem is more stable (Insam
189 and Haseiwandter, 1989; Anderson, 1994; Andrews et al., 2004).

190

191 2.5. Statistical analyses

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

195

196 3. Results and discussion

197

198 3.1. Soil organic carbon and pools

199 Pasture (PA) and cork oak forest (CO) showed significantly higher SOC and TEC contents ($p < 0.05$)
200 across ecosystems (Table 5). In particular, SOC and TEC were 2.08% and 1.60% respectively in
201 PA, and 2.10% and 1.66% in CO. In the other land uses, SOC was lower and ranged from 1.32 (FV)
202 to 1.54% (TV, GV), TEC from 0.95 (FV) to 1.15% (HC). The ranking was
203 $CO > PA > TV + GV > HC > FV$ for SOC content, $CO > PA > HC > TV + GV > FV$ for TEC.

204 No significant differences in SOC were found in the two vineyards ecosystems (TV and GV), and
205 SOC was 1.54% in both. The most likely explanations are: i) the yearly addition to TV of organic
206 carbon as organic fertilizer (200 kg C ha⁻¹ year⁻¹), and ii) the supplementary irrigation provided in
207 GV in case of water stress. In Mediterranean conditions this might promote microbial activity and
208 enhance SOC mineralization (Nuñez et al., 2007; Butenschoen et al., 2011; Arroita et al., 2013),
209 thus offsetting the positive effect of the grass cover (Lagomarsino et al., 2011; Muñoz-Rojas et al.,
210 2015). PA and HC, even if both grazed by sheep for some months during the year (Table 1),
211 showed significantly different SOC and TEC contents ($p < 0.05$), due to the differences of cropping
212 intensity and the lower soil disturbance by tillage in PA (Francaviglia et al., 2014; Muñoz-Rojas et
213 al., 2015). In particular, SOC content of these ecosystems is in agreement with the findings obtained

214 in Sardinia (Italy) by Salis et al. (2015), reporting 2.11% of SOC for a natural pasture grazed by
215 sheep, and 1.56% for a pasture annually ploughed and sowed for forage production.

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

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

233 Not humified carbon (NHC) ranged from 0.19 to 0.77% in FV and CO respectively (Table 5), with
234 significantly higher contents under pasture (PA) and cork oak forest (CO), indicating a higher labile
235 C fraction with a rapid turnover in comparison with the other ecosystems (Jenkinson and Ladd,
236 1981; McGill et al., 1986; Jenkinson and Parry, 1989; von Lützow et al., 2007). The ranking was
237 CO>PA>HC>GV+TV>FV, in agreement with SOC content. Pastures have been shown to transfer a
238 large amount of organic matter to soil (Fischer et al., 1994) mainly through the root systems, as well

239 woody plants are reported to be potential sources of recalcitrant organic materials in soils (Lorenz et
240 al. 2007).

241 Humin, i.e. not extractable organic carbon (NEC), did not show significant differences among the
242 ecosystems, and ranged from 0.28 to 0.48% in HC and PA respectively. The ranking was
243 PA>CO>GV+TV>FV>HC. The highest contents were found in PA (0.48%) and CO (0.44%),
244 indicating the presence of a C pool more stable and less affected by mineralization processes, and
245 preferentially stabilized in more stable forms chemically or physically protected (Camilli et al.,
246 2016).

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

254 Humification degree (DH), humification rate (HR) and humification index (HI) in the different
255 ecosystems are reported in Table 5. Humification parameters (DH, HR, and HI) are commonly
256 considered as an index of soil humification activity as well as of availability of non humified labile
257 fractions (Ciavatta et al., 1990; Vittori Antisari et al., 2010). DH ranged from 81.6 to 56.5%
258 respectively in FV and PA, was significantly different in PA and CO in comparison with GV, TV
259 and GV, while HC showed an intermediate value (63.5%) not significantly different from the other
260 ecosystems. Soil organic matter is well humified, as indicated by the DH values (56-82%), higher in
261 the two vineyards (71-72%) and in the former vineyards (82%); the higher is DH the higher is the
262 soil ability to humify the organic materials available, meaning a higher chemical and biological
263 stability of organic matter in the soil (Allison, 1973). Differences among land uses might be
264 ascribed to the effect of a reduced soil disturbance in PA and CO, as well as to an increase of C

265 inputs in these ecosystems. DH values are consistent with the reference value of 70-80% which is
266 commonly measured in the Italian soils (Benedetti et al., 2006). HR, indicating the fraction of
267 humified carbon in comparison with SOC, was higher in the two vineyards (about 52%) and in the
268 former vineyards (58%), in agreement with DH and with significant differences in comparison to
269 PA and CO ecosystems. HI was lower and significantly different in the two vineyards (0.41-0.44)
270 and in the former vineyards (0.25). The higher and significantly different values in PA and CO
271 (0.91-0.93) might indicate that mineralization prevails on humification, in agreement with DH and
272 HR values.

273

274 3.2. Soil biological fertility parameters

275 Microbial biomass carbon (C_{mic}) showed the highest and significantly different value (193.8 mg C
276 kg^{-1}) in the CO ecosystem (Table 6), followed by GV (156.3 mg C kg^{-1}), but in the latter case C_{mic}
277 value was not significantly different in comparison with PA and TV land uses (114.9 and 119.9 mg
278 C kg^{-1} respectively). The lowest and significantly different values were found in HC and FV (78.7
279 and 58.5 mg C kg^{-1} respectively). The ranking for C_{mic} was $CO > GV > TV > PA > HC > FV$. Similarly
280 to SOC pools, microbial biomass carbon (C_{mic}) increased in the less disturbed ecosystems,
281 reflecting mainly ranking already observed in SOC values. C_{mic} and its ratio to SOC (q_{mic}) can
282 indicate both a larger substrate availability to the soil microorganisms (Anderson and Domsch,
283 1989), and an increasing trend to stock organic C in the long term in the natural or less disturbed
284 systems, i.e. CO, and GV in this study.

285 C_{bas} (mg CO_2-C $kg^{-1} d^{-1}$) and the cumulated respiration after 25 days of incubation (C_{cum} in mg
286 CO_2-C kg^{-1}) were significantly higher in CO (C_{bas} 11.7, C_{cum} 486.3) and to a lesser extent in GV
287 (C_{bas} 4.8, C_{cum} 234.1). The rankings were $CO > GV > TV > FV > PA > HC$ for C_{bas} ,
288 $CO > GV > FV > TV > PA > HC$ for C_{cum} . We found the highest values of C_{bas} and C_{cum} in CO, and
289 might suppose that in this land use the higher C inputs from vegetation to soil were counterbalanced
290 by an increase in microbial respiration.

291 The metabolic quotient (qCO_2), showed values always high (Table 6), independently from the land
292 use intensity. qCO_2 was significantly different in CO and FV compared with the other land uses.
293 The ranking was $CO > FV > TV > GV > PA > HC$. It must be considered that for qCO_2 the less is better
294 (Insam and Haseiwandter, 1989; Anderson, 1994; Andrews et al., 2004), i.e. more favorable
295 conditions for microbial survival are present, while high values are indicative of an increase of CO_2
296 related to a microbial stress (Jenkinson and Ladd, 1981; Andersen, 2003). High qCO_2 values can be
297 ascribed also to changes in the bacterial-to-fungal ratio (Sakamoto and Oba, 1994; Landi et al.,
298 2000; Nannipieri et al., 2003).

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

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

309

310 *3.3. Soil biological fertility index*

311 To calculate the biological fertility index, soil organic matter (SOM) and microbial activity
312 parameters determined in the different ecosystems were used to evaluate their sustainability (Table
313 7). Data showed that the average scores of the parameters and BFI, and the lower and upper values
314 based on the standard deviation of the measured parameters. Cork oak forest ecosystem (CO)
315 showed the highest BFI (18 as average, range 16-20), and ranked in the medium-good class of soil
316 biological fertility commonly observed in in Italy (Renzi et al., 2017). Tilled vineyard (TV), no-

317 tilled grassed vineyard (GV) and pasture (PA) had lower BFIs (12 as average, range 11-15), ranking
318 in the pre stress-medium BFI class. The other land uses (FV and HC) had the lowest BFIs (10-11 as
319 average, range 9-12), and the corresponding soil biological fertility class was always typical of a
320 pre-stress (alarm) situation. This was particularly evident in hay crop (HC), the more disturbed
321 ecosystem due to the frequent tillage operations, which had the lowest BFI (10 as average, range 9-
322 12) denoting a low condition of biological fertility which highlights the need for a sustainable
323 management of pasturelands to avoid soil degradation if improperly managed (Papini et al., 2011).
324 Results confirmed the relevance of land use changes in maintaining soil biological fertility, as well
325 as the need to adopt sustainable practices at lower intensification management to avoid soil
326 degradation. In particular, pastures should be managed with the minimum soil disturbance by
327 tillage, allowing a higher resilience both of SOM and microbial activity parameters. Moreover,
328 supplementary irrigation in vineyards enhances SOC mineralization offsetting the positive effect of
329 the grass cover, and should be avoided.

330

331 **4. Conclusions**

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

339 The BFI index indicated a higher soil biological fertility under cork oak forest, and we can
340 reasonably suppose that the maintenance of this ecosystem can increase globally the soil biological
341 fertility level and the microbial diversity on the area. Pasture might improve its soil biological

342 fertility in the long-term, as well as the grassed vineyards provided that supplementary irrigation is
343 avoided.

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

347

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349

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355

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357

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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 2Main soil parameters in the 0-20 cm soil layer (mean \pm 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 \pm 34	120 \pm 14	50 \pm 44	Loamy Sand	5.1 \pm 0.1	0.99 \pm 0.07	34 \pm 4	12.67 \pm 2.16	0.22 \pm 0.08
GV (n=3)	800 \pm 6	115 \pm 13	85 \pm 14	Sandy Loam	6.2 \pm 0.3	0.93 \pm 0.05	30 \pm 14	15.03 \pm 1.97	0.75 \pm 0.18
FV (n=7)	791 \pm 36	101 \pm 31	108 \pm 37	Sandy Loam	6.0 \pm 0.3	1.07 \pm 0.05	6 \pm 2	18.08 \pm 5.50	0.24 \pm 0.10
HC (n=5)	733 \pm 5	131 \pm 9	136 \pm 5	Sandy Loam	5.6 \pm 0.4	1.73 \pm 0.45	35 \pm 22	16.94 \pm 4.55	0.51 \pm 0.24
PA (n=4)	732 \pm 12	135 \pm 8	133 \pm 5	Sandy Loam	5.5 \pm 0.4	2.08 \pm 0.15	26 \pm 8	17.17 \pm 3.73	0.44 \pm 0.08
CO (n=2)	761 \pm 29	116 \pm 3	123 \pm 26	Sandy Loam	5.7 \pm 0.2	1.69 \pm 0.22	5 \pm 3	17.83 \pm 2.29	0.36 \pm 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 C _{bas} (mg CO ₂ -C kg ⁻¹ soil d ⁻¹)	< 5	≥ 5 ≤ 10	> 10 ≤ 15	> 15 ≤ 20	> 20
Cumulative respiration C _{cum} (mg CO ₂ -C kg ⁻¹ soil)	< 100	≥ 100 ≤ 250	> 250 ≤ 400	> 400 ≤ 600	> 600
Microbial biomass carbon C _{mic} (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 C _{mic} ⁻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).

	I	II	III	IV	V
Fertility class	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 \pm SD). n=9 (3 replicates \times 3 periods).

Land use	SOC (%)	TEC (%)	HA+HF (%)	NHC (%)	NEC (%)	TEC/SOC	DH (%)	HR (%)	HI
TV	1.54 \pm 0.26a	1.11 \pm 0.13a	0.78 \pm 0.12ns	0.32 \pm 0.15a	0.43 \pm 0.18ns	0.73 \pm 0.10ns	71.0 \pm 12.1ab	51.3 \pm 9.6bc	0.44 \pm 0.24a
GV	1.54 \pm 0.25a	1.11 \pm 0.13a	0.79 \pm 0.12ns	0.32 \pm 0.12a	0.43 \pm 0.16ns	0.73 \pm 0.08ns	71.9 \pm 8.4b	51.8 \pm 4.0bc	0.41 \pm 0.16a
FV	1.32 \pm 0.25a	0.95 \pm 0.23a	0.76 \pm 0.13ns	0.19 \pm 0.18a	0.38 \pm 0.02ns	0.71 \pm 0.04ns	81.6 \pm 15.9b	57.8 \pm 9.7c	0.25 \pm 0.22a
HC	1.43 \pm 0.28a	1.15 \pm 0.22a	0.73 \pm 0.16ns	0.42 \pm 0.18a	0.28 \pm 0.13ns	0.81 \pm 0.08ns	63.5 \pm 14.1ab	50.3 \pm 7.2abc	0.65 \pm 0.43ab
PA	2.08 \pm 0.14b	1.60 \pm 0.31b	0.86 \pm 0.05ns	0.74 \pm 0.41b	0.48 \pm 0.27ns	0.77 \pm 0.13ns	56.5 \pm 16.3a	41.6 \pm 6.2a	0.91 \pm 0.59b
CO	2.10 \pm 0.20b	1.66 \pm 0.14b	0.89 \pm 0.23ns	0.77 \pm 0.45b	0.44 \pm 0.14ns	0.79 \pm 0.06ns	56.6 \pm 19.3a	43.9 \pm 13.2ab	0.93 \pm 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 6Soil microbial activity parameters in land uses (means \pm SD). n=9 (3 replicates \times 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 \pm 26.9ab	4.3 \pm 0.9b	189.1 \pm 28.5ab	1.77 \pm 0.54a	1.26 \pm 0.26bc
GV	156.3 \pm 39.8b	4.8 \pm 0.8b	234.1 \pm 41.8b	1.54 \pm 0.37a	1.54 \pm 0.26c
FV	58.5 \pm 6.4a	3.7 \pm 0.5ab	215.5 \pm 0.93ab	2.63 \pm 0.23ab	1.66 \pm 0.29c
HC	78.7 \pm 24.1a	2.1 \pm 0.3a	132.4 \pm 31.6a	1.43 \pm 0.33a	0.96 \pm 0.22ab
PA	114.9 \pm 48.1ab	3.2 \pm 1.7ab	142.4 \pm 21.5a	1.53 \pm 0.87a	0.68 \pm 0.45a
CO	193.8 \pm 62.4b	11.7 \pm 4.4c	486.3 \pm 28.7c	3.28 \pm 1.28b	2.36 \pm 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, qCO₂ 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, qCO₂ 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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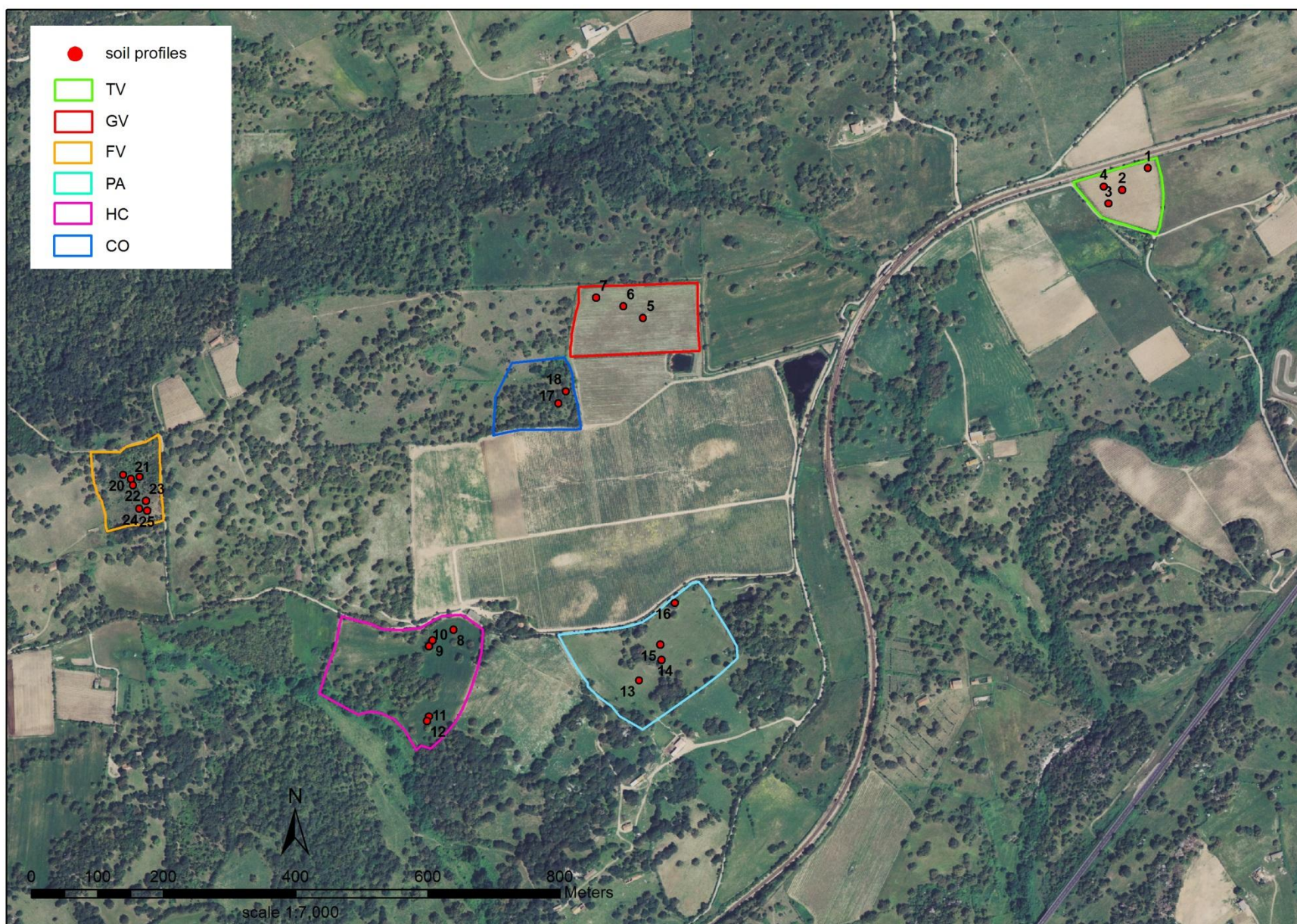


Fig. 1. Study area in northeast Sardinia (Italy).

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

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