



Microbial, physical and chemical indicators together reveal soil health changes related to land cover types in the southern European sites under desertification risk

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ABSTRACT

Soil microbial communities, which play a key role in the provision of essential ecosystem services, are significantly influenced by several physical and chemical soil properties that may change with land management. This study explores the effect of different land cover types (coniferous tree stands, broad-leaved stands, shrublands, pastures/grasslands and croplands) on physical, chemical and microbial properties (all contributing to soil health) in southern European areas under moderate-high desertification risk selected in Italy, Spain and Portugal. In sites that differ in land cover, we determined microbial biomass (C_{mic}), activity and indices of microbial metabolism including C_{mic}/C_{org} ratio, metabolic quotient (qCO_2) and quotient of mineralization (qM). Soil physical and chemical properties were also measured, comprising bulk density (BD), water content (WC), pH, cation exchange capacity (CEC), total organic C (C_{org}) and some of its labile fractions, extractable C (C_{ext}) and mineralizable C (C_{min}), total N content and C/N. Results showed that land cover type played a strong role in determining magnitude of microbial variables with biomass and activity being higher under coniferous tree cover than in other land covers, according to trends in WC, CEC, C_{org} , C_{ext} , C_{min} , N, C/N. Compared to land cover, aridity index had lower effect on investigated variables. In comparison to sites with higher C_{org} content, sites with lower C_{org} content (most croplands) tended to lose C more rapidly, as suggested by high qM values, except for Spanish acidic soils. Therefore, urgent actions must be taken to counteract the tendency of C-poorer soils to lose C, promoting land cover types that facilitate soil recovery by ensuring denser and more continuous soil cover over time. We also identified a minimum set of soil variables that provide information on soil health changes in both short term (microbial variables) and longer term (physical and chemical variables) in areas under desertification risk.

1. Introduction

Soil microbial community is a key component of the terrestrial ecosystem. It represents only a small part of soil organic matter (0.3–7 % of total organic C, as reported for a broad spectrum of sites by Anderson and Domsch, 1989), but it is the most active portion, being involved in ecosystem functions like organic matter decomposition, humification, and nutrient cycling (Jeffery et al., 2010; Pulleman et al., 2012). Thus, it provides several ecosystem services, such as nutrient, gas and climate regulation (through C sequestration) and water purification (Adhikari and Hartemink, 2016; Bünemann et al., 2018; Pereira et al., 2018; Saccà et al., 2017).

Besides soil physical and chemical properties, which provide

information about soil hydrologic characteristics (as water retention related to texture, BD, etc.), equilibrium between soil solution reaction (pH) and exchange sites (cation exchange capacity), and nutrient turnover (through total organic carbon and total nitrogen), microbial biomass and activity are useful to assess soil health (Doran and Parkin, 1996; Marzaioli et al., 2010a). The term soil health, which has been used interchangeably with soil quality in recent years (Evangelista et al., 2023), indicates "the ability of a soil to function within ecosystems and land use boundaries to sustain biological productivity, maintain or improve environmental quality, and promote plant and animal health" (Bünemann et al., 2018) and "the continued capacity of soils to support ecosystem services" (EC, 2020). Since "soil functions are context-dependent in time and space" (Evangelista et al., 2023) a relative approach to soil health should be

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adopted, by comparing, for example, soils within the same macroclimate, but differing for anthropic factors (as land use types, stress/disturbances, etc.) that may cause change in soil properties, so affecting its ability to function. Keeping this in mind, within a defined area, a relatively well functioning soil ("healthy") has higher reserve of water, organic matter and nutrients, lower bulk density, neutral pH and more abundant and active microbial community, compared to soils with lower soil health (Marzaioli et al., 2010a). All these soil properties are associated with high soil health according to both agronomic and environmental perspective because they assure plant growth and essential functions such as water infiltration, water and soil depuration, nutrient cycling and C sequestration (Bouma et al., 2021). The assessment of soil health over time and over space is recognized as a primary indicator of sustainable land management (EC, 2020).

To assess soil health, it is important to identify a set of sensitive soil properties that reflect its ability to function and can be used as indicators of its health status (Bünemann et al., 2018). Currently, the quantification of soil health is still dominated by the study of physical and chemical properties, despite the growing awareness of the importance of soil biological properties (Lehmann et al., 2020). Among these, a crucial role in the assessment of soil health is represented by the microbial community (Sharma et al., 2010), thanks to its critical role in mediating carbon (C), nitrogen (N) and other nutrient cycling processes in soils (Pulleman et al., 2012). Compared to higher organisms, microorganisms may provide more information on soil health because they respond quickly to environmental changes. This is due to more intimate relationship with their surroundings thanks to their higher surface to volume ratio (Nielsen and Winding, 2002). The microbial community is sensitive to several stress/disturbance factors, such as pollution (Marzaioli et al., 2010b), fire (Catalanotti et al., 2018; Giuditta et al., 2019; Rutigliano et al., 2013; Marfella et al., 2023), land use change (Marzaioli et al., 2010a; Zhang et al., 2019; Steinberger et al., 2022) as well as increased drought due to climate change (Bogati and Walczak, 2022). Therefore, microbial community changes may be useful indicators in areas where several strongly limiting factors may act simultaneously. This is the case of areas at desertification risk that could also be affected by land use types. The effects of land use on the microbial community could be due to the different contributions to the carbon pool by different plant cover types (annual and perennial crops, pastures, forests) through the production of litter and root exudates that differ in chemical composition (Cardoso et al., 2003) so affecting microbial biomass and activity (Zhong et al., 2020). Moreover, in agricultural tilled soil, where the oxidation processes are sped up, a reduction of the stable organic matter content can occur, determining a reduction of soil microbial biomass and basal respiration (Babujia et al., 2010).

Besides microbial biomass and activity, microbial performance can be assessed by microbial indices (Dilly, 2005; Dilly and Munch, 1998; Moscatelli et al., 2007), which readily respond to disturbance/stress factors and provide an effective early warning for the deterioration of soil health (Bastida et al., 2008; Wardle and Ghani, 1995). Among these, C_{mic}/C_{org} ratio (also called "microbial quotient"), which indicates the biologically active fraction of the soil organic C pool, is sensitive to changing soil conditions. When soil management change, microbial biomass decreases or increases faster than total organic C (Brookes, 1995). This means that C_{mic}/C_{org} is more sensitive to changes in carbon dynamics than the contents of C_{org} and C_{mic} alone (Dilly, 2003; Sparling, 1992; Woloszczyk et al., 2020). A valid index of the metabolic status of microbial community is metabolic quotient (qCO_2 , CO_2 -C per unit of C_{mic}), with higher values indicating generally more stressful conditions (Cardoso et al., 2013) and lower carbon use efficiency (Brookes, 1995). Moreover, the percentage of potentially mineralized C within the total organic C (qM) indicates the efficiency of microflora in metabolizing organic matter (Mocali et al., 2008) and its degradability. These microbial indices, together with microbial biomass and activity, are recognized as early signals of degradation process, with respect to change in total soil organic C (Bastida et al., 2008), which is an

important indicator of soil health but may reveal variation only over a long period of time (> 10 years in temperate region; Thoumazeau et al., 2020). Overall, microbial variables are known to be suitable in the evaluation of soil functioning in highly degraded areas due to the desertification process (Hu et al., 2016) because they allow to quickly identify ongoing changes and to take recovery action.

Southern Europe has been identified as particularly vulnerable to soil degradation, with the overall highest erosion rates within the EU, strong and increasing human pressures (Ferreira et al., 2022) and high sensitivity to desertification (Mirzabaev et al., 2019; Yassoglou and Kosmas, 2000). The last is mainly due to the high erosion rates, decrease in soil organic matter, compaction, salinization, landslides, contamination, sealing and decline in biodiversity (Montanarella, 2007) caused by a strong and increasing human pressures and high climate change vulnerability (ECA, 2018; Mirzabaev et al., 2019). Indeed, across Europe, these areas are most vulnerable to droughts, especially at annual to decadal scale (An et al., 2023). The risk of desertification is most serious in some southern regions of the Europe (particularly, Portugal, part of Spain and southern Italy, south-eastern Greece, Malta, Cyprus), where an increase of 177,000 km² of territory with a high or very high sensitivity to desertification in less than a decade was observed (ECA, 2018; Práválie et al., 2017).

Land use is a crucial factor affecting soil health and often has a direct influence on desertification (INTOSAI WGEA, 2013). A long history of unsustainable agriculture practices, land overexploitation, poor management of grazing areas and livestock and bad irrigation practices alters the physical, chemical and biological health of soils and compromises soil functions, enhancing the risk of desertification (Gibbs and Salmon, 2015). The land use/management may result in a mosaic of land cover types, ranging from managed croplands and pastures to shrublands and broad-leaved stands, as well as areas planted with coniferous trees, as observed in areas at desertification risk of Italy, Spain and Portugal (Grilli et al., 2021). These different land covers may affect soil health. An increase in organic C content was found in forests/grasslands vs croplands in Spanish soils (Rodríguez Martín et al., 2016) and in forests vs grasslands/croplands in Italian soils (Gardin et al., 2021). On the other hand, in central Spain, land cover types (grassland/shrubland, mixed shrubland-pine, and pine forest) did not affect soil organic C, but caused changes in microbial activity, with higher values in grasslands/shrublands than in forests (Ortiz et al., 2022). Marzaioli et al. (2010a), who observed improved soil quality (evaluated by 22 physical, chemical and microbial variables) in Southern Italy forests/shrublands/pastures compared to croplands, suggested that the development of a herbaceous cover on the soil surface was responsible for increased soil quality. The protective role of plant cover on soil may also result in a reduction of desertification risk.

Comprehensive knowledge of the status of soil properties/functions is a preliminary step in implementing strategies to counter the risk of desertification in these areas. Soil degradation is often closely related to soil health/quality (Bone et al., 2010). In areas at desertification risk, such as those of southern Europe, it is useful to establish the linkages between soil degradation and soil health indicators to quickly identify a suitable recovery strategy, also with the active engagement of stakeholders and local communities (MEA, 2005).

Numerous studies on soil health have been conducted at the plot (Andrews et al., 2002; Idowu et al., 2009; Marzaioli et al., 2010a; Hussain et al., 1999) and landscape scale (Karlen et al., 2008; Svoray et al., 2015), but few at broader spatial levels (Brejda et al., 2000a, 2000b; Fine et al., 2017). Therefore, this study aims: i) to investigate changes in soil health as derived from changes in physical, chemical and microbial variables in three large geographic areas of southern Europe under significant desertification risk, differing for land cover types (coniferous tree stands, broad-leaved stands, shrublands, pastures/grasslands, croplands) and for aridity index (AI), the latter being related to the desertification risk (Spinoni et al., 2015); ii) to investigate, through multivariate analysis, the overall relations among soil physical,

chemical and microbial properties in order to identify soil properties more suitable to evaluate the soil health changes.

We hypothesized that, in areas under desertification risk of southern Europe, i) within considered land cover types, croplands and pasture can worsen soil health, whereas tree cover can improve soil health, promoting recovery process, with more pronounced effects in areas with higher sensitivity to desertification; ii) a minimum set of physical, chemical and microbial indicators is necessary to identify soil health changes in areas differing for land cover and aridity index.

The overall results will help to understand if some land cover types considered in this study may be suitable to slow down degradation processes or promote recovery processes, so providing useful indications to restore severely degraded areas.

2. Material and methods

2.1. Study areas

Study areas were located in the regions the of Alentejo in Portugal (PT), the Extremadura in Spain (SP) and Sicily in Italy (IT) (Fig. 1A). These areas, included in the LIFE project Desert-Adapt (<http://www.desert-adapt.it>), were identified within regions of southern Europe considered to be at significant desertification risk, according to the Sensitive Desertification Index (SDI) calculated by Práválie et al. (2017). Based on the derived SDI map, which integrates three biophysical parameters, i.e., Climate Quality Index (CQI), Soil Quality Index and Vegetation Quality Index, we choose areas having from moderate ($1.3 < SDI < 1.4$ Spanish areas) to high ($1.4 < SDI < 1.6$ Italian and Portuguese areas) sensitivity to desertification (Práválie et al., 2017). In these areas, the combination of pressures generated by climate and land management (cultivation, extensive grazing) and extreme events like fires create the conditions to significantly increase the sensitivity to land degradation.

2.2. Experimental sites

In total, 38 study sites, located in remote areas far from urban-industrial centers, were chosen within 4 Italian areas (IT1-IT4), 3 Spanish areas (SP1-SP3) and 3 Portuguese areas (PT1-PT3) (Fig. 1 A; Table 1). The sites, whose size varied from 0.2 to 24 ha (Table 1), were grouped on the basis of the most representative land cover in 5 major groups (Fig. 1B): coniferous tree stands ($n = 3$), broad-leaved stands ($n = 3$), shrublands ($n = 9$), pastures/grasslands ($n = 11$) and croplands ($n = 12$) (Table 1). The selection of land covers was driven by the sites, municipalities and farmers and animal breeders available on the private and public lands of participants in the EU project Desert-Adapt. However, they are also quite representative of each analysed region.

The coniferous tree stands are close-canopy afforestation stands (>50 years old), two in Italy (*Pinus halepensis* Mill.) and one in Spain (*P. pinaster* Aiton). The broad-leaved stands are open-canopy re-forested stands, one in Italy with holm oak (*Quercus ilex* L.), and two in Portugal, one with eucalyptus (*Eucalyptus* sp.pl.; >30 years), and one agroforestry system with cork oak (*Quercus suber* L.). The last ecosystem is a typical Iberian agroecosystem, called “montado” in Portugal and “dehesa” in Spain, characterized by few trees per hectare (20–80 trees ha⁻¹; Pinto-Correia and Mascarenhas, 1999) generally represented by cork oak (*Q. suber*) and holm oak (*Q. ilex* subsp. *rotundifolia*) (Pinto-Correia et al., 2011; Pinto-Correia and Mascarenhas, 1999), and species-rich annual herbaceous vegetation, grasses and scattered shrubs (Bugalho et al., 2009). These systems resulted from centuries of human activity (mainly grazing) in the original broad leaved forests. The eleven pastures/grasslands included eight sites in Portugal and one site in Spain, representing a degraded condition of montado/dehesa systems where pasture pressure was very high and vegetation was dominated by herbaceous plants with few trees per hectare, as well as three Italian pasture sites. The nine shrublands included one area in Portugal, six areas in Spain, all

representing post-disturbance (deforestation, pasture abandonment, fires) stages of montado/dehesa systems, and two garrigue areas in Lampedusa Island of Italy. All 12 cropland sites (2 in Portugal, 1 in Spain, 9 in Italy) were tilled for seeding or weeding, except for the prickly pear stands (IT4-1 and IT4-2; Table 1). Further details on climate variables, soil classification and texture are reported in Table 1. All the sites have a warm temperate climate with hot and dry summer, according to Köppen-Geiger climate classification (Beck et al., 2018). According to their aridity index (AI; Table 1), calculated as the inter-annual average of the ratio between annual total precipitation and potential evapotranspiration (Spinoni et al., 2015), all sites were generally classified as semi-arid lands ($0.2 < AI \leq 0.5$), except for some Spanish sites, the shrubland stands SP1-1, SP1-2, SP2-2 and SP2-3 and the coniferous stand SP2-1 identified as humid lands ($AI > 0.75$; Table 1) and other Spanish sites (the shrubland stands SP3-2, SP3-3, the cropland SP3-1 and the pasture SP3-4), identified as sub-humid lands ($0.65 < AI \leq 0.75$; Table 1).

2.3. Soil sampling

Soil samplings were carried out in April-May 2018. In the southern Europe, the later spring season corresponds to the period of year in which conditions are the most favourable for soil microorganisms and values of microbial biomass and activity are generally more similar to mean annual values compared to those obtained in the other seasons (Marzaioli et al., 2010b). At each of the 38 sites, the soil sampling was carried out along a transect starting from the center, outwards (N-S), collecting soil from a minimum of 5 to a maximum of 10 sampling points or field replicates (241 sampling points in total), mainly depending on the size of the site and maintaining 100 m distance between sampling points. In each sampling point, a composite soil sample (Joergensen and Emmerling, 2006) was taken (after removing the litter, where present) consisting in four soil cores (diameter: 8 cm; depth: 10 cm) collected at the corners of a 25-m² square area, centered on the sampling point, and then mixed together. The top 10 cm of soil corresponds to the depth at which most of microbial biomass and activity are expected (Jandi et al., 2014). Indeed, microbial biomass and activity significantly decreased with increasing depth also in cultivated soils (Babujia et al., 2010; Marzaioli et al., 2022; Yang et al., 2016). Being the most active depth, the shallower soil layer was also the most sensitive to land management (Warren et al., 2019). Moreover, at the central point of each site, three undisturbed soil cores were collected within corers, which were covered at both ends with a plastic cap and sealed in plastic bags until the assessment of bulk density (BD; data already reported by Grilli et al., 2021).

2.4. Soil processing

All samples were placed in plastic bags, sealed tightly and stored in the field refrigerator and then shipped at the Ecology laboratory of University of Campania Luigi Vanvitelli within ten days of sampling; postage packages were kept cool (approx. < 4 °C) during transit by the insertion of cool packs. In the laboratory, soil samples were sieved (< 2 mm) and separated into two aliquots; the first was stored at 4 °C (for up to 2 weeks) for the determination of water content (WC), labile organic C (extractable and fast mineralizable C), soil microbial biomass and activity. The second aliquot, air-dried to constant weight, was used to measure soil texture. The air-dried aliquot was used to determine (by standard protocols) pH, cation exchange capacity (CEC), total organic C (C_{org}), nitrogen (N) content and C/N ratio that were already reported in Grilli et al. (2021) and have been used in this manuscript to correlate biological data to physical and chemical characteristics and to obtain a larger data set to perform multivariate analysis (as described in 2.6 section).



Fig. 1. Study areas (A) located in the Sicily region in Italy (IT), the Extremadura in Spain (SP) and the Alentejo region in Portugal (PT). B) Photos of some sites differing for land cover type and geographic region (acronyms as in Table 1).

Table 1
Site code, site size, number of soil replicates in each site (n), land cover, mean annual temperature (T)¹, total annual precipitations (P)¹, aridity index (AI)¹, soil type¹, percentage of sand, silt and clay and soil texture of 38 studied sites in Italian (A), Spanish (B) and Portuguese (C) sites. The climate data refer to the period 1976–2005. Crop typologies refer to the year of sampling.

Site code	Site size (ha)	n	Land cover	Mean annual T (°C)	Total annual P (mm)	AI	Soil type ²	Sand (%)	Silt (%)	Clay (%)	Soil texture
A) Italy											
IT1-2	15.3	10	Conifer tree stand	16.6	352	0.29	Leptosol	47.8 (± 0.7)	40.3 (± 0.8)	11.9 (± 0.7)	loam
IT1-4	4.1	5	Conifer tree stand	16.6	352	0.29	Leptosol	55.4 (± 2.0)	26.7 (± 0.9)	19.9 (± 0.2)	sandy-loam
IT2-5	1.2	5	Broad-leaved stand	14.7	484	0.43	Regosol	21.3 (± 1.8)	34.4 (± 1.7)	44.3 (± 0.1)	clay-loam
IT1-1	1.8	10	Shrubland	16.6	352	0.29	Leptosol	57.0 (± 1.3)	24.7 (± 0.4)	18.3 (± 1.7)	sandy-loam
IT1-3	0.9	5	Shrubland	16.6	352	0.29	Leptosol	65.3 (± 0.4)	16.3 (± 0.6)	18.4 (± 0.2)	sandy-loam
IT2-2	3.2	5	Pasture/Grassland	14.7	484	0.43	Regosol	17.5 (± 2.1)	40.9 (± 0.5)	41.5 (± 1.2)	silt-clay
IT3-1	7.7	5	Pasture/Grassland	13.5	486	0.42	Regosol	15.2 (± 1.4)	39.3 (± 2.5)	45.5 (± 0.9)	clay
IT3-2	1.9	5	Pasture/Grassland	13.5	486	0.42	Regosol	11.1 (± 3.9)	42.0 (± 1.4)	47.0 (± 1.1)	silt-clay
IT2-1	0.6	10	Cropland (olive grove)	14.7	484	0.43	Regosol	10.0 (± 0.8)	41.3 (± 0.2)	48.7 (± 0.6)	silt-clay
IT2-3	0.7	5	Cropland (sulla, oat)	14.7	484	0.43	Regosol	16.0 (± 4.9)	26.9 (± 3.8)	57.1 (± 1.1)	clay
IT2-4	1.5	5	Cropland (olive grove)	14.7	561	0.43	Regosol	17.4 (± 2.0)	42.0 (± 1.6)	40.7 (± 0.9)	silt-clay
IT3-3	1.1	5	Cropland (chickpeas)	13.5	486	0.42	Regosol	8.1 (± 0.6)	48.3 (± 3.0)	43.6 (± 2.4)	silt-clay
IT3-4	0.8	5	Cropland (olive grove)	13.5	486	0.42	Regosol	42.5 (± 2.6)	36.4 (± 0.5)	21.1 (± 0.8)	loam
IT4-1	1.5	5	Cropland (prickly pear)	14.9	561	0.49	Regosol	80.3 (± 1.6)	11.6 (± 2.5)	8.2 (± 0.9)	loamy-sand
IT4-2	0.6	5	Cropland (prickly pear)	14.9	561	0.49	Regosol	69.2 (± 1.1)	15.1 (± 1.6)	15.7 (± 0.6)	sandy-loam
IT4-3	0.4	5	Cropland (fruit)	14.9	561	0.49	Regosol	52.8 (± 1.2)	25.9 (± 0.7)	21.3 (± 0.4)	sandy-clay-loam
IT4-4	0.4	5	Cropland (bamboo plantation)	14.9	561	0.49	Regosol	54.2 (± 1.4)	28.5 (± 1.1)	17.3 (± 0.3)	loam
B) Spain											
SP2-1	15.6	10	Conifer tree stand	11.7	1427	1.42	Cambisol	73.1 (± 2.1)	17.4 (± 2.7)	9.6 (± 0.6)	sandy-loam
SP1-1	0.7	5	Shrubland	12.7	1204	1.13	Cambisol	65.2 (± 1.8)	25.4 (± 0.9)	9.4 (± 1.1)	sandy-loam
SP1-2	21.4	10	Shrubland	12.7	1204	1.13	Cambisol	73.0 (± 2.0)	24.2 (± 1.8)	2.8 (± 0.3)	loamy-sand
SP2-2	0.2	5	Shrubland	11.7	1427	1.42	Cambisol	18.3 (± 2.1)	40.2 (± 0.5)	41.6 (± 1.6)	silt-clay
SP2-3	1.6	5	Shrubland	11.7	1427	1.42	Cambisol	73.8 (± 1.0)	23.2 (± 0.2)	3.0 (± 0.3)	sandy-loam
SP3-2	2.3	10	Shrubland	14.3	783	0.67	Cambisol	26.5 (± 1.5)	68.8 (± 1.4)	4.7 (± 0.1)	silt-loam
SP3-3	8.7	5	Shrubland	14.3	783	0.67	Cambisol	16.5 (± 1.9)	40.4 (± 1.9)	43.2 (± 0.8)	silty-clay
SP3-4	2.0	5	Pasture/Grassland	14.3	783	0.67	Cambisol	41.7 (± 2.1)	54.6 (± 3.1)	3.7 (± 1.1)	sandy-loam
SP3-1	11.7	10	Cropland (fodders)	14.3	783	0.67	Cambisol	40.9 (± 2.7)	47.2 (± 4.1)	11.9 (± 1.4)	loam
C) Portugal											
PT1-1	24.2	10	Broad-leaved stand	16.1	501	0.38	Luvisol	51.5 (± 1.2)	36.8 (± 0.9)	11.7 (± 0.4)	loam
PT1-2	13	5	Broad-leaved stand	16.1	501	0.38	Luvisol	48.0 (± 0.9)	47.2 (± 1.6)	4.8 (± 0.7)	sandy-loam
PT3-3	2.0	5	Shrubland	16.1	511	0.38	Leptosol	61.8 (± 2.5)	32.5 (± 3.1)	5.7 (± 0.7)	silty-clay
PT1-3	1.8	8	Pasture/Grassland	16.1	511	0.38	Luvisol	61.8 (± 2.1)	28.6 (± 1.1)	9.7 (± 0.5)	sandy-loam
PT2-2	0.5	8	Pasture/Grassland	16.2	511	0.35	Leptosol	57.3 (± 1.3)	31.7 (± 0.4)	14.6 (± 0.5)	sandy-loam
PT2-3	6.9	5	Pasture/Grassland	16.2	511	0.35	Leptosol	40.2 (± 1.4)	45.8 (± 1.0)	13.6 (± 2.4)	sandy-loam
PT2-4	11.5	5	Pasture/Grassland	16.2	511	0.35	Luvisol	52.7 (± 0.7)	33.7 (± 3.1)	13.6 (± 2.4)	sandy-loam
PT2-5	9.1	5	Pasture/Grassland	16.2	456	0.35	Luvisol	53.7 (± 1.9)	33.2 (± 2.1)	13.1 (± 0.2)	sandy-loam
PT3-1	8.9	5	Pasture/Grassland	16.1	511	0.38	Leptosol	54.0 (± 0.5)	32.1 (± 13.9)	13.9 (± 0.3)	sandy-loam
PT3-2	9.9	5	Pasture/Grassland	16.1	511	0.38	Leptosol	65.1 (± 1.0)	27.2 (± 0.6)	7.6 (± 0.6)	sandy-loam
PT2-1	0.41	10	Cropland (lupine)	16.2	456	0.35	Leptosol	55.2 (± 3.8)	31.5 (± 2.3)	13.4 (± 1.1)	sandy-loam
PT2-6	12.9	5	Cropland (fodder)	16.2	456	0.35	Luvisol	60.1 (± 0.3)	28.0 (± 0.4)	11.9 (± 0.7)	sandy-loam

¹Data from Grilli et al. (2021). ²Reference Soil Groups (IUSS Working Group WRB, 2015).

2.5. Soil analyses

The particle-size distribution (expressed as percentage of sand, silt and clay) was determined on dry soil samples using the pipette method and the relative textural classes were identified according to USDA standards (Soil Survey Staff, 2014). Soil water content, microbial biomass and activity were determined on soil samples stored at 4 °C within 3 weeks from soil sampling, with this storage time showing no significant changes in the original contents (ISO 18400-206, 2018; Černohlávková et al., 2009; Meyer et al., 2019).

Soil water content was evaluated gravimetrically (Allen, 1989), while soil microbial C (C_{mic}) was determined by the fumigation-extraction method (Vance et al., 1987). In particular, C_{org} was extracted with a 0.5 M solution of K_2SO_4 from fumigated and non-fumigated soil samples and later determined by wet oxidation with a 0.4 N $K_2Cr_2O_7$ solution at 160 °C followed by titration of the $K_2Cr_2O_7$ excess with a 0.04 N Fe_2SO_4 solution. The microbial biomass, expressed as microbial C (C_{mic}), was calculated from the difference between organic carbon in fumigated and non-fumigated soil samples using the equation of Vance et al. (1987). Organic C determined on the 0.5 M K_2SO_4 extracts of unfumigated samples corresponds to soil-extractable C or C_{ext} (Joergensen and Mueller, 1995) and includes the compounds that are readily decomposed by soil microorganisms (Haynes, 2005). This represents only a part of total soil organic carbon (C_{org}) that was determined by wet sulphochromic oxidation followed by $FeSO_4$ titration (Grilli et al., 2021).

Microbial activity was determined as soil respiration, evaluated by alkali NaOH trap of CO_2 evolved from soil samples during an incubation of 10 days in standard conditions (at the dark, 20 °C, 55% of soil water holding capacity, determined gravimetrically as Allen, 1989) according to ISO 16072 (2002) - Section 5.2. A pre-incubation at 20 °C for about 3 days was applied to all samples to settle the soil microbial community following disturbance of sampling and sieving, allowing the initial carbon flush to diminish (Pell et al., 2006). Further, 50 ml-glass beakers containing fresh soil samples (5 g) were placed in 500 ml-jars containing 10 ml of 0.1 N NaOH at the bottom; the jars were tightly sealed and then incubated for 10 days. Evolved CO_2 from soil was monitored about every 3 days between the 1st and the 10th day of incubation by opening the jars and, after titration, re-incubating soil samples after addition of a new NaOH solution for CO_2 absorption to ensure that NaOH was available to bind the CO_2 . The excess NaOH was titrated with 0.05 M HCl after precipitating the carbonate with a 0.75 N $BaCl_2$ solution and using phenolphthalein as indicator (ISO 16072, 2002 - Section 5.2, modified according to Stinca et al., 2020). For each sample, the following variables were determined: i) mean respiration (R; $mg\ CO_2-C\ kg^{-1}\ d.w.\ d^{-1}$) in the whole incubation period; ii) basal respiration (BR) recorded in the last incubation step (7th-10th day of incubation). Moreover, the mineralizable C ($g\ CO_2-C\ kg^{-1}\ d.w.$) was calculated by fitting each cumulated CO_2-C evolved against incubation time using first-order pool kinetics models from Riffaldi et al. (1996):

$$C = C_0 \times (1 - e^{-kt}) \quad (1)$$

where C is the cumulative C mineralized after time t ($g\ CO_2-C\ kg^{-1}\ d.w.$), t is the time from start of incubation (days), C_0 ($g\ CO_2-C\ kg^{-1}\ d.w.$) is potentially mineralizable C, i.e., the asymptotic maximum quantity of CO_2-C produced, k is the mineralization rate constant (day^{-1}).

From C_{mic} , C_{org} and respiration data, several indices were calculated: i) the C_{mic}/C_{org} ratio (%); ii) the metabolic quotient (qCO_2), representing the metabolic status of the microbial community (Anderson and Domsch, 1993), calculated from basal respiration (BR) and C_{mic} ($g\ CO_2-C\ kg^{-1}\ C_{mic}\ d^{-1}$); iii) the quotient of C mineralization (qM), calculated from the asymptotic maximum quantity of evolved CO_2-C (C_0), derived from Eq. 1, and organic carbon (C_{org}) and expressed as $CO_2-C\ %\ C_{org}$ (Riffaldi et al., 1996; Stinca et al., 2020). The last index expresses the ability of the soil to mineralize the organic matter during the 10 days

of incubation (Dommergues, 1960).

2.6. Data analysis

A normality test (Kolmogorov-Smirnov) was performed on all data set before applying parametric tests; all data (apart from pH and bulk density) did not show a normal distribution and they were normalized by the \log_{10} transformation (Sokal and Rohlf, 2012).

To evaluate if variability among considered geographic regions (Italy, Spain and Portugal) (Table 1) affected the soil response to land cover, first the two-way ANOVA, followed by the Bonferroni test when required, was performed for each variable using as factors land cover (tree stands, shrublands, pastures/grasslands, croplands) and geographic region (encompassing differences in mean aridity index, AI: 0.37, 0.40 and 1.07, respectively, in Portugal, Italy and Spain). The two land covers "coniferous tree stands" and "broad-leaved stands" were grouped in the same land cover (tree stands) to have the same numbers of land cover in each geographic region (Spain lacked broad-leaved stands and Portugal lacked of coniferous tree stands; Fig. 1B).

Since two-way ANOVA showed an interaction between land cover and geographic region for most variables (Table S1), to better highlight the effect of land cover, the three geographic region (Italy, Spain and Portugal) were also separately analysed. In particular, one-way ANOVA with the Type III hypothesis test for adjusted sums of squares, followed by the Student-Newman-Keuls test when required, was applied to evaluate the significance ($P < 0.05$) of differences among different land cover types. Type III hypothesis test for adjusted sums of squares was applied to consider the differences in the number of sampling points (5–10) of different sites. It is widely used in studies with unbalanced datasets (Hector et al., 2010).

In addition, to obtain an overall response of selected soil indicators to land cover but also to the aridity index, the multivariate analysis was carried out using the sites of all geographic areas together. In particular, the Principal Component Analysis (PCA) was applied to a matrix including 38 sites and 10 variables (BD, WC, pH, CEC, C_{org} , N, C_{ext} , C_{min} , C_{mic} , R). In the biplot resulting from PCA analysis, the scores of sites (red dots) and loadings of soil variables (red vectors) were shown along the principal components (axes 1 and 2), suggesting that the further away these vectors were from a PC origin, the more influence they had on the principal components. By the Pearson coefficient, the correlations were assayed between two axes of the biplot deriving from PCA and both the 10 variables included in the matrix and chemical and microbial indices (C/N , C_{mic}/C_{org} , qCO_2 , qM), soil texture (as % of sand, silt and clay) and climate variables (precipitations, temperature and aridity index). Pearson coefficient was also calculated to highlight the relationships among all variables (except correlations among microbial indices and soil variables used to calculate them, i.e., qCO_2 and C_{mic}).

Moreover, to check the similarity between sites, Cluster Analysis, i.e., agglomerative hierarchical clustering (AHC), was performed, using Euclidean distance and Ward's method on the matrix of site's factor scores obtained from PCA. The resulting dendrogram provided a visual representation of the distribution of the sites, with sites that join together sooner being more similar to each other than those that join together later.

All statistical tests were performed using XLSTAT (Addinsoft, New York, USA).

3. Results

3.1. Changes in physical, chemical and microbial properties within studied areas

By analyzing all study areas through two-way ANOVA, the effect of both land cover and geographic region was found for most considered variables, together with a significant interaction among factors (Table S1). Generally main differences in the most soil variables were

found among tree stands and all other land cover types.

To better understand the role of land cover in regulating soil properties, these were compared also within each geographic region (Italy, Spain and Portugal), which differed from each other in the aridity index, soil type and texture, etc. (Table 1). Significant variations among land covers were observed for all considered physical and chemical variables (Table 2). In detail, soil pH generally showed lower values in coniferous tree stands than in other land cover types both in Italian and Spanish areas (Table 2A,B), the only ones where coniferous stands were found. Soil cation exchange capacity (CEC), total organic carbon content (C_{org}), its labile and mineralizable fractions (C_{ext} and C_{min}), total N content (N) and C/N ratio and, limited to Spanish sites, WC content, had significantly (P < 0.05) higher values in the coniferous tree sites than in the soils of other sites (Table 2 A,B). This trend was not affected by climatic conditions; indeed, it was found also comparing sites located in the same areas (IT1 in Italy, SP2 in Spain) of coniferous tree stands and so having the same climatic conditions (i.e., shrublands IT1-1 and IT1-3, in Italy;

shrublands SP2-2 and SP2-3, in Spain; Table 1), or sites characterized by higher rainfall and lower aridity (high AI; i.e., the broad-leaved stand, croplands and pasture in IT2, IT3 and IT4, vs the coniferous tree stand IT1-2 and IT1-4, in Italy; Table 1). An opposite trend was observed for the bulk density, showing in conifer tree stands values about half as much as in other stands (Table 2A,B).

Moreover, within Italian sites, significant (P < 0.05) lower values of soil C_{org} were found in croplands than in shrublands (besides coniferous tree stands; Table 2A); while within Spanish sites significantly higher values of BD in pastures and lower values of C/N in pasture and cropland soils compared to other soils were found (Table 2B). In the Portuguese area, the lowest values of WC, CEC, C_{org}, C_{ext} and N contents and the highest values of BD were observed in cropland soils compared to other sites (with differences not always significant with respect to shrublands and pastures); moreover, broad-leaved stands generally showed higher values of pH, compared to shrublands, and higher CEC, C_{org}, C_{ext} and N content, compared to croplands (Table 2C).

Table 2

Mean values (± standard deviations) of bulk density (BD)¹, water content (WC), pH¹, cation exchange capacity (CEC)¹, content of total organic C (C_{org})¹, total N (N)¹, C/N ratio¹, extractable C (C_{ext}), mineralizable C (C_{min}), microbial C (C_{mic}), respiration (R), C_{mic}/C_{org} ratio, metabolic quotient (qCO₂) and quotient of mineralization (qM) in Italian (A), Spanish (B) and Portuguese (C) sites, with indication of replicate number (n) for each land cover. Results of one-way ANOVA to assay significant differences among land cover types in each country were reported in the last column (* P < 0.05; ** P < 0.01; *** P < 0.001; N.S.: not significant); different letters in apex indicated significant differences among land covers evaluated by the Student-Newman-Keuls test.

	Coniferous tree stands	Broad-leaved stands	Shrublands	Pastures/Grasslands	Croplands	P _{land cover}
A) Italy	(n = 15)	(n = 5)	(n = 15)	(n = 15)	(n = 50)	
BD (g d.w. cm ⁻³)	0.7 (± 0.1) ^a	1.3 (± 0.03) ^b	1.2 (± 0.1) ^b	1.3 (± 0.2) ^b	1.2 (± 0.2) ^b	***
WC (%)	17.5 (± 5.5) ^{ab}	18.8 (± 6.5) ^a	7.7 (± 2.1) ^b	18.0 (± 5.2) ^{ab}	13.1 (± 9.3) ^{ab}	***
pH	7.9 (± 0.2) ^a	8.2 (± 0.1) ^{ab}	8.3 (± 0.1) ^b	8.3 (± 0.2) ^b	8.1 (± 0.3) ^{ab}	***
CEC (cmol kg ⁻¹ d.w.)	34.7 (± 6.2) ^a	23.0 (± 7.7) ^{ab}	19.7 (± 9.4) ^b	18.0 (± 7.5) ^b	14.0 (± 9.8) ^b	***
C _{org} (g kg ⁻¹ d.w.)	128.6 (± 27.1) ^a	18.8 (± 2.4) ^{bc}	30.0 (± 10.0) ^b	18.3 (± 10.4) ^c	14.7 (± 10.8) ^c	***
N (g kg ⁻¹ d.w.)	6.7 (± 2.5) ^a	2.1 (± 0.4) ^{bc}	3.4 (± 1.4) ^b	2.1 (± 1.2) ^c	1.9 (± 1.0) ^{bc}	***
C/N	22.5 (± 10.7) ^a	2.9 (± 1.7) ^b	6.3 (± 2.5) ^b	9.4 (± 2.8) ^b	8.4 (± 4.3) ^b	***
C _{ext} (g kg ⁻¹ d.w.)	0.8 (± 0.3) ^a	0.4 (± 0.01) ^b	0.2 (± 0.1) ^b	0.3 (± 0.1) ^b	0.3 (± 0.1) ^b	***
C _{min} (g kg ⁻¹ d.w.)	1.4 (± 0.5) ^a	0.9 (± 0.1) ^{ab}	0.7 (± 0.5) ^b	0.6 (± 0.3) ^b	0.7 (± 0.6) ^b	***
C _{mic} (mg kg ⁻¹ d.w.)	747.1 (± 355.2)	405.1 (± 119.5)	665.3 (± 198.0)	583.1 (± 284.3)	428.2 (± 362.8)	N.S.
R (mg CO ₂ -C kg ⁻¹ d.w. d ⁻¹)	107.7 (± 35.9) ^a	66.1 (± 6.5) ^{ab}	63.7 (± 28.9) ^b	48.4 (± 22.2) ^b	49.7 (± 24.2) ^b	***
C _{mic} /C _{org} (%)	0.7 (± 0.3) ^a	2.1 (± 0.6) ^b	2.9 (± 0.7) ^b	2.8 (± 1.6) ^b	3.6 (± 2.5) ^b	***
qCO ₂ (g CO ₂ -C kg ⁻¹ C _{mic} d ⁻¹)	106.5 (± 47.2) ^a	25.4 (± 4.6) ^{ab}	51.8 (± 15.1) ^a	18.7 (± 22.4) ^b	111.7 (± 122.8) ^a	**
qM (CO ₂ -C % C _{org})	1.2 (± 0.5) ^a	4.6 (± 0.3) ^{bc}	2.5 (± 1.1) ^b	4.4 (± 3.1) ^{bc}	8.3 (± 9.3) ^c	***
B) Spain	(n = 10)	Not included	(n = 40)	(n = 5)	(n = 10)	
BD (g d.w. cm ⁻³)	0.6 (± 0.03) ^a		1.1 (± 0.2) ^b	1.5 (± 0.1) ^c	1.2 (± 0.1) ^b	***
WC (%)	54.6 (± 7.8) ^a		19.9 (± 9.5) ^b	23.4 (± 8.3) ^{bc}	27.4 (± 2.2) ^c	***
pH	4.5 (± 4.5) ^a		5.2 (± 5.3) ^b	5.0 (± 5.1) ^b	5.2 (± 5.2) ^b	***
CEC (cmol kg ⁻¹ d.w.)	26.0 (± 5.6) ^a		13.0 (± 4.1) ^b	10.7 (± 1.2) ^b	14.3 (± 1.0) ^b	***
C _{org} (g kg ⁻¹ d.w.)	184.4 (± 26.2) ^a		36.2 (± 20.4) ^b	21.7 (± 6.2) ^b	33.9 (± 6.2) ^b	***
N (g kg ⁻¹ d.w.)	7.0 (± 2.1) ^a		3.1 (± 1.4) ^b	4.3 (± 0.5) ^{bc}	4.3 (± 0.8) ^c	***
C/N	32.7 (± 18.0) ^a		14.3 (± 9.8) ^b	5.0 (± 1.0) ^c	7.8 (± 0.9) ^c	***
C _{ext} (g kg ⁻¹ d.w.)	0.5 (± 0.1) ^a		0.2 (± 0.1) ^b	0.1 (± 0.02) ^b	0.1 (± 0.01) ^b	***
C _{min} (g kg ⁻¹ d.w.)	5.8 (± 3.4) ^a		1.0 (± 0.8) ^b	0.5 (± 0.2) ^b	0.7 (± 0.1) ^b	***
C _{mic} (mg kg ⁻¹ d.w.)	1206.0 (± 696.4) ^a		259.4 (± 87.1) ^b	234.5 (± 65.9) ^b	344.2 (± 138.4) ^b	***
R (mg CO ₂ -C kg ⁻¹ d.w. d ⁻¹)	216.1 (± 35.7) ^a		45.1 (± 16.1) ^b	34.1 (± 5.4) ^b	55.3 (± 10.4) ^b	***
C _{mic} /C _{org} (%)	0.7 (± 0.3)		1.0 (± 0.6)	1.0 (± 0.1)	1.0 (± 0.5)	N.S.
qCO ₂ (g CO ₂ -C kg ⁻¹ C _{mic} d ⁻¹)	142.5 (± 43.8)		143.4 (± 145.7)	70.5 (± 27.1)	18.2 (± 9.0)	N.S.
qM (CO ₂ -C % C _{org})	2.8 (± 1.8)		4.2 (± 4.4)	2.1 (± 1.4)	1.9 (± 0.5)	N.S.
C) Portugal	Not included	(n = 15)	(n = 5)	(n = 41)	(n = 10)	
BD (g d.w. cm ⁻³)		1.3 (± 0.2) ^a	1.3 (± 0.1) ^a	1.3 (± 0.1) ^a	1.5 (± 0.1) ^b	***
WC (%)		21.1 (± 9.4) ^a	23.1 (± 3.4) ^a	19.9 (± 6.5) ^a	13.5 (± 5.3) ^b	***
pH		6.1 (± 0.4) ^a	5.6 (± 0.2) ^b	5.9 (± 0.3) ^{ab}	5.9 (± 0.3) ^{ab}	*
CEC (cmol kg ⁻¹ d.w.)		11.0 (± 2.6) ^a	7.9 (± 0.9) ^{ab}	8.8 (± 4.6) ^{ab}	7.0 (± 1.5) ^b	*
C _{org} (g kg ⁻¹ d.w.)		21.9 (± 15.9) ^a	13.5 (± 3.2) ^{ab}	17.4 (± 8.9) ^a	9.5 (± 3.5) ^b	***
N (g kg ⁻¹ d.w.)		3.6 (± 1.8) ^a	2.1 (± 0.2) ^{ab}	2.6 (± 1.3) ^{ab}	1.7 (± 0.5) ^b	***
C/N		5.8 (± 1.6) ^{ab}	6.5 (± 1.0) ^{ab}	7.1 (± 2.1) ^a	5.6 (± 1.9) ^b	***
C _{ext} (g kg ⁻¹ d.w.)		0.2 (± 0.1) ^a	0.1 (± 0.02) ^{ab}	0.2 (± 0.1) ^a	0.1 (± 0.04) ^b	*
C _{min} (g kg ⁻¹ d.w.)		0.4 (± 0.2)	0.4 (± 0.1)	0.5 (± 0.2)	0.6 (± 0.5)	N.S.
C _{mic} (mg kg ⁻¹ d.w.)		195.6 (± 74.2)	325.3 (± 103.4)	246.7 (± 166.1)	198.7 (± 101.4)	N.S.
R (mg CO ₂ -C kg ⁻¹ d.w. d ⁻¹)		24.7 (± 4.6) ^a	37.7 (± 4.1) ^b	39.3 (± 11.0) ^b	39.3 (± 12.3) ^b	***
C _{mic} /C _{org} (%)		1.1 (± 0.5) ^a	2.7 (± 0.6) ^b	1.4 (± 0.7) ^a	2.4 (± 0.9) ^b	***
qCO ₂ (g CO ₂ -C kg ⁻¹ C _{mic} d ⁻¹)		77.6 (± 63.3)	70.1 (± 30.6)	111.5 (± 105.9)	132.5 (± 104.7)	N.S.
qM (CO ₂ -C % C _{org})		2.8 (± 1.9) ^a	3.1 (± 0.8) ^{ab}	3.7 (± 2.0) ^{ab}	7.7 (± 5.7) ^b	**

¹Data from Grilli et al. (2021)

According to the trend observed for chemical variables, microbial biomass (C_{mic}) and respiration (R) showed higher values in coniferous stands than in all other stands both in Italian and in Spanish sites (Table 2A,B), with not significant differences for C_{mic} in Italian sites; an opposite trend was found for C_{mic}/C_{org} showing lower values in coniferous stands (with a significant difference only in Italian stands). Moreover, Italian cropland soils showed the highest values of qM (significantly only compared to coniferous tree stands and shrublands soils) and higher values of qCO_2 than pastures soils (Table 2A). In Spanish soils, besides observing higher values of C_{mic} and respiration in coniferous tree stand compared to other stands, no other differences among land cover occurred (Table 2B). In Portuguese sites, although no significant differences in soil microbial biomass (C_{mic}) were found in all

considered land cover types, lower values of soil respiration were found in broad-leaved stands compared to other land cover types (Table 2C); moreover, cropland soils generally had the highest values of C_{mic}/C_{org} ratio (together with shrubland) and qM compared to other sites, whereas broad-leaved stands showed the lowest values of C_{mic}/C_{org} (Table 2C).

3.2. Overall relationships among physical, chemical and microbial variables

Principal Component Analysis (PCA; Fig. 2A) and Cluster Analysis (Fig. 2B) were applied on soil physical and chemical properties, microbial biomass and respiration of areas from all considered geographic regions together. PCA explained 72.67% of the variance (54.40% axis 1,

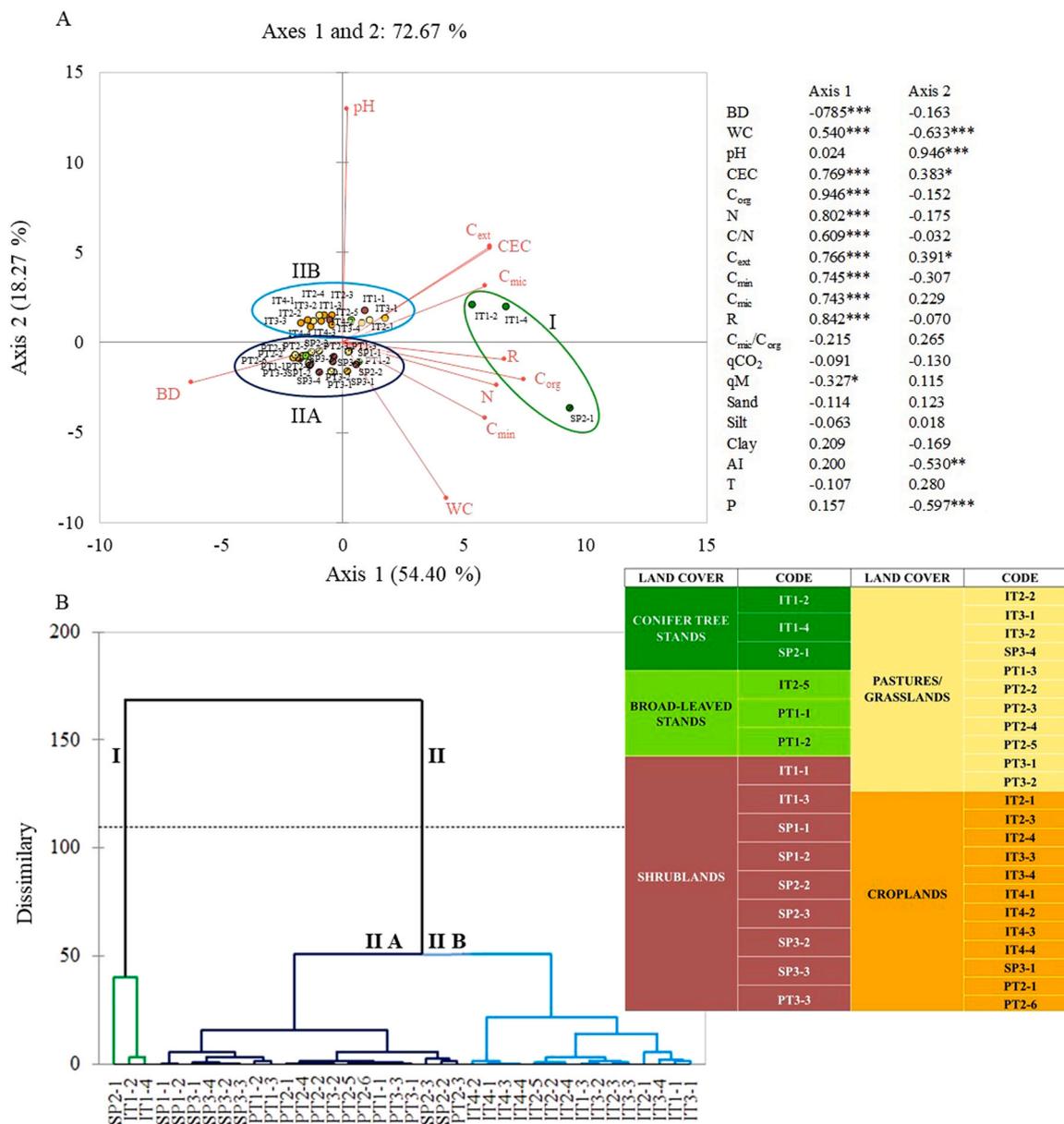


Fig. 2. Biplot deriving from the Principal Component Analysis - PCA (A) and dendrogram deriving from Cluster Analysis (B) referring to 38 sites from Italy (IT), Spain (SP) and Portugal (PT) (acronyms as legend in B) and 10 variables. In the biplot, the 38 sites are indicated in black and the following 10 variables are in red: bulk density (BD), water content (WC), pH, cation exchange capacity (CEC), content of organic C (C_{org}), total nitrogen (N), extractable C (C_{ext}) and mineralizable C (C_{min}), microbial C (C_{mic}) and respiration (R). The top right table (in A) reports the results of Pearson correlation ($n = 38$) of biplot axes with variables included in matrix as well as with microbial indices (C_{mic}/C_{org} ratio, metabolic quotient or qCO_2 , quotient of mineralization or qM), C/N ratio, percentage of sand, silt and clay, aridity index (AI), mean annual temperature (T) and total annual precipitation (P). The dotted line in the Cluster analysis (B) represents the automatic truncation, leading to three groups (I, IIA, IIB), corresponding to those delimited with ellipses in PCA biplot.

18.27% axis 2). Axis 1 of biplot deriving from PCA was positively correlated with WC, CEC, C pools (C_{org} , C_{ext} , C_{min}), N content, C/N, C_{mic} , respiration and negatively correlated with BD and qM (Fig. 2A). Axis 2 of the biplot was positively correlated with pH, CEC, C_{ext} , and negatively correlated with WC, aridity index (AI) and precipitations (P). In the biplot deriving from PCA (Fig. 2A) and the dendrogram deriving from Cluster Analysis (Fig. 2B), sites appeared separated into three main clusters: cluster I, including coniferous tree stands (only found in Italian and Spanish areas); cluster IIA, including all other Spanish (excluding coniferous tree stand) and all Portuguese stands; cluster IIB, including all other Italian sites (excluding coniferous tree stands). Compared to cluster II, Cluster I included sites with the best soil characteristics, i.e., with the highest values of CEC, total C_{org} and its labile fractions (C_{ext} , C_{min}), N content, C_{mic} , respiration and the lowest value of bulk density (Fig. 2A), so having the highest soil health. Moreover, it showed, on average, the highest values of soil C/N and the lowest values of qM (as derived, respectively, by positive and negative correlations of these indices and axis 1). Within cluster II, IIA cluster showed the worst characteristics, mainly due to lower pH (5–6.1 vs 8.1–8.3 of cluster IIB), but also to lower CEC (7.0–14.3 vs 14.0–23.0 cmol kg⁻¹ d.w. of cluster IIB) and C_{ext} (0.1–0.2 vs 0.2–0.4 g kg⁻¹ d.w. of cluster IIB). Therefore, cluster IIB (including most Italian sites) had intermediate soil characteristics between cluster I and IIA, even if its soils were drier of IIA soils, as suggested by the negative correlations of axis 2 with WC, aridity index (AI) and precipitations (Fig. 2A).

Overall, data confirmed that, compared to all other land cover types, coniferous tree stands had the best soil properties that can guarantee the best functioning of soil. On the contrary, neither clear differences occurred among soils from croplands, pastures, shrublands and broad-leaved stands of Italian sites (all in cluster IIB, Fig. 2), nor for sites with different land covers (except for Spanish coniferous tree stand) of Spanish and Portuguese areas (all in cluster IIA, Fig. 2).

It has to be underlined that the Italian sites appeared separated from Spanish and Portuguese sites along axis 2 of the biplot, according to the positive correlation of axis 2 with pH (higher in Italian sites than in Spanish and Portuguese), which was, in turn, negatively correlated with soil water content and precipitation amounts, but not affected by percentage of sand, silt or clay (Table 3). On the contrary, the separation of different geographic areas did not appear to depend on aridity index (AI) because most Spanish (higher AI) and all Portuguese (lower AI) sites were comprised in the same cluster (IIA). However, a negative correlation between the aridity index and axis 2 occurred, but it was mainly due to the overall AI trend (0.29–1.42), regardless of geographic areas (0.29–0.49 in Italian sites; 0.67–1.42 in Spanish sites; 0.35–0.38 in Portuguese sites; Table 1). To note that biplot axes were not affected by percentages of sand, silt and clay, neither of these variables was correlated with soil water content.

Changes in C_{mic} and respiration reflected variations in physical and chemical soil properties among all 38 considered sites. Indeed, both C_{mic} and soil respiration, positively correlated to each other, were both positively correlated with WC, CEC, total C_{org} , C_{ext} , C_{min} (the C pools being positively correlated to each other), N content and C/N (CEC being positively correlated with organic C pools, N content and C/N) and negatively with BD, but were not influenced by soil pH (Table 3). It has to be noted that BD was negatively correlated with C_{org} , C_{ext} , C_{min} and N contents and C/N (Table 3).

C_{mic} was also negatively correlated with the quotient of mineralization (qM) (Table 2), the latter corresponding to the mineralizable C as percentage of total organic C_{org} .

It has to be underlined that notwithstanding differences in average annual climatic conditions occurring among studied sites (annual temperature from 11.7° to 16.6°C, annual precipitations from 352 to 1427 mm, Aridity Index from 0.29 to 1.42; Table 1), neither C_{mic} and respiration nor microbial indices were correlated to these variables. The coniferous tree stands, which clustered together in PCA and Cluster Analysis and showed the best soil properties related to its functioning,

Table 3 Pearson correlation (n = 38) between considered variables: bulk density (BD), water content (WC), pH, cation exchange capacity (CEC), total organic C (C_{org}), total nitrogen (N), C/N ratio, extractable C (C_{ext}), mineralizable C (C_{min}), microbial C (C_{mic}), respiration (R), C_{mic}/C_{org} , quotient of mineralization (qM), percentage of sand, silt and clay, aridity index (AI), mean annual temperature (T) and total annual precipitations (P).

	BD	WC	pH	CEC	C_{org}	N	C/N	C_{ext}	C_{min}	C_{mic}	R	C_{mic}/C_{org}	qCO ₂	qM	Sand	Silt	Clay	AI	T
WC	-0.219																		
pH	-0.130	-0.498*																	
CEC	-0.491*	0.268	0.330*																
C_{org}	-0.765***	0.533**	-0.161	0.645***															
N	-0.664**	0.556**	-0.204	0.571**	0.794***														
C/N	-0.528**	0.193	-0.117	0.480**	nd	nd													
C_{ext}	-0.637***	0.204	0.356*	0.734**	0.696**	0.600***	0.479**												
C_{min}	-0.457*	0.484**	-0.195	0.368*	0.726**	0.385*	0.592**	0.392*											
C_{mic}	-0.557***	0.356*	0.269	0.684**	0.601***	0.500*	0.401*	0.439*	0.475**										
R	-0.579***	0.358*	0.110	0.545**	0.786**	0.520*	0.514**	0.545**	0.901***	0.566***									
C_{mic}/C_{org}	0.111	-0.274	0.316 ¹	-0.142	nd	-0.299	-0.293	-0.254	nd	nd	-0.086	-0.127							
qCO ₂	0.054	-0.223	-0.121	-0.310	-0.063	-0.306	0.130	-0.009	0.342*	nd	nd	0.307	0.688***						
qM	0.263	-0.441**	0.182	-0.372*	nd	-0.571***	-0.195	-0.212	nd	-0.351*	0.107	0.295	-0.269	0.042					
Sand	0.243	0.019	0.179	0.019	-0.201	-0.013	-0.450**	-0.008	-0.211	-0.005	-0.106	0.295	-0.269	0.046	-0.676***				
Silt	-0.064	-0.128	-0.030	-0.126	-0.016	-0.147	0.166	0.047	0.017	-0.121	-0.060	-0.107	0.380*	0.046	-0.844***	0.177			
Clay	-0.295	0.069	-0.209	0.074	0.288	0.139	0.481**	-0.008	0.271	0.104	0.190	-0.323	0.081	-0.097	-0.844***	0.080	0.532**		
AI	-0.179	0.333*	-0.536**	-0.023	0.293	0.020	0.551***	-0.126	0.515**	0.007	0.255	-0.193	0.308	0.124	-0.445**	0.074	-0.320	-0.848***	
T	0.138	-0.274	0.214	0.023	-0.126	0.118	-0.381*	0.125	-0.365*	-0.080	-0.106	0.012	-0.124	-0.145	0.198	0.074	-0.320	-0.848***	
P	-0.142	0.348*	-0.607***	-0.083	0.267	0.023	0.509**	-0.181	0.477**	-0.046	0.211	-0.208	0.300	0.106	-0.428**	0.068	0.517**	nd	-0.825***

* = P < 0.05; ** = P < 0.01; *** = P < 0.001; ¹P = 0.053; nd = correlation between these variables was not taken into account.

included both Spanish and Italian stands that strongly differed for annual precipitations (1427 vs 352 mm) and Aridity Index (AI, 1.42 vs 0.29).

4. Discussion

4.1. Main factors affecting soil physical, chemical and microbial features

Land cover significantly affected soil properties as found analyzing data from different geographic region both together and separately. According to our hypothesis, data analysis within each region clearly showed that close-canopy coniferous tree stands (limited to Italian and Spanish sites) had the best soil physical, chemical and microbial properties (lower bulk density, higher cation exchange capacity, higher contents of total organic C and its labile fractions, C_{ext} and C_{min} , and N, higher C_{mic} and respiration), corresponding to the best soil functioning and, consequently, to the highest soil health, compared to all other stands (broad-leaved, shrubland, pasture and cropland stands). This trend was not influenced by soil texture and was observed independently by climatic conditions. This result suggested that land cover was the main factor affecting the analyzed soil properties. The large difference in soil C_{org} (negatively correlated with BD) between coniferous and broad leaved stands, observed in Italian areas and also reported by Chiti et al. (2012) in Spanish forests, could be explained in part by organic C accumulation due to the lower decomposition rate of the needle, compared to leaf litter (Devi, 2021a; Wang et al., 2020). This may be determined by higher litter C/N ratio and lignin content observed in coniferous than in broad-leaved forests (Zhang et al., 2020; Han et al., 2015) which slows down decomposer activity. Consistent with this, the higher C/N ratio observed in soils from Italian and Spanish coniferous stands than in soils from other land covers could result in lower decomposition rate of soil organic matter and, consequently, in higher C_{org} content. The low decomposition rate in coniferous stand, also due to lower soil pH compared to other stands, may be further slowed down by the water limitation conditions for litter and soil occurring in southern Europe areas for late spring – early autumn period. The highest values of labile organic C (C_{ext} , C_{min}) in coniferous tree stands, compared to other stands of the same geographic region, indicates an excess of available labile C due to a high input compared to losses by microbial mineralization, leaching or erosion. Lower microbial consumption rates of these pools, compared with inputs, and possible adsorption of labile pools on stable organic matter contribute to increasing the permanence time of this organic matter on the soil. Moreover, the low decomposition rate of needle compared to leaf litter likely increases the persistence of the litter (that we excluded from the samples) on the soil providing soil cover and protecting the soil from erosion processes. Higher soil organic matter generally leads to an increase in water retention, even if soil texture may be the most important factor regulating water content in fine-textured soil (Rawls et al., 2003). However, among soils considered in this study, only Spanish coniferous tree stand showed higher water content values compared to other stands.

The higher availability of C pools, together with higher N content and CEC and lower BD in coniferous tree stands compared to other stands, also explained higher microbial growth and activity, which have been demonstrated to be sensitive to different land cover (Evangelou et al., 2021; Liu et al., 2018; Marzaioli et al., 2010a).

The average reduction of soil C_{mic} and respiration observed in croplands (57% and 71%, respectively), compared to coniferous tree stands of the same geographic region, was consistent with that observed by Islam and Weil (2000) who found a similar reduction in microbial biomass (about 40%) compared to the forest soils, but not on soil respiration. The reduction in C_{mic} and activity in croplands could be explained by the fact that all cropland sites of this study were generally tilled for seeding or weeding (Grilli et al., 2021). Tillage may reduce microbial biomass by modifying resource availability for microorganisms (Six et al., 2006). The increase in soil aeration by tillage (Khan

et al., 1996) favors the decomposition of organic matter, so reducing its availability for microorganisms (Lal, 1993) and lowering soil respiration (Moraru and Rusu, 2012). Moreover, tillage physically disrupts fungal hyphae (Evans and Miller, 1990) and alters microbial community composition (Li et al., 2021).

The average reduction in C_{mic} and respiration observed in pasture, shrubland and broad-leaved compared to coniferous tree stands (respectively, of 66%, 60% and 68%, for C_{mic} , and 73%, 70% and 75% for respiration), may reflect the difference in plant cover (Singh et al., 2021) that was much denser in the coniferous stands, thus protecting the soil.

Whereas Spanish cropland generally did not differ from other land covers of the same region (except for coniferous tree stand), Italian croplands showed lower values of C_{org} , compared to shrublands (besides coniferous tree stand), and Portuguese croplands showed lower values of WC, CEC, C_{org} , C_{ext} , N and higher BD, compared to other land covers of the same geographic region. This is in accordance with Marzaioli et al. (2010a) who observed the same results by comparing croplands, shrublands, grazing lands, coniferous forest and mixed forests in southern Italy. On the other hand, Portuguese broad-leaved stands showed higher soil pH vs shrublands, and higher CEC, C_{org} , C_{ext} and N contents vs croplands.

4.2. Overall relationships among soil variables and sites to identify soil health changes

Results of PCA and Cluster analysis, applied to all variables and all sites together, confirmed that land cover was the main factor affecting soil health, overshadowing aridity (derived from the aridity index), as shown by the clear separation of coniferous tree stand soils (cluster I) from all other sites (cluster II). At a lower level, the separation between Italian sites other than coniferous stands (cluster IIB) and Spanish (except coniferous stand) and Portuguese sites together (cluster IIA), was mainly due to pH. This latter resulted moderately alkaline in Italian sites, from slightly to moderately acidic in Portuguese sites and from strongly to very strongly acidic in Spanish sites (after classification of USDA-NRCS, 2004). The lowest pH values observed in Spain soils may be related to the highest precipitations, as suggested by the negative correlation between these variables. Abundant precipitation may reduce soil pH by removing base cations (Ng et al., 2022). The high pH values in Italian soils, compared to Portuguese and Spanish soils with the same land cover, explained the higher values of CEC in these soils (Graber et al., 2017); indeed, a positive correlation between pH and CEC was found.

At a lower extent, Italian soils differed from Spanish and Portuguese soils for higher values of CEC, C_{ext} and C_{mic} , all suggesting higher soil health (Andrews et al., 2004), notwithstanding Italian sites were more arid than Spanish sites (lower precipitations and aridity index). The very low pH values reduced the overall soil health of Spanish soil, making them more similar to Portuguese soils, despite Spanish sites had lower desertification risk (moderate vs high) and lower aridity (higher aridity index, AI, Table 1) compared to Portuguese and Italian sites.

C_{mic} and soil respiration (positively correlated to each other) were sensitive to changes in physical and chemical properties and quickly respond to difference of land cover. Indeed, previous studies provided evidence that C_{mic} responds more quickly to disturbance/stress due to land management than C_{org} (Babur and Dindaroglu, 2020; Gupta et al., 1994) thanks to its faster turnover rate (1–2 years; Jenkinson and Ladd, 1981), which makes this labile soil C pool a more sensitive indicator of changes in soil processes, such as biogeochemical cycles and soil structure stabilization (Babur and Dindaroglu, 2020; Haynes, 2008). Like to soil microbial biomass, soil respiration, performed in laboratory standardized conditions of temperature and moisture, was found to be sensitive to soil changes linked to land use (Han et al., 2015; Marzaioli et al., 2010a; Singh et al., 2021). Therefore, compared to physical and chemical properties, which require long times to respond to anthropic

activities/disturbances, microbial biomass and soil potential respiration could provide more immediate information on soil health changes, making them useful for short-term monitoring of land management activities (Nielsen and Winding, 2002). By adding to these variables also indices of microbial metabolism (qCO_2 , qM and C_{mic}/C_{org} ratio), it is possible to better understand the direction of ongoing processes in which microorganisms are involved. High value of qCO_2 indicates a low microbial efficiency to store C that is typical of stress/disturbance conditions, in which “repairing damage by disturbances requires diverting energy from growth and production to maintenance” (Odum, 1985). Singh et al. (2021) found lower qCO_2 in tree-based systems (plantations and agroforestry systems) than pasture, shrubland and cropland in Central India, in areas characterized by hot-dry summers and cold winters. However, in our study, there was no clear relation between qCO_2 and land cover types in all considered areas or physical and chemical variables, as observed also by other authors (Wardle and Ghani, 1995).

The soil C_{mic}/C_{org} ratio, representing the importance of soil microorganisms as a sink for mobile C in soils (Klose et al., 2004) was, in average, lower in coniferous tree stands than in other land covers, while it showed relatively higher values in cropland soils (except for Spanish soils). A high C_{mic}/C_{org} ratio also indicates soil labile C accumulation and favourable environment for microbial growth, while a low C_{mic}/C_{org} indicates a reduced availability for soil microorganisms (Cheng et al., 2013; Joergensen and Emmerling, 2006), due to prevalence of recalcitrant C compounds in the soil C pools under coniferous forests (Cheng et al., 2013). The different trend observed in Spanish soils, where no significant difference among land cover types occurred for C_{mic}/C_{org} , suggested that only a low fraction of total organic C (about 1%) was accumulated in microbial biomass, independently by land cover, probably because acid condition of Spanish sites limited the C immobilization in the microbial biomass (relatively to total C_{org}). Indeed, a weak positive correlation ($P = 0.05$) between the C_{mic}/C_{org} ratio and pH was found, according to other authors (Anderson and Domsch, 1993; Serna-Chavez et al., 2013).

The quotient of mineralization (qM) showed higher values in soils with lower content of organic matter (C_{org} , C_{min} , C_{ext}) and C_{mic} which clustered on the left of PCA biplot. Indeed, qM was negatively correlated with axis 1 of PCA biplot. In the Italian sites, the lowest quotient of mineralization (qM) found in the coniferous tree stands compared to other land cover types was in accordance to the high fraction of recalcitrant C compound (low C_{mic}/C_{org} ratio) and suggested that these soils tended to conserve more C than soil from other land cover types. On the contrary, the Italian and Portuguese cropland soils showed the highest values of qM compared to soil of all other land covers of the respective geographic region. This indicates that cropland soils, showing lower C pools (C_{org} , C_{ext} , C_{min} , C_{mic}) tended to loss C more quickly. This trend was not found in Spanish sites.

In soils from croplands, and, to a lesser extent, soils from pastures, shrublands and broad-leaved stands of the study areas, the important ecosystem services (as nutrient, water and climate regulation and C sequestration; Adhikari and Hartemink, 2016) provided by soil microbial community could be compromised by low organic C pools, since soil organic carbon was identified as the most important driver for global distribution patterns of microbial community (Wan et al., 2021). This is confirmed not only by the results of our study (showing significant positive correlations of C_{mic} and respiration with C_{org}) but also by several other studies carried out in alpine grasslands (Chen et al., 2016), forests and croplands (Wan et al., 2021), temperate coniferous and tropical forests (Fierer et al., 2009).

Data suggest that soils already poorer in C tend to lose C more quickly, following a positive feedback pattern that lead them into an irreversible decline. Therefore, urgent actions are needed to avert this risk. To address this alarming condition observed in studied soils, site-specific Desertification Adaptation Models (DAMs) are being applied in private and public lands selected within the LIFE Project Desert-Adapt, to improve land quality, soil conservation, plant support and,

consequently, socio-economic development.

This study also reveals that most measured soil variables were sensitive to changes in land cover in areas at desertification risk. However, among them, a selection may be made to reduce the dataset and make more easily replicable our study. In particular, to evaluate the soil health changes in areas at desertification risk, we propose the assessment of the following minimum data set (MDS): bulk density (BD), water content (WC), pH, C_{org} , C_{ext} , N, C/N, C_{mic} , respiration and qM . Bulk density reflects the degree of soil compaction and affects water and solute movement, and soil aeration (Arshad et al., 1996). WC provides information on climatic conditions in the days before sampling. Soil pH influences the availability of soil nutrients (Smith and Doran, 1996). Contents of total organic carbon (C_{org}), as pH, always included in MDS reported in literature, influence many critical soil functions (Andrews et al., 2004). Labile organic C (C_{ext}) and total N contents are widely used as indicators of soil processes and are particularly sensitive to disturbance (Bünemann et al., 2018). The C/N ratio is highly correlated to ecological processes of immobilization and mineralization and is considered as the essential factor influencing the equilibrium of C and N cycling (Zhang et al., 2015). Moreover, soil microbial biomass (C_{mic}) and respiration have been shown to be responsive to short-term of changes of soil processes. Potentially mineralized C (qM) reflects the efficiency of microflora in metabolizing organic matter (Mocali et al., 2008). The chosen properties were also selected in different studies on soil quality/health. For example, pH, C_{org} and N contents showed their sensitivity in soil quality evaluation in degraded region of the Qinghai–Tibetan Plateau of China (Dong et al., 2012). Moreover, C_{mic} and respiration allowed to compare the effect on soil of several agronomic management practices (Nunes et al., 2020) as well as the effect of different land-use types of Northeast India (forests, agroforests, grassland, agricultural lands; Devi et al., 2021b).

In this minimum data set we included soil properties providing information on soil health changes on the both short term (microbial variables), and longer term (physical and chemical variables) in order to obtain information on main factors influencing soil health in areas at desertification risk.

In the proposed minimum data set, we did not include CEC, although it is an important soil property, because it is strongly correlated with soil C_{org} , so it showed the same variations of C_{org} . Similarly, we propose to avoid using two available C pools (C_{min} and C_{ext}) and to choose C_{ext} because it is more sensitive to land use changes; moreover, C_{min} has already considered in the calculation of qM that was, among microbial indices, the most sensitive to land cover changes. Finally, the percentage of sand, silt and clay are very important soil properties, however, they did not appear very sensitive to the investigated factors.

4.3. Conclusion

In line with our first hypothesis, coniferous tree stands were identified as the land cover type with the highest soil health, compared to other land covers, in areas of southern Europe with a moderate-high sensitivity to desertification. Moreover, only under coniferous cover, the C storage in microbial biomass and in soil was favored over C losses, as suggested by the low quotient of mineralization (qM), which revealed the direction of the ongoing process, as well as by the highest C pools. Other land cover types showed less marked differences from each other, even if cropland sites had the worst soil health, mainly in Portuguese sites, as well as the highest quotient of mineralization (except for Spanish croplands) and so higher C loss risk, suggesting that urgent actions are necessary to recover them. Our results can provide useful support and indications for management strategies to restore severely degraded areas in the southern Europe ecosystem. However, further studies on the same sites or on other sites with similar characteristics are necessary to confirm our observations.

This study also showed that a minimum data set of microbial, physical and chemical properties allows to evaluate soil health changes

in areas under desertification risk, by providing information on both short term (microbial variables) and long term (most physical and chemical variables). It also allowed to highlight that land cover was the main regulating factor of the study areas, overcoming the aridity index.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.pedobi.2023.150894](https://doi.org/10.1016/j.pedobi.2023.150894).

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