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REGULAR ARTICLE

Holm oak (*Quercus ilex* L.) rhizosphere affects limestone-derived soil under a multi-centennial forest

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Abstract

Background and Aims The activity of roots and associated microorganisms plays a key-role in soil formation and evolution, but we lack of knowledge on the quality and extent of the "rhizosphere effect" in the different soil horizons. The aim of this study was to assess the interactions between rhizosphere processes and genetic horizons in a forest (*Quercus ilex* L.) soil developed from limestone. Specifically, we tested (a) if the rhizosphere effect was significant in all the horizons of the soil profiles, and (b) if the intensity of the rhizosphere effect was associated to structure, composition and activity of the microbial community.

Methods Bulk and rhizosphere soils were characterized by physical, mineralogical, chemical and biological (microbial activity and community structure) analyses.

Results Throughout the soil profile, the rhizosphere processes affected properties like particle-size distribution and soil structure, mineralogy, pH, and organic C and total P content. Conversely, amounts of exchangeable Ca, Mg and K, iron oxides, available P, and total nitrogen showed no significant change. As for the microbial community, its structure and metabolic activity differed between rhizosphere and bulk only in the core of the solum (2Bwb and 3Bwb horizons).

Conclusions The main processes controlling the intensity of the rhizosphere effect on the soil horizons were root activity, soil faunal perturbation and slope dynamics. While root activity impacted the whole soil profile, although to a lesser extent at depth, the influence of fauna and slope was confined atop the profile. It follows that long-term changes due to root activity and associated microbial community were more strongly expressed in the core of the solum, not at the surface, of this limestone-derived soil.

Keywords Mineral weathering · Rhizodeposition · Soil microbial community · Basal respiration · Plfa · Nutrient cycling

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Introduction

During its development, the soil undergoes continuous modifications that involve fluxes of energy, chemical elements and water (Richter et al. 2007) driven by pedogenetic factors such as climate, parent material, biota, topography and time. Among these forces, plant

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roots and associated microorganisms trigger some of the most important biological processes controlling weathering reactions through the production of organic acids, the biocycling of cations, the formation of secondary minerals, and the modification of soil hydrology (Kelly et al. 1998). Indeed, roots and associated microbes are able to modify living and non-living soil constituents such as microbial communities, nutrient status and minerals (Curl and Truelove 1986; Courchesne and Gobran 1997; Jordan et al. 2008) via the production and release of CO_2, organic acids, ligands, protons and enzymes (Drever 1994; Gobran et al. 1998, Cocco et al. 2013).

Most of the reactions involving soil, plants, and root-associated microorganisms occur in the ecto-rhizosphere (hereinafter "rhizosphere"), the thin soil layer surrounding roots and affected by the activity of roots themselves, and the microorganisms; the thickness of this soil portion usually ranges from less than one to a few millimetres (Lynch 1990; Hinsinger et al. 2003, 2005; Turpault et al. 2007). The rhizosphere represents a physico-chemical milieu that differs from the bulk soil (Curl and Truelove 1986) because of rhizodeposition processes that enrich the soil close to the roots with highly bioavailable and low-molecular weight carbon compounds (Toal et al. 2000). Accordingly, the microbial communities harboured in the rhizosphere are usually more abundant, about ten- to hundred-fold higher than those of the surrounding bulk soil (Huang and Gobran 2005). The high microbial activity, together with processes such as root nutrient uptake, organic acid release, redox reactions, and CO_2 production, affect soil acidification and mineral weathering (e.g., Hinsinger et al. 2003; Richter et al. 2007) and make the rhizosphere a driving component of soil formation.

The extent of soil development promoted by rhizospheric processes is however very much dependent on the type of bedrock (e.g., De Vries and Breeuwsma 1987; Fujii et al. 2008; Cocco et al. 2013). For example, compared to soils developed from non-carbonaceous parent materials, rhizosphere processes take longer to impact calcareous soils because these latter are less susceptible to acidification as carbonate dissolution neutralises the activity of protons. However, a significant physico-chemical differentiation of the rhizosphere from the bulk soil may develop even in soils derived from calcareous parent materials, when soil-forming mechanisms are particularly intense or have lasted long enough (Cocco et al. 2013). Further, in calcareous soils, the weathering processes controlled by the rhizosphere microbial community are crucial in making available plant nutrients such as phosphates, iron, and manganese (Marschner 1997; Lambers et al. 2009), and play a key role in driving the soil development.

Notwithstanding the importance of the activity of roots and associated microorganisms in the soil formation and evolution, only few works considered the quality and the extent of the so-called "rhizosphere effect" in the different soil horizons (e.g., Richter et al. 2007; Cocco et al. 2013).

To appraise the rhizosphere dynamics and reveal its influence on soil formation in limestone-derived soils, it would be ideal to carry out studies on soils that have constantly been covered by the same trees for a duration that is long enough to be relevant to the time-scale of pedogenic processes. Such conditions exist in the Umbria region (Italy), where a Holm oak (*Quercus ilex* L.) forest has been maintained and preserved for at least eight centuries within the Franciscan monastery of Assisi located on the slope of a limestone massif. In this context, the aim of this study was to assess the interactions between rhizosphere processes and genetic horizons in soils developed from calcareous parent material. Specifically, we tested (a) if the rhizosphere effect was significant in all horizons of highly developed soil profiles, and (b) if the intensity of the rhizosphere effect was associated to the structure, composition and activity of the microbial community. Our approach was multivariate and was based on a thorough evaluation of the physical, mineralogical, chemical and biological differences existing between the bulk and the rhizosphere of Holm oak in a limestone-derived soil under a multicentennial forest.

Material and methods

Study site and soil sampling

The Holm oak Forest (43°03′47″ N, 12°39′07″ E) stands within the "Eremo delle Carceri", a small hermitage built in the XIII century inside an already present forest of Holm oaks (*Quercus ilex* L.), in the municipality of Assisi (Perugia, Italy). The forest occupies a NW facing slope of Mount Subasio at elevations from 820 to 880 m above sea level and has slopes from 25 to 30 %. The mean annual precipitation in the area is 1271 mm, whereas the mean annual air temperature is 13.6 °C.

The woodlot, which spans about 6 ha, is composed of Holm oaks about 20 m tall and with a breast-height mean diameter of about 36 cm. The site has been conserved by monks, who have been permanently living in the monastery since the XIV century. Other than Holm oaks, the soil hosts sparse autochthonous trees of sycamore maple (*Acer pseudoplatanus* L.), elder (*Sambucus nigra* L.) and chestnut (*Castanea sativa* Miller). The shrub layer of the understory comprises common spindle (*Euonymus europaeus* L.), butcher's broom (*Ruscus aculeatus* L.), elmleaf blackberry (*Rubus ulmifolius* Schott), common ivy (*Hedera helix* L.) and seedlings of sycamore maple, European hop hornbeam (*Ostrya carpinifolia* Scop.) and manna ash (*Fraxinus ornus* L.). The herbaceous layer hosts green hellebore (*Helleborus viridis* L.), *Galium spp.*, *Senecio spp.*, *Cyclamen spp.*, yellow sweet clover (*Melilotus officinalis* L.) and various orchids.

The soil of the forest has been developed from massif limestone and was classified as fine loamy, mixed, mesic, non-acid Udic Haplustoll (Soil Survey Staff 2014). The soil undergoes medium to intense sheet erosion as indicated by the presence of discontinuous and thin Oi and Oe horizons. A further cause of erosion and soil disturbance is the activity of wild pigs.

During the autumn of 2010, three profiles located at 40–50 cm from the stem of 100 to 140 years old Holm oaks with a breast-height diameter of 43–45 cm were dug to a depth of 70–80 cm. The three trees were situated at the vertexes of a triangle with an area of about 80 m^2. The profiles were morphologically described (see Table 8) according to the manual of Schoeneberger et al. (2012). The profiles revealed the polycyclic nature of the soil, which contained the materials from at least four landslides superimposed one over the other. The 5BCb horizon represented the remnants of the soil developed from the in situ bedrock. For every profile, large amounts (at least 3 kg) of each horizon were collected and the samples were kept field-moist in a portable refrigerator. Once in the laboratory, the rhizosphere of Holm oak was isolated from each soil sample by picking up the roots together with the adhering soil, according to the method of Corti et al. (2005). Coarse and medium roots (diameter size larger than 2 mm) were discarded. The soil particles loosely adhering to the roots were detached by gentle shaking and added to the bulk soil. The soil material strictly adhering to the roots was reduced to a thickness of about 3–6 mm (Corti et al. 2005; Cocco et al. 2013) by using a dissecting knife, and the material removed was added to the bulk soil. The 3–6 mm soil layer still adhering to the roots was considered as rhizosphere, and then recovered by shaking and gentle brushing of the roots (Cocco et al. 2013; Massaccesi et al. 2015). During this operation, the root fragments were removed by using tweezers under a magnifying lens. Aliquots of field moist rhizosphere and bulk soil were stored at 4 °C for the microbial biomass C and basal respiration analyses, and at −20 °C for the phospholipid fatty acid analysis. The remaining soil material was air-dried and sieved through a 2-mm mesh and used for physical, mineralogical and chemical analyses.

Particle-size distribution and mineralogy

Particle-size distribution was determined after maintaining the samples submerged in deionised water for 24 h and after the dissolution of organic cements by NaClO solution at 6 % of active chlorine adjusted to pH 9 with HCl. The sand was retrieved by wet sieving at 0.05 mm, while silt was separated from the clay by sedimentation after dispersion in 0.01 M NaOH solution at 20 °C.

The mineralogical investigation was accomplished on powdered specimens with a Philips PW1830 X-ray diffractometer, using the Fe-filtered Kα1 radiation produced by a Co anode, and operating at 35 kV and 25 mA. Diffractograms were acquired in the range 3–80 °2θ, with a step size of 0.02 °2θ and scanning speed of 1 s per step (see details in the Supplementary Materials).

Chemical analyses

The soil pH was determined potentiometrically in water and in 1 M KCl solution (solid:liquid ratio of 1:2.5). Total organic C content (TOC) was estimated by K-dichromate digestion, heating the suspension at 180 °C for 30 min (Nelson and Sommers 1996). To measure water extractable organic C (WEOC), 1 g of sample was placed into a plastic container, submerged with distilled water (solid:liquid ratio 1:10) and shaken overnight with an orbital shaker (140 rpm). The mixture was left to rest for a while, centrifuged at 1400 *g* for 10 min, and then filtered through Whatman 42 filter paper. The resulting solutions were analysed with a TOC-500A (Shimatzu, Tokyo, Japan) analyser after the addition of a few drops of concentrated H_3PO_4 to eliminate carbonates.

Total N content was determined by a Carlo Erba EA1110 (Carlo Erba Instruments, Milan, Italy) dry combustion analyzer. To determine the different forms of inorganic N (NO_3^-N, NO_2^-N, NH_4^+-N), specimens were treated with 2 M KCl solution at a 1:10 soil:liquid ratio, shaken for 1 h with an orbital shaker (140 rpm) and the suspensions were filtered through a Whatman 42 filter paper. The amount of the inorganic N forms was measured on soil extracts by the FOSS Fiastar™ 5000 system (Hillerod, Denmark) (Application Note 520 method). The organic N content was calculated as the difference between total N and the sum of N in form of inorganic compounds.

To determine the exchangeable Ca, Mg, K and Na, 2 g of sample were placed into centrifuge tubes, submerged with 0.2 M $BaCl_2$ solution (solid:liquid ratio of 1:10), and shaken for 10 min (Corti et al. 1997). The mixture was left to rest, gently shaken for few seconds to re-suspend the sediments and then centrifuged. The extracted solutions were filtered through Whatman 42 filter paper, and analysed by atomic absorption with a Shimadzu AA-6300 spectrophotometer (Tokyo, Japan).

Iron was sequentially extracted from the samples with: 1) 0.1 M Na-acetate solution at pH 5 to extract the Fe bound to carbonates (Loeppert and Suarez 1996), 2) 0.1 M hydroxylamine hydrochloride in 0.01 M HNO_3 solution to estimate the labile Fe, namely the Fe of the easily reducible Fe-oxyhydroxydes (Berna et al. 2000), 3) NH_4-oxalate/oxalic acid solution at pH 3.0 in the dark to recover the non-crystalline Fe-oxyhydroxydes and Fe associated to organic matter (Blakemore et al. 1981), and 4) 0.25 M hydroxylamine hydrochloride in 0.25 M HCl solution to obtain crystalline Fe-oxyhydroxydes (Berna et al. 2000). The Fe in the extracts was determined by a Shimadzu AA-6300 atomic absorption spectrophotometer..

Available and total P were determined by the Olsen method (Olsen et al. 1954) and the ignition method (Kuo 1996), respectively.

Microbial biomass C, basal respiration and PLFA analysis

The amount of microbial biomass C was determined by the fumigation-extraction method (Vance et al. 1987), after 20 days of incubation at 25 °C and at 50 % of the total water holding capacity. Basal respiration was measured by alkali (1 M NaOH solution) absorption of the CO_2 developed during the incubation period followed by titrating the residual OH with a standardised acid. The specific microbial respiration (qCO_2), which expresses the CO_2-C evolved per unit of microbial biomass and time (μg CO_2-C mg^{-1} C_{mic} h^{-1}) was calculated according to Anderson and Domsch (1993).

Soil microbial community structure was assessed by analysing the ester-linked phospholipid fatty acid (PLFA) composition (Bardgett et al. 1996), on a HP 5890 Series II gas-chromatograph, equipped with a 5970 MSD detector and Supelco SP 2331 column (60 m, 0.25 mm I.D., 0.20 μm D.F.). The peak identification was accomplished by comparing retention times with known standards (FAME Mix and BAME Mix, Supelco Analytical, USA). Concentration of each PLFA was obtained by comparing the peak area of each identified fatty acid with that of methyl nonadecanoate (C19:0) added to the samples as internal standard. All identified PLFAs were summed to obtain the total extractable PLFAs, which is considered one of the indicator of living microbial biomass. The different PLFAs were used as markers to quantify the relative abundance of specific cell types (Fierer et al. 2003). These microbial groupings are considered approximate since some PLFAs are not specific for a single microbial type (Zelles 1997). Gram-positive bacteria were identified by summing i15:0, a15:0, i16:0, i17:0 and a17:0 PLFAs, while the Gram-negative bacteria were accounted by summing 16:1, cy17:0, 17:1ω9c and 18:1ω7 PLFAs (Federle 1986; Fierer et al. 2003). The total bacterial biomass was calculated by the sum of the PLFAs attributed to Gram-positive and Gram-negative bacteria. The 18:2ω6 PLFA was taken as a marker for fungi (Federle 1986). The 16:1ω5 PLFA was used as an indicator for arbuscular mycorrhizal fungi (AMF) (De Deyn et al. 2011). The ratio between total bacteria and total fungal biomass was taken as an indicator of changes in the relative abundance of these two microbial groups (Bardgett et al. 1996). Actinomycetes were identified by the 10Me17:0 and 10Me18:0 PLFAs (Zelles et al. 1994; De Deyn et al. 2011), whereas the 20:2 PLFA was used as biomarker for protozoa (Fierer et al. 2003).

Replicates and statistics

A single determination was performed on all the horizons of the three profiles for particle-size distribution, mineralogy, pH, exchangeable cations, extractable Fe-forms, available and total P, and PLFA analysis. The values from

the three profiles were then averaged per horizon ($n = 3$). A double determination (2 aliquots for each horizon of the three profiles) was run for TOC, WEOC, total N, NO_3^-N, NO_2^-N, NH_4^+-N, organic N, microbial biomass C and basal respiration, and the two values per sample were averaged. These averages were used to calculate the mean for each horizon ($n = 3$). In all cases, the standard error was calculated for $n = 3$. The data were tested for the normality of the distribution and the homogeneity of the variances by Shapiro-Wilk and Levene tests, respectively, and log-transformed if necessary. The logarithmic transformation was selected by the maximum likelihood procedure devised by Box and Cox (1964), as implemented in the boxcox function of the package MASS (Venables and Ripley 2002) in the R statistical environment (R Core Team 2014). Two-way ANOVA were performed (see Table 9), and the comparison of means was assessed by Tukey HSD post-hoc test ($P < 0.05$).

To test for differences in microbial community structure between bulk and rhizosphere and among the horizons, as quantified by the relative abundance of all PLFA peaks, we performed a Permutational Multivariate Analysis of Variance (PERMANOVA). Non-metric multidimensional scaling (NMDS) was used to provide a graphical representation of results.

All the statistical analyses were performed using R (R Core Team 2014).

Results

Particle-size distribution, mineralogy, and pH

In both bulk and rhizosphere, sand was the most abundant fraction when the particle-size distribution was determined after water treatment (Table 1). The rhizosphere had a greater amount of sand in the upper soil portion (A-2Bwb2 horizons), while the contrary happened more in depth. When the particle-size analysis was performed after dissolution of organic cements, a decrease in sand with a corresponding silt and clay increase occurred for both bulk and rhizosphere (Table 1). Generally, the bulk contained more sand than the rhizosphere, while the silt content was higher in the rhizosphere; the clay abounded in the rhizosphere of the AB and 2Bwb1 horizons and in the bulk of the horizons underneath.

Quartz was the most abundant primary mineral, and it was higher in the rhizosphere than in the bulk of the A,

Table 1 Particle-size distribution in water and after NaClO treatment, and ratio between sand content after NaClO and water treatment (S_{NaClO}/S_{water}) for the bulk (B) and rhizosphere (R) materials of the soil under Holm oak (*Quercus ilex* L.), Eremo delle Carceri forest, Mount Subasio (Assisi, Italy). Numbers in parentheses are the standard errors ($n = 3$)

	Particle-size distribution in water						Particle-size distribution after NaClO						S_{NaClO}/S_{water}	
	Sand		Silt		Clay		Sand		Silt		Clay			
	B	R	B	R	B	R	B	R	B	R	B	R	B	R
	g kg^{-1}													
A	666 (12)cd	769 (7)a	266 (7)ef	191 (9)ij	68 (6)ab	40 (4)cf	290 (6)a	226 (1)bc	413 (3)h	456 (4)fg	297 (8)e	318 (4)de	0.44	0.29
AB	607 (12)ef	798 (10)a	327 (8)ad	167 (7)j	66 (3)ac	35 (4)df	265 (6)ab	182 (9)de	398 (6)hi	405 (8)h	337 (11)ce	413 (8)ab	0.44	0.23
2Bwb1	584 (3)f	675 (10)cd	347 (5)b	282 (8)df	69 (2)ab	43 (6)cf	316 (3)a	151 (2)ef	372 (6)i	424 (8)gh	312 (9)de	425 (6)a	0.54	0.22
2Bwb2	702 (14)c	749 (6)b	224 (9)gi	215 (12)hi	74 (6)a	36 (7)ef	218 (7)cd	122 (3)gh	407 (11)h	649 (6)c	375 (11)ac	229 (5)f	0.31	0.16
3Bwb	678 (11)cd	595 (10)ef	255 (7)fg	376 (9)a	67 (7)ac	29 (3)f	136 (9)fg	96 (3)i	495 (5)e	774 (2)ab	369 (9)bc	130 (3)h	0.20	0.16
4Bwb	632 (5)de	605 (8)ef	312 (2)be	337 (7)ac	56 (3)ae	58 (5)ad	102 (3)hi	105 (3)hi	484 (3)ef	728 (4)b	414 (10)ab	167 (5)g	0.16	0.17
5BCb	686 (11)c	656 (7)cd	245 (9)fh	286 (7)cf	69 (8)ab	58 (2)ad	104 (5)hi	80 (6)j	551 (4)d	824 (6)a	345 (9)cd	96 (3)i	0.15	0.12

For each parameter, mean values with different letters significantly differ for $P < 0.05$

AB, 3Bwb and 5BCb horizons (Table 2). Plagioclases with albitic composition (X-ray diffraction lines from 0.318 to 321 nm) were present in the A to 3Bwb horizons, while anorthitic plagioclases (X-ray diffraction lines from 0.322 to 0.324 nm) prevailed in the 4Bwb and 5BCb horizons. Plagioclases were higher in the rhizosphere than in the bulk in the 2Bwb1 horizon, and the reverse occurred in the 4Bwb and 5BCb horizons. Gypsum was present in greater amounts in the bulk throughout the soil (6–13 %), with the exception of the 4Bwb horizon, where it occurred as traces. Calcite was present in similarly small amounts (< 5 %) in both fractions from the A to the 4Bwb horizon, and in larger amount in the 5BCb horizon. Traces of micas were detected only in the rhizosphere of the deepest horizons. The two soil fractions displayed similar contents of clay minerals, which amounted to 16–26 % of the total mineral assemblage and were represented by hydroxy-interlayered vermiculite, vermiculite and kaolinite.

The pH values in water and in KCl (Table 3) increased with depth for both bulk and rhizosphere. The pH_{H2O} ranged from sub-acidic values in the upper horizons to sub-alkaline values at depth, and was significantly lower in the rhizosphere than in the bulk only in the 2Bwb1 horizon. The rhizosphere also showed lower pH_{KCl} values in the upper soil portion (A–2Bwb1 horizons).

Exchangeable cations and extractable forms of Fe

No significant difference was found in the amount of exchangeable cations between the bulk soil and the rhizosphere (Fig. 1). Calcium was the most abundant exchangeable element and its content showed negligible variations along the profile, ranging from 25 to 26 cmol(+) kg^{-1} in the A horizon to 33 cmol(+) kg^{-1} in the 5BCb horizon. The amount of Mg tended to decrease with depth with values always lower than 1.6 cmol(+) kg^{-1}. Potassium content decreased from the A to the horizons underneath assuming values always lower than 2.6 cmol(+) kg^{-1}, while Na was rather constant throughout the profile at around 1.1–1.4 cmol(+) kg^{-1}.

The amount of extractable Fe forms showed no significant difference between bulk and rhizosphere, with the only exception for the higher content of carbonate-bound Fe in the rhizosphere than in the bulk of the AB horizon (Fig. 2). The largest amount of Fe was in the form of non-crystalline oxyhydroxydes and of Fe

Table 2 Semi-quantitative estimation of the mineralogical composition for the bulk and rhizosphere materials of the soil under Holm oak (*Quercus ilex* L.), Eremo delle Carceri forest, Mount Subasio (Assisi, Italy). Numbers in parentheses are the standard errors ($n = 3$)

	Quartz		Plagioclases		Gypsum		Calcite		Micas		Clay minerals	
	Bulk	Rhizosphere	Bulk	Rhizosphere	Bulk	Rhizosphere	Bulk	Rhizosphere	Bulk	Rhizosphere	Bulk	Rhizosphere
	%											
A	54 (2)de	66 (2)ab	9 (1)bc	9 (1)bc	9 (1)ab	4 (1)cd	5 (1)bc	5 (1)bc	-	-	23 (1)a	16 (2)a
AB	58 (2)cd	66 (2)ab	7 (1)c	9 (1)bc	9 (2)ab	3 (1)d	3 (1)cd	3 (0)cd	-	-	23 (2)a	19 (1)a
2Bwb1	62 (1)ad	62 (1)ad	5 (1)d	11 (1)ab	9 (1)ab	4 (0)cd	4 (1)cd	2 (0)de	-	-	20 (2)a	21 (2)a
2Bwb2	60 (1)ad	60 (2)ad	11 (2)ab	11 (1)ab	7 (1)b	tr	3 (1)cd	4 (1)cd	-	-	19 (2)a	25 (2)a
3Bwb	51 (1)e	57 (2)cd	11 (1)ab	14 (1)a	13 (1)a	tr	4 (1)cd	3 (1)cd	-	-	21 (2)a	26 (2)a
4Bwb	65 (2)ac	68 (2)a	12 (1)a	5 (1)d	tr	tr	tr	tr	-	tr	22 (3)a	26 (2)a
5BCb	44 (2)f	62 (1)ad	12 (1)a	4 (1)d	6 (1)bc	tr	14 (2)a	11 (1)ab	-	tr	24 (4)a	23 (2)a

tr: traces (< 1 %)

For each parameter, mean values with different letters significantly differ for $P < 0.05$

Table 3 Values of pH in water and in 1 M KCl solution, and contents of total organic C (TOC) and water-extractable organic C (WEOC) for the bulk and rhizosphere materials of the soil under Holm oak (*Quercus ilex* L.), Eremo delle Carceri forest, Mount Subasio (Assisi, Italy). Numbers in parentheses are the standard errors ($n = 3$)

	pH in H_2O		pH in KCl		TOC		WEOC	
	Bulk	Rhizosphere	Bulk	Rhizosphere	Bulk	Rhizosphere	Bulk	Rhizosphere
	g kg^{-1}							
A	6.47 (0.06)df	6.29 (0.02)ef	5.68 (0.04)d	5.38 (0.06)ef	56.16 (7.47)ab	64.36 (6.08)a	0.50 (0.05)a	0.49 (0.05)a
AB	6.34 (0.16)ef	6.04 (0.21)f	5.42 (0.11)e	5.13 (0.05)f	41.26 (5.67)ad	49.66 (8.56)ac	0.38 (0.02)ac	0.41 (0.01)ab
2Bwb1	6.58 (0.14)ce	6.20 (0.15)f	5.55 (0.03)de	5.29 (0.12)f	37.39 (1.79)bd	39.81 (0.87)ad	0.32 (0.00)ce	0.41 (0.00)ab
2Bwb2	6.55 (0.24)ce	6.34 (0.06)ef	5.55 (0.04)de	5.38 (0.05)ef	31.51 (1.99)cd	37.56 (4.09)bd	0.25 (0.00)ef	0.32 (0.01)bd
3Bwb	7.00 (0.07)bd	6.93 (0.14)be	6.26 (0.17)bc	6.06 (0.16)cd	23.63 (0.65)ef	36.03 (3.23)bd	0.20 (0.01)f	0.27 (0.03)de
4Bwb	7.53 (0.13)ab	7.15 (0.09)ac	6.38 (0.11)bc	6.25 (0.03)bc	24.89 (1.57)ef	30.21 (0.64)cd	0.24 (0.01)ef	0.30 (0.00)ce
5BCb	7.80 (0.03)a	7.60 (0.09)ab	6.98 (0.11)a	6.68 (0.14)ab	22.12 (1.40)f	33.03 (5.02)cd	0.23 (0.02)ef	0.28 (0.00)de

For each parameter, mean values with different letters significantly differ for $P < 0.05$

associated to organic matter, followed by crystalline Fe-oxyhydroxydes, easily reducible Fe-oxyhydroxydes, and Fe bound to carbonates. All these Fe forms showed no significant trend with depth.

Organic C, N, and P

The TOC and WEOC contents decreased with depth in the bulk and the rhizosphere (Table 3). Both fractions had a similar TOC content in the upper part of the profile (A-2Bwb2 horizons), whereas it was higher in the rhizosphere than in the bulk of the horizons underneath. The amount of WEOC was significantly higher in the rhizosphere than in the bulk in the 2Bwb and 3Bwb horizons.

The concentration of NO_2^-N was always below the detection limit. The content of total, inorganic (NO_3^-N plus NH_4^+-N) and organic N showed no significant difference between bulk and rhizosphere in all the horizons and, for both soil fractions, tended to decrease with depth (Table 4). For both bulk and rhizosphere, NO_3^-N and NH_4^+-N represented negligible portions of total N, from 0.33 to 0.86 % for NO_3^-N, and from 0.49 to 1.35 % for NH_4^+-N. Thus, the organic N was the most represented N pool.

The available P content was extremely low in both fractions (from 5 to 11 mg kg^{-1}), whereas the total P ranged from 706 to 898 mg kg^{-1} (Table 5). The total P showed no trend with depth and was more abundant in the rhizosphere than in the bulk of the AB, 2Bwb1, 2Bwb2, 3Bwb horizons.

Microbial biomass C and basal respiration

In the bulk soil, the microbial biomass C (C_{mic}) content (Table 6) increased from the A to the 2Bwb1 horizon and then decreased with depth. In the rhizosphere, after an increase from the A to the AB horizon, the C_{mic} showed a decreasing trend with depth until the 3Bwb horizon, followed by a trend inversion in the two deepest horizons. The amount of C_{mic} was similar in bulk and rhizosphere of the A and AB horizons, but was higher in the bulk of the 2Bwb and 3Bwb horizons, and in the rhizosphere of the 4Bwb and 5BCb horizons. The CO_2-C evolved during the incubation experiment (ΣCO_2-C) by the rhizosphere was always higher than that of the bulk, with the exception of the A and 4Bwb horizons where respiration was similar in the two soil fractions (Table 6). The C_{mic}/TOC ratio showed no significant difference between the two fractions, with the exception of the 3Bwb horizon where the rhizosphere had a lower C_{mic}/TOC proportion than the respective bulk, although in the 2Bwb2 and 3Bwb the rhizosphere respired more organic C (ΣCO_2-C/TOC) than the bulk (Table 7). This latter results were supported by the comparison of the evolved CO_2 with WEOC (ΣCO_2-C/WEOC) in the rhizosphere of the 2Bwb2 and 3Bwb horizons, where the proportions were the highest (Table 7).

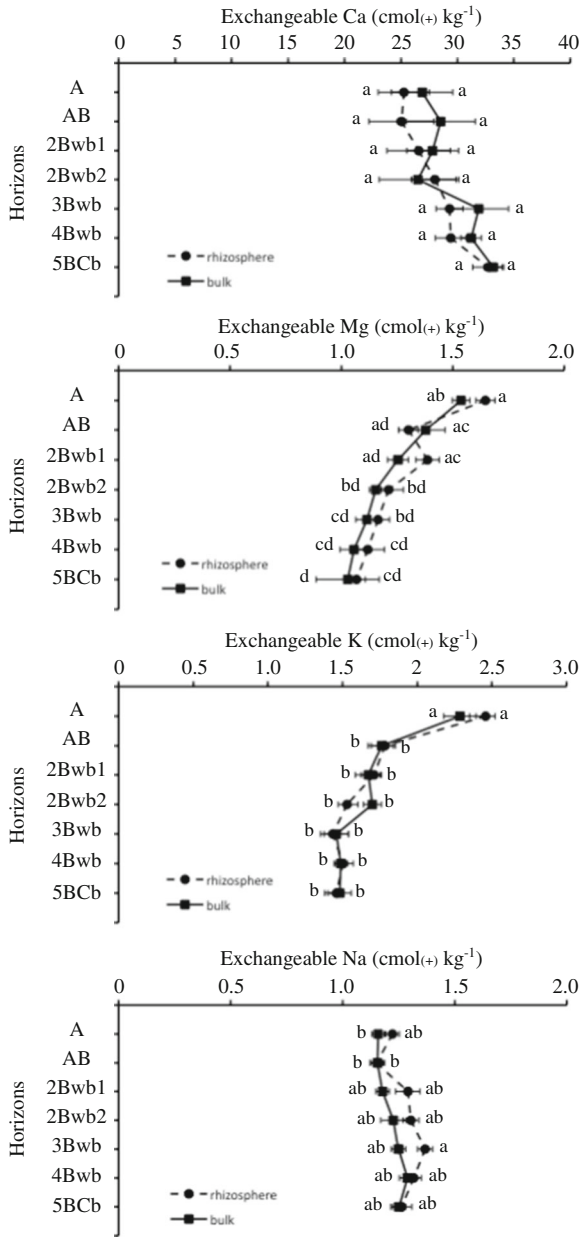

Fig. 1 Exchangeable cations extracted from the bulk and rhizosphere of the soil under Holm oak (*Quercus ilex* L.), Eremo delle Carceri forest, Mount Subasio (Assisi, Italy). Error bars are the standard errors (n = 3) and, for each graphs, mean values with different letters significantly differ at $P < 0.05$

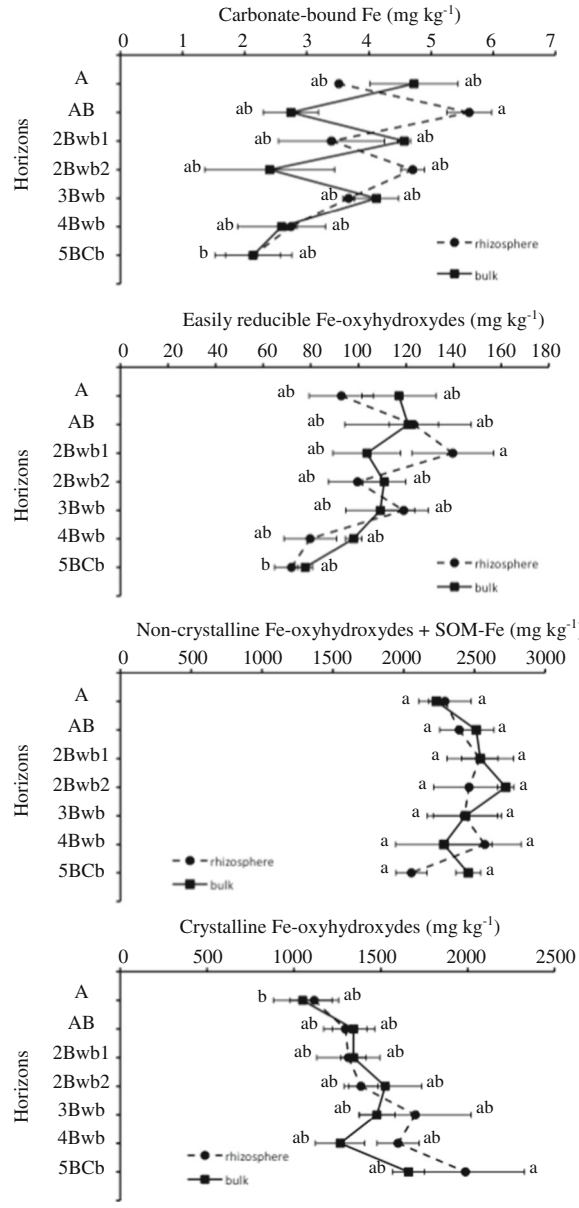

Fig. 2 Sequentially extracted Fe from the bulk and rhizosphere materials of the soil under Holm oak (*Quercus ilex* L.), Eremo delle Carceri forest, Mount Subasio (Assisi, Italy). Error bars are the standard errors (n = 3) and, for each graphs, mean values with different letters significantly differ at $P < 0.05$

The qCO_2 values of the rhizosphere were higher than those of the bulk in the 2Bwb and 3Bwb horizons, and similar in the two fractions of the A, AB and 5BCb horizons; the bulk showed a qCO_2 greater than the rhizosphere only in the 4Bwb horizon.

Phospholipid fatty acid (PLFA) analysis

The NMDS analysis of the relative abundance of all identified PLFAs showed that horizons (PERMANOVA: $F_{6.56} = 2.40$, $R^2 = 0.200$, $p = 0.014$*) and bulk or rhizosphere (PERMANOVA: $F_{1.56} = 4.78$, $R^2 = 0.07$, $p = 0.015$*) affected the microbial

Table 4 Contents of total N, NO_3^-N, NH_4^+-N, and organic N for the bulk and rhizosphere materials of the soil under Holm oak (*Quercus ilex* L.), Eremo delle Carceri forest, Mount Subasio (Assisi, Italy). Numbers in parentheses are the standard errors ($n = 3$)

	Total N		NO_3^-N		NH_4^+-N		Organic N	
	Bulk	Rhizosphere	Bulk	Rhizosphere	Bulk	Rhizosphere	Bulk	Rhizosphere
	g kg^{-1}							
A	4.60 (0.27)a	4.15 (0.09)ab	0.04 (0.01)a	0.02 (0.01)ab	0.03 (0.00)a	0.02 (0.00)ab	4.53 (0.27)a	4.11 (0.09)ab
AB	3.75 (0.38)ab	3.96 (0.34)ab	0.02 (0.01)ab	0.02 (0.01)ab	0.02 (0.00)ab	0.02 (0.00)ab	3.71 (0.38)ab	3.92 (0.34)ab
2Bwb1	2.91 (0.20)ad	3.01 (0.03)ac	0.02 (0.00)ac	0.01 (0.00)ac	0.02 (0.00)ab	0.02 (0.00)ab	2.87 (0.20)ad	2.98 (0.03)ac
2Bwb2	2.00 (0.31)ae	2.40 (0.25)ae	0.01 (0.00)ac	0.01 (0.00)bc	0.01 (0.00)bc	0.02 (0.00)ab	1.98 (0.31)ae	2.37 (0.24)ae
3Bwb	1.08 (0.31)ce	1.47 (0.10)be	0.00 (0.00)b	0.00 (0.00)bc	0.01 (0.00)c	0.02 (0.01)ab	1.07 (0.31)ce	1.45 (0.09)be
4Bwb	1.03 (0.39)de	1.63 (0.49)ae	0.01 (0.00)b	0.00 (0.00)bc	0.01 (0.00)c	0.02 (0.00)ab	1.01 (0.39)de	1.61 (0.49)ae
5BCb	0.94 (0.33)e	1.26 (0.44)ce	0.01 (0.00)b	0.01 (0.00)bc	0.01 (0.00)bc	0.01 (0.00)ab	0.92 (0.33)e	1.24 (0.44)ce

For each parameter, mean values with different letters significantly differ for $P < 0.05$

community structure. Indeed, pairwise contrasts revealed that the structure of the microbial community was significantly different between rhizosphere and bulk (Fig. 3a). Also the microbial community harboured in the AB horizon was significantly different from that of the 3Bwb and 4Bwb horizons, while that of the other horizons showed similarities to both the extremes (Fig. 3b). The effects of horizons and of bulk or rhizosphere (Fig. 3c) appeared largely driven along the NMDS1 by the relative abundance of i15:0 and a15:0 (Gram-positive bacteria), 16:1 (Gram-negative bacteria), 10Me16:0 (actinomycetes), and 14:0, 15:0, 20:0 (non-specific PLFAs) fatty acids; along the NMDS2, the effect was explained by the 17:0 and br18:0 (non-specific PLFAs), and 20:2 (protozoa) fatty acids.

Table 5 Contents of available and total P for the bulk and rhizosphere materials of the soil under Holm oak (*Quercus ilex* L.), Eremo delle Carceri forest, Mount Subasio (Assisi, Italy). Numbers in parentheses are the standard errors ($n = 3$)

	Available P		Total P	
	Bulk	Rhizosphere	Bulk	Rhizosphere
	mg kg^{-1}			
A	7 (1)a	7 (1)a	820 (38)ab	820 (33)ab
AB	7 (1)a	10 (1)a	706 (49)b	868 (43)a
2Bwb1	7 (1)a	11 (1)a	708 (25)b	865 (6)a
2Bwb2	10 (4)a	8 (1)a	761 (29)b	869 (13)a
3Bwb	6 (1)a	6 (0)a	717 (32)b	865 (7)a
4Bwb	6 (1)a	9 (0)a	789 (43)ab	839 (41)ab
5BCb	5 (0)a	8 (1)a	898 (33)a	854 (33)a

For each parameter, mean values with different letters significantly differ for $P < 0.05$

The PLFA analysis confirmed a different distribution of microbial groups in the rhizosphere and bulk along the profile (Fig. 4). In the 2Bwb1 and 2Bwb2 horizons, the bulk had a larger bacterial population than the rhizosphere, although no significant difference between bulk and rhizosphere occurred for the Gram-positive and Gram-negative bacteria. Only in the 2Bwb1 horizon, the rhizosphere was more densely colonized by fungi than the bulk. As for the distribution of other microorganisms estimated by the PLFA analysis, the actinomycetes was found in higher amount in the rhizosphere than in the bulk of the A horizon, whereas no difference between bulk and rhizosphere was found for AMF and protozoa. The non-specific PLFAs, namely the compounds that are common components of microbial cell walls, represented about 15 % of the total PLFAs throughout the soil for the bulk, but they reached values of up to 30 % in the rhizosphere of the 2Bwb1 and 2Bwb2 horizons.

Discussion

The rhizosphere effect on physical, mineralogical and chemical properties

The finer particle-size distribution measured after dissolution of organic cements than after water

Table 6 Microbial biomass C content and amount of CO_2 evolved during 20 days of incubation ($\sum CO_2$-C) for the bulk and rhizosphere materials of the soil under Holm oak (*Quercus ilex* L.), Eremo delle Carceri forest, Mount Subasio (Assisi, Italy). Numbers in parentheses are the standard errors ($n = 3$)

	Microbial biomass C		ΣCO_2-C	
	Bulk	Rhizosphere	Bulk	Rhizosphere
	μg g^{-1}			
A	273.5 (14.2)bc	265.4 (18.8)bc	355.4 (19.9)b	331.1 (15.7)b
AB	341.0 (4.5)ab	375.8 (9.7)a	227.7 (18.7)c	327.1 (12.5)b
2Bwb1	357.9 (8.8)a	247.4 (14.0)c	228.0 (3.0)c	325.2 (11.3)b
2Bwb2	233.9 (21.3)c	167.6 (4.4)d	205.0 (8.1)cd	485.5 (5.7)a
3Bwb	158.1 (17.7)d	88.2 (1.6)e	163.1 (5.0)d	360.8 (17.6)b
4Bwb	113.3 (2.6)e	176.6 (1.4)d	182.9 (3.1)cd	193.3 (11.4)cd
5BCb	106.7 (3.4)e	160.0 (3.0)d	214.5 (7.4)c	311.8 (17.5)b

For each parameter, mean values with different letters significantly differ for $P < 0.05$

treatment (Table 1) indicated that aggregates were mainly cemented by organic compounds. Judging by the ratio between the content of sand after NaClO and after water treatments (Table 1), the NaClO treatment was generally more effective in the rhizosphere than in the bulk. As the NaClO oxidizes the labile organic matter (e.g., Kleber et al. 2005; Favilli et al. 2008), we ascribed the major effect of the treatment on the rhizosphere to a higher amount of labile organics in this soil fraction. This hypothesis was supported by two facts: 1) the generally higher concentration of WEOC in the rhizosphere (Table 3), and 2) the rhizosphere is enriched of labile carbon released by rhizodeposition processes (e.g., Gregory 2006; Stockmann et al. 2013; Agnelli et al. 2014).

The mineralogical assemblage showed few differences and the main contrasts were the greater

Table 7 Percentage of total organic C present as microbial biomass C (Cmic/TOC), percentage of the respired C over total organic C and water-extractable organic C (ΣCO_2-C/TOC and ΣCO_2-C/WEOC, respectively), and metabolic quotient (qCO_2) for the bulk and rhizosphere materials of the soil under Holm oak (*Quercus ilex* L.), Eremo delle Carceri forest, Mount Subasio (Assisi, Italy). Numbers in parentheses are the standard errors ($n = 3$)

	Cmic/TOC		ΣCO_2-C/TOC		ΣCO_2-C/WEOC		qCO_2	
	Bulk	Rhizosphere	Bulk	Rhizosphere	Bulk	Rhizosphere	Bulk	Rhizosphere
	%						μg CO_2-C mg^{-1} Cmic h^{-1}	
A	0.49 (0.09)bd	0.42 (0.07)cd	0.64 (0.10)ce	0.52 (0.04)e	72.74 (9.10)cd	68.53 (5.40)cd	2.71 (0.18)cd	2.61 (0.29)ce
AB	0.85 (0.13)ab	0.77 (0.15)ac	0.56 (0.06)de	0.67 (0.10)be	60.84 (3.91)d	80.42 (2.20)bd	1.39 (0.13)g	1.81 (0.11)fg
2Bwb1	0.96 (0.02)a	0.62 (0.05)ac	0.61 (0.02)ce	0.82 (0.01)ae	71.88 (1.31)cd	79.40 (3.30)bd	1.33 (0.02)g	2.75 (0.25)cd
2Bwb2	0.74 (0.02)ac	0.45 (0.06)bd	0.65 (0.04)ce	1.30 (0.14)a	81.08 (3.31)bd	151.01 (5.68)a	1.85 (0.19)eg	6.04 (0.16)a
3Bwb	0.67 (0.06)ac	0.29 (0.03)d	0.69 (0.02)ce	1.17 (0.16)ab	81.87 (4.10)bd	134.59 (21.50)a	2.17 (0.21)df	8.57 (0.29)a
4Bwb	0.45 (0.02)bd	0.62 (0.04)ac	0.74 (0.06)ae	0.69 (0.05)be	77.21 (3.89)cd	64.40 (3.99)cd	3.36 (0.09)bc	2.28 (0.14)df
5BCb	0.51 (0.05)bd	0.50 (0.08)bd	1.02 (0.10)ac	0.96 (0.22)ad	91.46 (5.93)bc	113.58 (6.53)ab	4.19 (0.09)b	4.07 (0.16)b

For each parameter, mean values with different letters significantly differ for $P < 0.05$

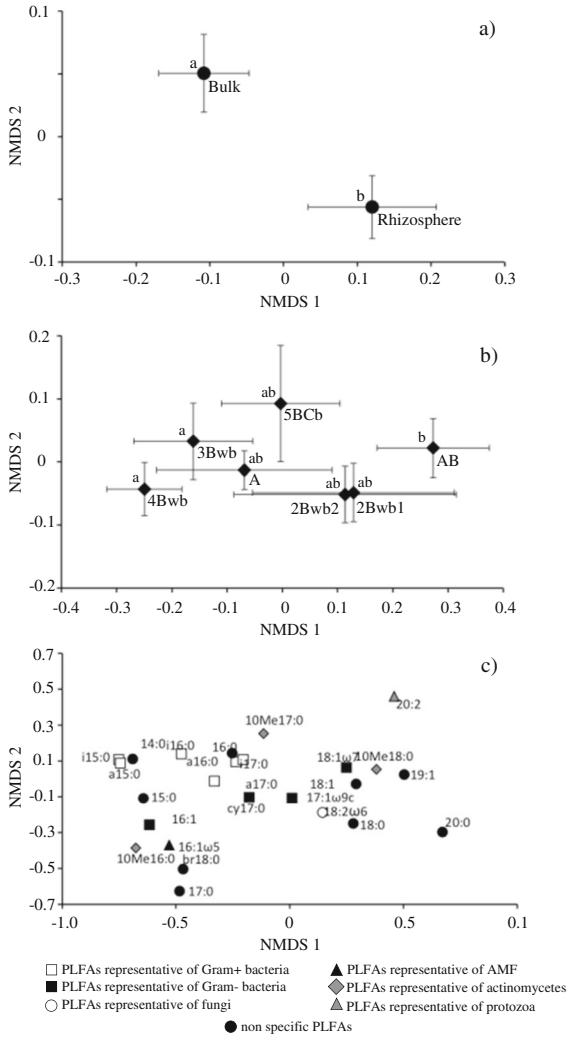

Fig. 3 Two-dimensional non-metric multidimensional scaling (NMDS) plots showing differences in the microbial community structure among rhizosphere and bulk (plot a) and horizons (plot b) of the soil under Holm oak (*Quercus ilex* L.), Eremo delle Carceri forest, Mount Subasio (Assisi, Italy). The symbols represent the centroids of all samples from each variable (rhizosphere, bulk, horizons), and the bars show the standard error along each NMDS axis. Groups with centroids labelled with a common letter were not deemed to be different in pairwise post hoc tests (PERMANOVA) using the Bonferroni correction to maintain an overall $\alpha = 0.05$. Plot c shows NMDS scores for PLFAs

content of quartz (A, AB, 3Bwb and 5BCb horizons) and albitic plagioclases (2Bwb1 horizon) of the rhizosphere. These features are viewed as the result of a higher weathering intensity in the soil close to the roots, with a consequent relative enrichment of the most resistant minerals in the rhizosphere (Courchesne and Gobran 1997). In the 4Bwb and 5BCb horizons, the lower plagioclases content of the rhizosphere was ascribed to their anorthitic composition. The alteration rate of anorthitic plagioclases is known to be faster than that of albitic plagioclases, and a preferential weathering of calcic plagioclases has been reported by several authors (e.g., Clayton 1986; White et al. 2008). As anorthitic plagioclases represent a Ca source for plants, the rhizosphere is depleted of these Ca-bearing minerals because of the high plant-induced mineral weathering (Drever 1994). The indirect quartz enrichment in the rhizosphere was partly caused by the dissolution of gypsum, which was the most weatherable mineral of this soil, and consequently it was selectively removed in the rhizosphere of all the horizons. The modest differences in mineralogy between bulk and rhizosphere were attributed to: 1) the long-term presence of the Holm oak stand on this soil, so that most of the bulk was progressively transformed, at least for components with high inertia like minerals, to rhizosphere soil (Richter et al. 2007), 2) the buffering capacity of calcite, which can neutralize the protons and organic ligands released by roots (van Breemen et al. 1983; Gobran et al. 1998; Cocco et al. 2013), and 3) the distribution of the indirect mineral enrichment effect of the rhizosphere over several resistant mineral, thus producing many non significant differences..

The long-lasting rhizosphere effect and the high buffer capacity of the calcite could also be responsible for the slight pH_{H20} differences between the bulk and the rhizosphere. However, the lower rhizosphere pH_{KCl} values observed from the A to the 2Bwb1 horizons suggested that the acidifying process induced by root activity was gradually deepening into the soil profile. Rhizosphere acidification, other than via the CO_2 produced by root respiration (Richter et al. 2007), is induced by the low molecular weight organic acids released by plants to overcome nutrient deficiency (Hinsinger et al. 2003; Sandnes et al. 2005; Lemanceau et al. 2009), and by the uptake of nutrients in cationic form by roots (Haynes 1990). The high root uptake of basic cations by the oaks has to be considered one of the main

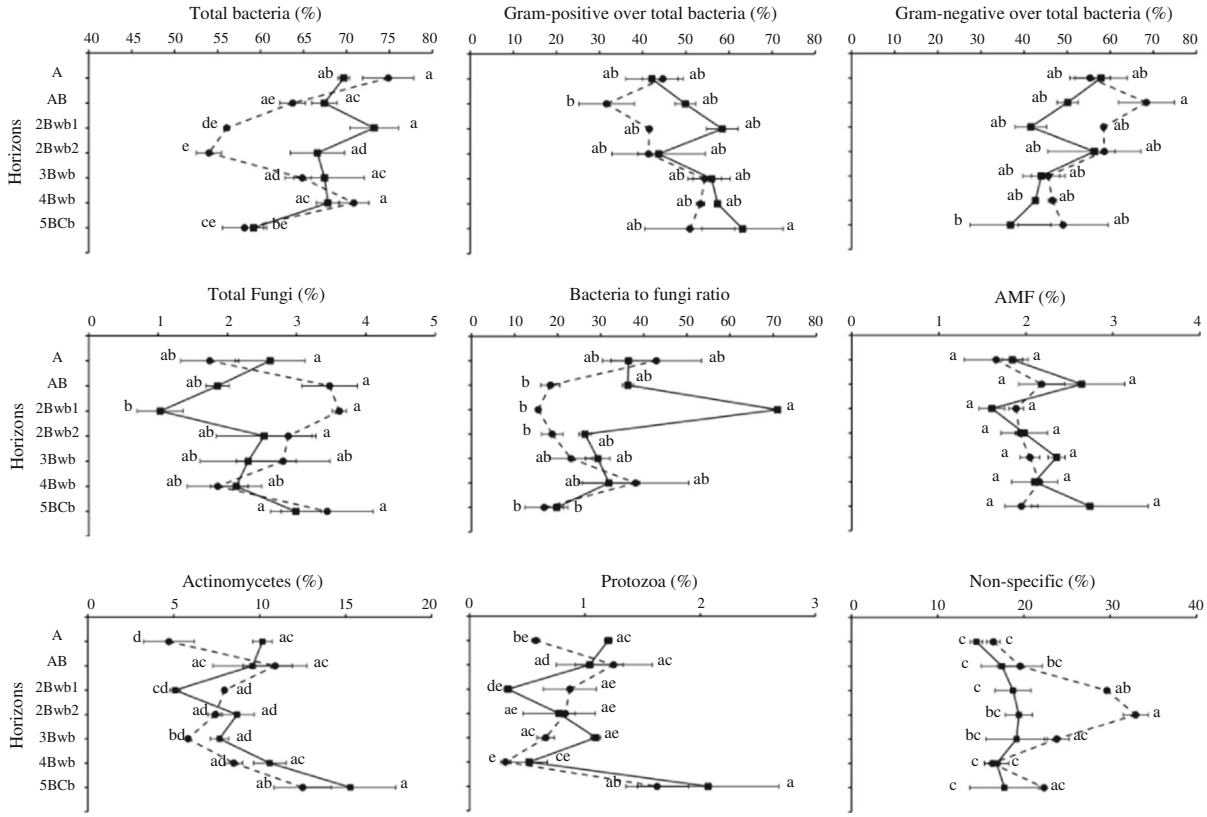

Fig. 4 Distribution of the different microbial groups as revealed by PLFA analysis for the bulk (—■—) and rhizosphere (- - ● - -) of the soil under Holm oak (*Quercus ilex* L.), Eremo delle Carceri forest, Mount Subasio (Assisi, Italy). Error bars are the standard errors ($n = 3$) and, for each graphs, mean values with different letters significantly differ at $P < 0.05$

driving forces that generated the relative homogenisation of the minerals and of the exchange complex between the bulk and the rhizosphere (Dijkstra and Smits 2002; Jobbágy and Jackson 2004). This uptake of cations and the consequent release of H^+ produced the acidity responsible for the weathering of primary minerals such as carbonates, gypsum and plagioclases, with the associated release of nutrients. Further, the cations absorbed by roots and allocated to the aboveground biomass are cycled back to the soil surface through litter degradation and leaching (Madeira and Ribeiro 1995). With time, the biocycling of nutrients should foster a redistribution of the exchangeable cations in the whole soil mass, therefore reducing the differences between the bulk and rhizosphere components.

The absence of contrasts between bulk and rhizosphere in the abundance of Fe forms was attributed primarily to the transformation of the bulk in rhizosphere by the root activity over the long period of continuous oak growth on this soil. Even in a well aerated soil like that under study, Fe may undergo reduction in the rhizosphere because of the high rate of oxygen consumption by root respiration and the microbial degradation of rhizodeposits (Richter et al. 2007; Lemanceau et al. 2009). Further, when Fe supply is limited, as it is often the case in calcareous

soils (Gogorcena et al. 2001), the acidification induced by oak roots can reduce Fe^{III} to Fe^{II} through the stimulation of Fe^{III} reductase activity (Toulon et al. 1992; Schmidt 1999). Such reduction in the rhizosphere should render Fe more soluble and favour its diffusion from the rhizosphere to the bulk. Here, because of a more oxidative soil environment and of a higher pH, Fe^{II} is oxidized back to Fe^{III} and precipitates as non-crystalline Fe-oxyhydroxides or is strongly chelated by organics.

The TOC content was higher in the rhizosphere than in the bulk only in the three deepest horizons while the N content, both in inorganic and organic forms, did not differ between the two soil components. This is somewhat in contrast with studies reporting that the rhizosphere is enriched in both C and N (e.g., Gobran et al. 1998; Turpault et al. 2005; Cocco et al. 2013). Our observations can be explained with a high N uptake by roots and an exudation of relatively N-poor labile C compounds such as carbohydrates (sugars and mucilage), aliphatic or aromatic organic acids, phenols, and fatty acids (Colin-Belgrand et al. 2003) in the rhizosphere. The abundance of labile compounds in the rhizosphere is reflected by the higher amount of WEOC in the rhizosphere than in the bulk of at least the 2Bwb and 3Bwb horizons.

The amount of available P was low in both bulk and rhizosphere probably because of the presence of relatively high quantities of the most active Fe-oxyhydroxydes (easily reducible and non-crystalline + SOM-Fe), which accounted to about 2100–2800 mg kg^{-1} (Fig. 2) and are able to strongly retain P through ligand exchange reactions. A minor contribution in P retention was likely exerted by carbonates that, judging from the low amount of carbonate-bound Fe (Fig. 2), should be poorly active. The P retention due to phosphates adsorption on Fe-oxyhydroxydes and coprecipitation with Ca ions (Hinsinger 2001) could be also responsible for the lack of an evident available P depletion in the rhizosphere. Indeed, Hinsinger and Gilkes (1996) reported that, in the ryegrass rhizosphere, the available P concentration shows a depletion in the immediate vicinity of the roots (at less than 0.5 mm) and a progressive accumulation until ca. 2.5 mm from the root surface, where no rhizosphere effect was detected. As our rhizosphere soil had a thickness of 3–6 mm, it is probably that the lower amount of available P in the soil closer to the roots was enriched by mixing this soil with that farther, so reducing differences among bulk and rhizosphere.

The greater total P content in the rhizosphere than in the bulk of the AB, 2Bwb and 3Bwb horizons might be due to a higher microbial immobilisation of orthophosphate in the soil close to the roots (Richardson and Simpson 2011). The P immobilisation in the microbial biomass is a process able to protect labile P forms from reaction with minerals, and control the amount of P in soil solution (Seeling and Zasoski 1993; Olander and Vitousek 2004). However, the higher total P content of the rhizosphere was not supported by a corresponding higher C_{mic} content (Table 6). Hence, at least for the 2Bwb and 3Bwb horizons, the major P immobilisation in the rhizosphere was related to its microbial community structure and activity (see below).

The rhizosphere effect on microbial activity and community structure

The microbial activity was noticeable in the rhizosphere of the 2Bwb2 and 3Bwb horizons (Tables 6, 7) where it is triggered by a larger availability of WEOC, whose consumption probably caused the mineralisation of more stable organic pools through a priming effect. The rhizosphere priming effect driven by the exudation of organics has been considered as responsible for the microbially-mediated release of nutrients through organic matter cycling (Kuzyakov 2002). Further, as showed by PERMANOVA analysis, the microbial community structure significantly varied along the soil. The PLFA analysis showed that in the 2Bwb1 and 2Bwb2 horizons the rhizosphere hosted a lower bacterial population than the bulk,

while only in the 2Bwb1 horizon the rhizosphere had a larger proportion of fungi and a lower bacterial-to-fungal biomass ratio than the bulk. At the soil-root interface, plant-fungi association improves nutrient availability through the production of hydrolytic and oxidative enzymes (Myers and Leake 1996; Cairney and Burke 1998; Hinsinger 2001; Finlay 2008), even though neither available P nor N concentrations support this circumstance. Also in this case, the sampling of a rhizosphere 3–6 mm thick has probably obliterated differences of these nutrients between bulk and rhizosphere. Indeed, the abundance of AMF, which are known to enrich the rhizosphere with organic C and N (e.g., Finlay 2008; Jones et al. 2009), did not differ between bulk and rhizosphere throughout the soil. This result could be due to the fact that the PLFA marker of AMF (16:1ω5) is not strictly specific to these organisms since it has been also found in bacteria (Nichols et al. 1986). Because of this, the 16:1ω5 fatty acid of bacterial source could have masked the real distribution of AMF in the rhizosphere. In the 2Bwb1 horizon, the larger proportion of fungi, which are important in the degradation of complex rhizodeposits (Paterson et al. 2006; Buée et al. 2009), indicated that at least the rhizosphere of this horizon was colonized by an efficient microbial community able to take advantage of the organic substrates supplied by the roots (Massaccesi et al. 2015). Further, since by the analysis of PLFAs it is not possible to distinguish the ecto-mycorrhizal by the saprophytic fungi as they are identified by the same PLFA (Karliński et al. 2007), it could be argued that the higher proportion of fungi found in the rhizosphere of the 2Bwb1 horizon is partly due to mycorrhizal fungi. The higher presence of fungi (both saprophytic and mycorrhizal) may further influence the microbial activity directly through the growth and degeneration of the hyphal network, and indirectly through the stimulation of the rhizodeposition processes (Marschner et al. 2005. The occurrence of a specific rhizosphere microbial community structure (2Bwb horizons) and activity (2Bwb and 3Bwb horizons) was considered as indicative of adaptation to the changes induced by the roots through acidification and rhizodeposition (e.g., Joergensen et al. 1990; Lavahum et al. 1996).

Conclusions

The findings of this multivariate study were that the Holm oak root system affected the whole soil profile over time, and that the presence and activity of roots influenced many properties of this limestone-derived soil. However, as this soil is made of materials superimposed one over the other because of landslides, it could be expected that some reverse depth-trends may occur along the soil for many properties, as it often happens in buried ancient surfaces. In our case, the depth-trend of the soil chemical properties and the few differences between bulk and rhizosphere indicated that pedogenesis under the same vegetation for such a long time has homogenized many of the soil features, especially those not directly linked to the microbial biomass. However, although the thickness of the sampled rhizosphere might have contributed to weaken the differences between the two fractions, the intensity of the rhizosphere effect varied throughout the profile. In particular, the more expressed changes occurred in the 2Bwb and 3Bwb horizons (roughly from 12 to 55 cm of depth), where the rhizosphere microbial community had a different structure and activity with respect to those of the bulk.

The main processes that differentiated horizons in terms of rhizosphere effect were root activity, soil faunal perturbation and slope dynamics. While root activity impacted the whole soil profile, although to a lesser extent at depth, the role of fauna and slope on soil disturbance was mostly confined atop the profile, thus explaining the smaller rhizosphere effect in the A horizon. It follows that, on this limestone-derived soil, long-term changes due to rhizosphere effect were more strongly expressed in the core of the solum, not at the surface.

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Compliance with ethical standards This research benefited of funds from the Majella National Park (Call n. 1, 14.02.2006).

Conflict of interest The authors declare that they have no conflict of interest.

Appendix

Table 8 Morphological description of the soil under Holm oak (*Quercus ilex* L.), Eremo delle Carceri forest, Mount Subasio (Assisi, Italy). For symbols see legend

	Depth*	Mean thickness	Colour[a]	Texture[b]	Structure[c]	Consistency[d]	Plasticity[e]	Roots[f]	Skeleton[g]	Boundary[h]	Other observations
	cm	cm									
Oi	7/3–5/1	2.0	-	-	-	-	-	0		cw	Undecomposed leaves and stems of Holm oak, *R. aculeatus*, *H. elix*, acorns
Oe	5/1–0	2.7	-	-	-	-	-	0		cw	
A	0–6/7	6.3	10YR 2/2-7.5YR 3/2	sl	2f sbk-2f cr	mfr, wso	wps	2mi,vf,f,m 3mi,vf,f,m,co	1–3 %, mm&cm	cw	Abundant mesofauna; clasts are pitted
AB	6/7–12/16	7.3	10YR 3/3-5YR 4/3	sicl-sil	2f,m sbk-2f sbk&cr	mfr-fi, wso	wps	2mi,vf,f; 1 m,co 3mi,vf,f,m,co	5–30 %, mm&cm-dm	cw	Few mesofauna
2Bwb1	12/16–19/30	10.3	7.5YR 3/3-7.5YR 3/3	sicl-sil	2f sbk-3f,m sbk	mfi, wss-so	wps-p	2mi,vf,f; 1 m,co 3mi,vf,f; 2 m,co	10–15 %, mm-cm&dm	cw	
2Bwb2	19/30–28/45	10.0	2.5YR 3/3-7.5Y 3/3	sicl	3f,m abk&sbk-3f,m sbk	mfi, wss-so	wps-p	3mi,vf,f; 1 m,co 2mi,vf,f,m; 1co	10–15 %, mm-cm&dm	cs	
3Bwb	28/45–37/55	9.3	2.5YR 3/4-7.5YR 3/3	sil	2f sbk-3f,m sbk	mfi, wss-so	wps-p	3mi,vf,f; 1 m,co	5–20 %, mm&cm&dm	cw-cs	Some clasts are flintstone
4Bwb	37/55–47/61	8.0	5YR 3/3-7.5YR 3/3	sil	3f,m sbk	mfi, wso	wps-p	3mi,vf,f,m; 1co 1mi,vf,f; 2 m,co	5–40 %, mm&cm-dm	cw	Some clasts are flintstone
5BCb	47/61–74/84+	-	5YR 3/3-7.5YR 3/3	sil	1f,m sbk-2f sbk	mfr-fi, wss-so	wps-p	2mi,vf,f,m; 1co	75–80 %, cm&dm	-	Some clasts are flintstone

*Numbers separated by slash (/) indicate the range of depths observed in the three profiles, while the hyphen (−) means "from (what is before the sign) to (what is after the sign)"

[a] moist and crushed, according to the Munsell Soil Color Charts

[b] sl = sand loam, sil = silt loam, sicl = silty clay loam

[c] 1 = weak, 2 = moderate, 3 = strong; f = fine, m = medium; cr = crumb, abk = angular blocky, sbk = subangular blocky

[d] m = moist, fr = friable, fi = firm; w = wet, ss = slightly sticky, so = non-sticky

[e] w = wet, p = plastic, ps = slightly plastic

[f] 0 = absent, 1 = few, 2 = plentiful, 3 = abundant; mi = micro, vf = very fine, f = fine, m = medium, co = coarse

[g] mm = dimension of the clasts is less than 1 cm; cm = dimension of the clasts is less than 10 cm; dm = dimension of the clasts is from 10 to 20 cm

[h] c = clear; w = wavy, s = smooth

Table 9 F-values from two-way ANOVA for the analyzed soil properties. The asterisks indicate the significance levels: * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

Soil properties	F-value			Soil properties	F-value		
	Soil fraction (bulk soil and rhizosphere)	Horizon	Soil fraction x Horizon		Soil fraction (bulk soil and rhizosphere)	Horizon	Soil fraction x Horizon
$Sand_{water}$	59.31***	42.51***	49.29***	ΣCO_2-C	265.82***	37.32***	29.59***
$Silt_{water}$	27.62***	55.06***	58.82***	Cmic/TOC	14.08***	7.82***+	4.76**
$Clay_{water}$	82.85***	2.78*	5.11***	Σ CO_2-C/TOC	10.55**	7.24***	4.43**
$Sand_{NaClO}$	348.64***	263.86***	23.03***	Σ CO_2-C/WEOC	29.40***	16.96***	7.83***
$Silt_{NaClO}$	1289.45***	439.72***	84.68***	qCO_2	148.50***	60.63***	48.48***
$Clay_{NaClO}$	1054.01***	204.19***	315.06***	Exchangeable Ca	1.18	2.56	0.28*
Quartz	65.38***	19.88***	10.51***	Exchangeable Mg	2.37	13.73***	0.38
Plagioclases	4.03	10.33***	24.60***	Exchangeable K	0.01	29.83***	0.74
Gypsum	728.21***	102.13***	45.88***	Exchangeable Na	7.90**	4.60**	0.78
Calcite	2.04	47.52***	1.84	Carbonate-bound Fe	1.52	3.56**	2.92**
Clay minerals	0.02	1.23	3.08	Easily reducibleFe	0.18	3.90**	1.23
pH_{H2O}	12.40***	35.44***	0.36	Non-crystalline + SOM Fe	0.39	0.85	0.77
pH_{KCl}	20.91***	69.88***	0.31	Crystalline Fe	0.84	3.54**	0.47
TOC	19.57***	18.60***	1.10	Bacterial PLFAs	13.85***	9.62***	6.84***
WEOC	35.20***	43.20***	1.99	Gram + Bacteria PLFAs	4.82*	2.72*	1.24
Total N	2.40	14.70***	0.41	Gram– Bacteria PLFAs	5.83*	2.77*	0.79
NH_4^+-N	37.66**	3.43**	1.27	Fungal PLFAs	6.23*	1.67	3.81**
NO_3^-N	1.80	11.99***	0.29	Bacterial/Fungal PLFAs	7.72**	2.30	4.29**
Organic N	2.21	14.37***	0.40	AMF PLFAs	1.42	2.04	0.78
Available P	6.52*	2.28	1.26	Actinomycetes PLFAs	4.03	7.25***	3.93**
Total P	2.21	14.37***	0.40	Protozoa PLFAs	0.45	10.29***	3.35*
Cmic	3.77	150.24***	30.33***	Not specific PLFAs	20.12***	5.96***	1.86*

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