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Rhizosphere effect of three plant species of environment under periglacial conditions (Majella Massif, central Italy)

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ABSTRACT

The chemical, physical and biological processes occurring in the rhizosphere can influence plant growth by modifying root associated microorganisms and nutrient cycles. Although rhizosphere has been widely investigated, little is known about the rhizosphere effect of pioneer plants in soils of periglacial environments. The knowledge of the processes controlling soil–plant relationships in these severe environments may help understanding the ecological evolution of newly deglaciated surfaces. We selected three plants [*Helianthemum nummularium* (L.) Mill. subsp. *grandiflorum* (Scop.), *Dryas octopetala* (L.), and *Silene acaulis* (L.) Jacq. subsp. *cenisia* (Vierh.) P. Fourn.] that sparsely occupy deglaciated areas of central Apennines (Italy), with the aim to assess changes between rhizosphere and bulk soil in terms of physical, chemical, and biological properties. The three plants considered showed to have different rhizosphere effect. *Helianthemum* induced a strong rhizosphere effect through a synergistic effect between root activity and a well adapted rhizosphere microbial community. *Dryas* did not foster a microbial community structure specifically designed for its rhizosphere, but consumes most of the energetic resources supplied by the plant to make nutrients available. Conversely to the other two species, *Silene* produced slight soil changes in the rhizosphere, where the microbial community had a structure, abundance and activity similar to those of the bulk soil. The ability to colonize harsh environments of *Silene* is probably linked to the shape and functions of its canopy rather than to a functional rhizosphere effect.

This study showed that the rhizosphere effect differed by species also under high environmental pressure (periglacial conditions, poorly developed soil), and the activity of roots and associated microbial community is decisive in modifying the soil properties, so to create a suitable environment where plants are able to grow.

1. Introduction

In the rhizosphere, the soil in proximity to the root, processes like rhizodeposition, intense microbial activity, root nutrient uptake, redox reactions, and CO_2 production induce modifications of soil components and properties (Hinsinger et al., 2003). The chemical, physical and biological differentiation of the rhizospheric soil with respect to the rest of the soil is called “rhizosphere effect”, which has been investigated in many ecosystems, including those with environmental constraints and nutrient-poor soils (e.g., Hinsinger et al., 2005; Teixeira et al., 2010). However, little is known about the rhizosphere effect of pioneer plants in young and poorly developed soils from periglacial environments (Wookey et al., 2009).

Periglacial environments are those affected by severe frost action that dominates geomorphic processes, and amount to about 25% of the Earth's land surface. The knowledge of the rhizosphere effect of pioneer plants in these environments is the basis in understanding how soil–plant relationships respond to environmental constraints. In general, arctic and alpine plants have a higher proportion of their biomass below-ground than trees and bushes from other ecosystems (Jackson et al., 1996; Körner, 2003), and this relatively high below-ground biomass increases the proportion of rhizosphere soil (Hinsinger et al., 2005; Finzi et al., 2015). Indeed, in poorly developed soils of cold areas, the presence of

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vascular plants strongly modifies the soil properties and the structure and function of the soil microbial community (Yergeau et al., 2007). In this areas, rhizospheric processes resulting from soil–plant–microbes interactions may improve the ability of plants to overcome abiotic disturbances such as freezing, high soil daily and seasonal temperature excursions, freeze–thaw and wet–dry cycles, excessive drainage, and strongly oligotrophic conditions (e.g., Tscherko et al., 2004, 2005; Edwards et al., 2006; Ciccazzo et al., 2014). The amount of energy supplied by the plants in form of exudates to rhizosphere heterotrophic microbial community is key for stimulating rhizospheric processes (Kuzyakov, 2002; Wookey et al., 2009; Ciccazzo et al., 2014; Jorquera et al., 2014). In fact, most arctic and alpine vascular plants allocate 10–30% of net carbon fixation to establish mycorrhizal associations (Read et al., 2004; Cripps and Eddington, 2005), although allocation patterns of these energetic resources depend on plant species and soil nutrient availability (Högberg et al., 2003; Wardle et al., 2004; Wookey et al., 2009). Hence, different plants colonizing the same soil might differently shape a specific rhizosphere microbial community depending on the quantity and quality of their root exudates (Haichar et al., 2008; Huang et al., 2014).

Our research focuses on the rhizosphere effect of three plant species [*Helianthemum nummularium* (L.) Mill. subsp. *grandiflorum* (Scop.) Sch. and Th., *Dryas octopetala* (L.), and *Silene acaulis* (L.) Jacq. subsp. *cenisia* (Vierh.) P. Fourn.] that sparsely occupy soils of deglaciated areas actually submitted to periglacial conditions (central Apennines, Italy). These soils are characterized by environmental constraints such as harsh climatic and nutritional conditions. Specifically, we tested the following hypotheses:

(i) the physical and chemical soil properties differ in rhizosphere versus bulk soil for the three plant species;
(ii) the microbial community structure and abundance, and microbial respiration differ in rhizosphere versus bulk soil within and among the three plant species.

To this aim, we investigated physical, chemical and microbiological properties of both rhizosphere and bulk soil, and the results were compared with those of the adjacent bare soil.

2. Materials and methods

2.1. Site description

The study site is located in one of the highest mountains of central Apennines (Italy), the Majella massif (Fig. 1) and, in particular, in the Cannella Valley, whose altitude ranges from 1900 to 2750 m, and has a southeast orientation. The mean annual precipitation is about 2100 mm (mostly snow) and the mean annual air temperature is 2.3 °C. January is the coldest month, with an average temperature of −4.3 °C, whereas August is the warmest month, with an average temperature of 11.4 °C (Corti et al., 2012). The area, that experienced a relatively recent glacier recession initiated about 12,700 and ended about 11,000 years before present (Giraudi, 2004), is mantled by thick morainic deposits (till) mostly made of limestone, from which the present soils developed. The area is covered by sparse vegetation mostly made of *H. nummularium* (L.) Mill. subsp. *grandiflorum* (Scop.) Sch. and Th., *D. octopetala* L., *S. acaulis* (L.) Jacq. subsp. *cenisia* (Vierh.) P. Fourn., *Carex kitaibeliana* Degen ex Bech., *Anthyllis vulneraria* L. subsp. *maura* (Beck) Maire, *Campanula scheuchzeri* Vill., *Minuartia verna* (L.) Hiern subsp. *verna*, *Trifolium pratense* L. subsp. *semipurpureum* (Strobl) Pign., with spots covered by *Salix retusa* L. and rare dwarf mountain pines (*Pinus mugo* Turra). Where the vegetation forms a rather continuous mat, the soils are loamy-skeletal, mixed, frigid Oxyaquic Haplocryolls (SSS, 2010), while in the bare areas the soils are loamy-skeletal, mixed, frigid Oxyaquic Cryorthents (SSS, 2010). In both cases, the soil is frozen for meters from December to February/March.

The plant species chosen for this study differ for their aboveground and belowground traits. *H. nummularium* subsp. *grandiflorum* is an evergreen trailing plant with loose terminal clusters of bright yellow, saucer-shaped flowers that is rather common in dry and base-rich soils. *D. octopetala* forms dense mats with trailing branches bearing adventitious roots that inhabits particularly well-drained mineral soils (Blaschke, 1991), and that colonizes young soils developed on moraines, especially where nitrogen is scarce (Schwintzer and Tjepkema, 1990). Finally, *S. acaulis* subsp. *cenisia* is a cushion-forming gynodioecious plant with a taproot system that generally grows on wind-exposed ridges, rocky slopes and open alpine grasslands, and can survive extreme temperature from −80 to 60 °C (Larcher et al., 2010).

2.2. Soil sampling and sample preparation

During July 2011, at about 2455 m above sea level, within an area of about 1600 m^2 (40 × 40 m) we selected three plots with a mean diameter ranging from 5 to 10 m; in each plot all the three plants were present at once. As a control, for each plot a bare soil was also located at least at 1.5 m from each plant. The three individual plants for each species were chosen among those showing the maximum development. For *Helianthemum* we considered plants forming patches of 1.5–1.8 m of diameter, with an estimated age of at least 30–32 years (obtained by the annual ring counting of basal stems). For *Dryas*, we took into consideration plant mats with a diameter of about 1 m, with an estimated age of at least 18–22 years (obtained by the annual ring counting of basal stems). In the case of *Silene*, we selected fully healthy cushions with a diameter comprised between 35 and 40 cm. According to Benedict (1989) and McCarthy (1992), cushions of *S. acaulis* have a growth rate ranging from 0.06 to 3 cm yr^{-1}, even though the maximum rate of 2–3 cm yr^{-1} is reached in the intermediate part of their life, which can attain 350 years (Beschel, 1958). Because of this, we estimated the age of the selected cushions to be more than 50 years.

Within each plot, a soil profile was opened under each plant and in the bare soil. The soil morphological descriptions (Schoeneberger et al., 1998) are reported in the Appendix. From the A horizon (A1 plus A2 in the case of the profiles under the plants) of each profile, a large amount of sample (at least 2 kg) was collected and stored at the field moist conditions in a portable refrigerator. Once in the laboratory, the rhizosphere was isolated according to the method of Corti et al. (2005) from each soil samples by picking up the roots together with the adhering soil. 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, considered as rhizosphere, was recovered by shaking and gentle brushing of the roots. During this operation, the root fragments were removed by using tweezers under a magnifying lens. Aliquots of rhizosphere, bulk and bare soil at field moist conditions were sieved through a 4-mm mesh and stored (for a period not exceeding four weeks) at 2 °C for the biological analyses: microbial biomass C content, basal respiration, and microbial community structure. The remaining soil samples were air-dried and sieved through a 2-mm mesh.

2.3. Physical and chemical analysis

The available water content (AWC) was calculated by difference between the amount of water retained by the soil at 33 kPa and at 1500 kPa, which was determined by pressure plate extractor

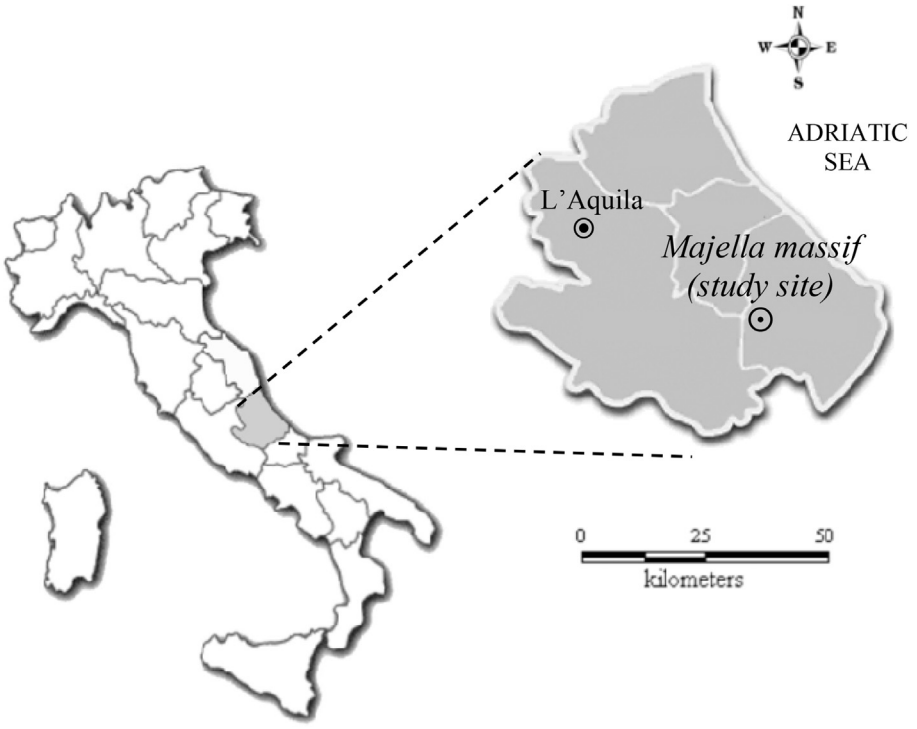

Fig. 1. Map of Italy with magnification of the Abruzzo region and indication of the study site.

(Soilmoisture Equipment Corp., Santa Barbara, CA). The soil pH was determined potentiometrically in water (solid:liquid ratio of 1:2.5) after one night of equilibration using a Thermo Scientific™ Orion™ 2-Star Benchtop pH-meter. Potentially plant-available P and organic P was determined by the Olsen method (Olsen et al., 1954) and the ignition method (Kuo, 1996), respectively. To determine the exchangeable Ca, Mg, K and Na, 2 g of each sample were placed into a centrifuge tube, submerged with 0.2 M $BaCl_2$ solution (solid/liquid ratio of 1:10) and shaken for about 10 min (Corti et al., 1997). The mixture was left to rest for a while and then gently shaken for few seconds to re-suspend the sediments and then centrifuged. The extracted solution was filtered through Whatman 42 filter paper, and analyzed by atomic absorption with a Shimadzu AA-6300 (Tokyo, Japan) spectrophotometer.

Iron was sequentially extracted from the samples with 1) 0.1 M Na-acetate 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 to estimate the "labile Fe", namely the Fe forming the easily reducible Fe–Mn oxy-hydroxides (Berna et al., 2000), 3) NH_4-oxalate/oxalic acid at pH 3.0 in the dark to recover the Fe of the non-crystalline Fe oxy-hydroxides plus that bound to organic matter (Blakemore et al., 1981), and 4) 0.25 M hydroxylamine hydrochloride in 0.25 M HCl to obtain the Fe of the crystalline Fe oxy-hydroxides (Berna et al., 2000). The Fe in the extracts was determined by a Shimadzu AA-6300 atomic absorption spectrophotometer.

The total organic C content (TOC) and total N were determined by a dry combustion analyzer (EA-1110, Carlo Erba Instruments, Milan, Italy). Prior to analysis, the specimens were treated with 0.1 M HCl to eliminate inorganic C. Water extractable organic C (WEOC) was obtained according to Agnelli et al. (2014) with the following procedure: 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 suspension was left to rest for a while, centrifuged at 1400 *g* for 10 min, and then filtered through a 0.45 μm membrane filter. The obtained supernatant solution received a few drops of concentrated H_3PO_4 to eliminate carbonates and was analyzed for organic C by a TOC-5000A analyser (Shimadzu Corp., Tokyo, Japan). On the same solution, the amount of water extractable N (WEN), that comprises part of the inorganic N forms (NH_4^+–N and NO_3^-–N) and the most soluble organic N forms, was determined by dry combustion analyzer.

The inorganic N was extracted by treating the samples with 2 M KCl solution (solid:liquid ratio 1:10); the suspension was shaken for 1 h with an orbital shaker (140 rpm), and filtered through Whatman 42 filter paper. The different forms of inorganic N (NH_4^+–N and NO_3^-–N) were measured on soil extracts by a FOSS Fiastar™ 5000 system (Hillerod, Denmark). The organic N content was obtained by the difference between the total N and inorganic N content.

2.4. Microbial biomass C, basal respiration

The amount of microbial biomass-C (C_{mic}) was determined by the fumigation-extraction method (Vance et al., 1987), after 62 days of conditioning at 25 °C and at 50% of their total water holding capacity. During this incubation period, basal respiration was measured by alkali (1 M NaOH solution) absorption of the CO_2 developed and titration of the residual OH^- with a standardized HCl solution. Basal respiration was expressed as the cumulative amount of CO_2–C evolved during the experiment.

2.5. Soil microbial community structure

The abundance and structure of soil microbial community in the rhizosphere, bulk and bare soil were assessed by analyzing the ester-linked phospholipid fatty-acids (PLFAs), which are specific membrane components of living cells and are not found in storage products or in dead cells. Specifically, the technique was used to measure the relative abundance of active fungi and bacteria (Bardgett et al., 1996), which are responsible for 90–95% of total

heterotrophic metabolism in most soils (Petersen and Luxton, 1982). Lipids were extracted from soil samples, fractionated and quantified using the procedure described by Bardgett et al. (1996). The analyses of phospholipid fatty acid methyl esters were run on an 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.). Separated fatty-acid methyl-esters were identified by chromatographic retention time and mass spectral comparison using the BAME and FAME mix qualitative standard (Supelco Analytical, USA), which ranged from C11 to C20. 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 an internal standard. Fatty-acid nomenclature was designed as described by Frostegård et al. (1993). Total extractable PLFAs were used as an indicator of living biomass, and single PLFAs were used as markers to quantify the relative abundance of specific cell types (Fritze et al., 2000; Fierer et al., 2003). Gram-positive bacteria were identified by summing i15:0, a15:0, i16:0, i17:0 and a17:0 fatty acids, while the Gram-negative bacteria were accounted by summing the fatty acids 16:1, cy17:0, 17:1ω9c and 18:1ω7 (Federle, 1986; Frostegård et al., 1993; Fierer et al., 2003; Massaccesi et al., 2015). The total bacterial biomass was calculated by the sum of the PLFAs attributed to Gram-positive and Gram-negative bacteria. The fatty acid 18:2ω6 was used as a marker for saprophytic fungi (Federle, 1986), while the fatty acid 16:1ω5 was used as an indicator for arbuscular mycorrhizal fungi (AMF) abundance (De Deyn et al., 2011). Although this latter fatty acid is not strictly specific to AMF, it was used as an indicator for their abundance in soil (e.g., Olsson, 1999; Chung et al., 2007; De Deyn et al., 2011). The ratio between fungal and bacterial PLFAs was taken as an indicator of changes in the relative abundance of these two microbial groups in the samples (Bardgett et al., 1996). Actinomycetes were identified by the 10Me17:0 and 10Me18:0 fatty acids (Kroppenstedt, 1985; De Deyn et al., 2011), whereas the fatty acid 20:2 was used as biomarker for protozoa (Fierer et al., 2003).

2.6. Statistical analysis

To test the extent of the effects of plant species and soil fractions (rhizosphere and bulk soil) on soil properties, we performed a redundancy analysis (RDA). The RDA model was tested for significance by using 999 random permutations. The adjusted R^2, that measures the variance explained by the RDA model, was used to estimate the variance of the ordination axes. Moreover, to investigate the variations of rhizosphere and bulk soil properties under the three plant species, a principal component analysis (PCA) was performed. All the data were standardized prior RDA and PCA by subtracting the mean of each variable and dividing by the standard deviation.

To test the differences in microbial community structure, as quantified by the relative abundance of all PLFA peaks, we performed a two-way permutational multivariate analysis of variance (PERMANOVA) on row dataset (Anderson, 2001), and non-metric multidimensional scaling (NMDS) was used to provide a graphical representation of results. For this analysis, we used the relative abundance of PLFA so that results reflected changes in community structure that were independent by changes in biomass. Changes in biomass were quantified through other metrics (e.g., total PLFA, total fungi).

The effects of plant species and soil fraction (rhizosphere or bulk soil) on the abundance of the identified microbial groups and soil properties were analyzed using analysis of variance (two-way ANOVA) (Table I of the Supplementary data). The comparison of means was assessed by Fisher post-hoc test at $P < 0.05$. The statistical analyses were performed using R (R Core Team, 2014).

3. Results

3.1. RDA and PCA

The RDA plots (Fig. 2a, b) showed that the plant species effect (Permutation test, $F = 3.973$, $P = 0.001^{***}$) explained about 27% of the total variance, whereas the soil fraction effect (Permutation test, $F = 3.198$, $P = 0.004^{**}$) explained about 18% of the total variance. The PCA scatter plot (Fig. 3) showed the variation of rhizosphere and bulk soil properties under the three plant species, and identified two axes that explained about 37 and 19% of the variation, respectively. The PCA indicated that differences between rhizosphere and bulk soil of the three species occurred, although to a different extent: *Helianthemum* was the species with the greatest differences between rhizosphere and bulk soil, followed by *Dryas* and, then, *Silene*. Further, PCA showed that bulk soil and rhizosphere of *Silene* and the bulk soil of *Dryas* were closer and more similar to the bare soil than the others.

PCA-axis 1 appeared to be positively driven by microbial community, total N, TOC content and exchangeable Ca and Mg, whereas it was negatively driven by pH and NO_3^-–N (Fig. Ia of the Supplementary data). PCA-axis 2 was mainly associated with positive relationships to CO_2–C/WEOC ratio and qCO_2, and with negative relationships to C_{mic}/TOC ratio, available P and C_{mic} contents (Fig. Ib of the Supplementary data). The PCA scores of the *Helianthemum* rhizosphere were placed on the right side of PCA-axis 1, indicating a strong positive relationship with the soil properties driving axis 1. Conversely, the bare soil and the bulk soil of *Silene* were the most negatively associated with axis 1. A relationship with the soil properties that positively drove axis 2 was indicated for the rhizosphere of *Dryas* and the bulk soil of *Helianthemum*.

3.2. Available water content (AWC), pH, and available and organic P

The *Helianthemum* rhizosphere had an AWC higher than that of the bulk soil (Table 1), while rhizosphere and bulk soil of *Dryas* showed similar values. Conversely, *Silene* rhizosphere had an AWC lower than that of the bulk soil, analogous to that of bare soil.

The pH values of the rhizosphere of *Helianthemum* and *Dryas* were lower than those of the respective bulk and bare soil (Table 1), while *Silene* showed no difference among rhizosphere, bulk and bare soil. Only for *Helianthemum*, the available P content showed a significant difference between rhizosphere and bulk soil, with the lowest value in the latter (Table 1). *Dryas* and *Silene* exhibited a higher amount of organic P in the rhizosphere than in the bulk and bare soil, while under *Helianthemum* organic P was similar in both soil fractions.

3.3. Exchangeable cations and extractable forms of Fe

For all the soils, Ca was the most abundant exchangeable cation (Table 2). The amount of exchangeable cations in the rhizosphere was higher than that in the bulk soil for Mg, K and Na under *Dryas*, and only for Ca under *Silene*. The quantity of exchangeable cations of the bulk soils was often similar to that of bare soil.

Only in a few cases the rhizosphere differed from the bulk soil in terms of extractable Fe forms (Table 2). For all the plants, the most represented Fe form was that of the non-crystalline and organic matter bound Fe-oxy-hydroxides. Significant differences between rhizosphere and bulk soil were found for the labile Fe under *Helianthemum*, non-crystalline and organic matter bound Fe-oxy-hydroxides for *Silene*, and crystalline Fe-oxy-hydroxides for *Dryas* and *Silene*.

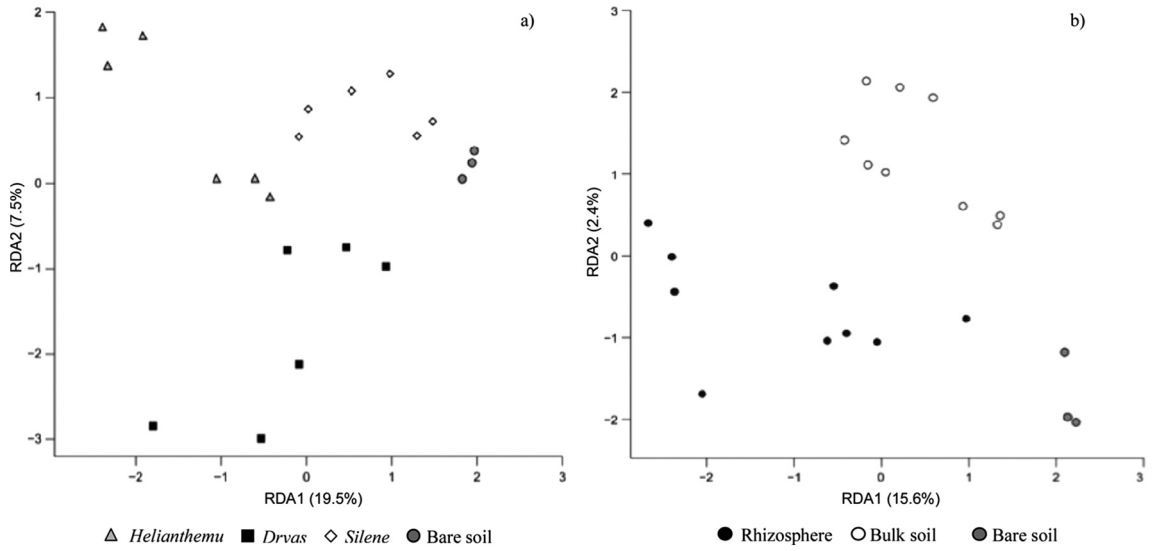

Fig. 2. Redundancy analysis (RDA) ordination plots: a) plant species and b) soil fractions (rhizosphere and bulk soil) effects on soil properties. Cannella valley, Majella massif (Italy).

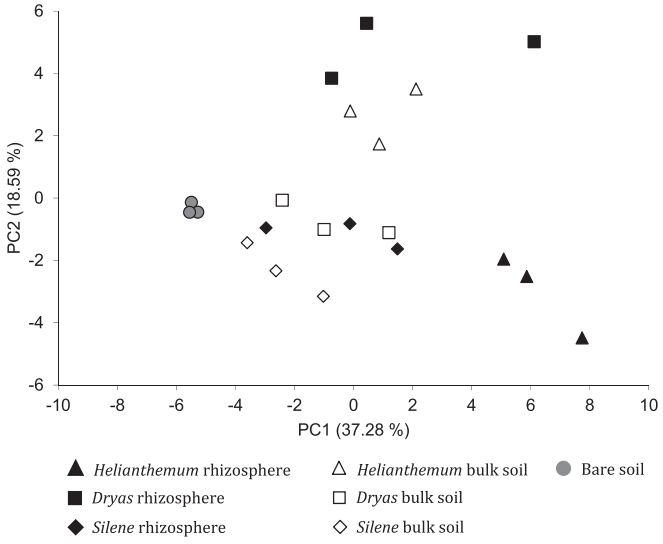

Fig. 3. Variation of rhizosphere and bulk soil properties under the three plant species tested as analyzed by principal component analysis (PCA) using standardized data.

3.4. Nitrogen, total and water soluble organic C, microbial biomass C and basal respiration

In all the samples, WEN represented a negligible portion of the total soil N, which was constituted by organic N for 99.1% in the bare soil and for at least 99.6% in the rhizosphere and bulk soil of the three plants (Table 3). In terms of organic N, the rhizosphere had a higher organic N content than the bulk soil for *Helianthemum* and *Dryas*. Under *Silene*, the total N of the rhizosphere was higher than that of the bulk soil. The bare soil and the bulk of the three plants showed similar contents of total and organic N.

The TOC concentration was similar in the rhizosphere and bulk soil of the three plants, while the WEOC content was always more abundant in the rhizosphere than in the bulk, with the highest concentration in the *Helianthemum* rhizosphere (Table 4). The bulk soil of *Helianthemum* contained more TOC than the bare soil.

The microbial biomass C (C_{mic}) had a greater concentration in the rhizosphere than in the bulk soil of the three plants (Table 4), with the highest value in the rhizosphere of *Helianthemum* (more than 7 fold-higher than the respective bulk soil). Surprisingly, the C_{mic} content of the bare soil was similar to that of the rhizosphere and higher than the bulk soil of *Dryas*. The CO_2 evolved during the basal respiration experiment ($\sum CO_2$–C) was higher in the rhizosphere than in the bulk soil for the three plants, with the highest values recorded in the rhizosphere of *Dryas*. The bare soil and the bulk soil of *Silene* showed the lowest amount of $\sum CO_2$–C. The rhizosphere of *Helianthemum* showed the largest percentage of C_{mic}/TOC (Fig. 4a) and the lowest percentage of organic C consumed during the incubation experiment with respect to WEOC ($\sum CO_2$–C/WEOC) (Fig. 4b). For *Dryas*, the percentage of C_{mic}/TOC was similar in both rhizosphere and bulk soil, but the rhizosphere showed the largest $\sum CO_2$–C/WEOC percentage and qCO_2 value.

3.5. Microbial community abundance and structure

Interactions between plant species and soil fractions (PERMANOVA, $F_{2,20} = 3.35$, $R^2 = 0.246$, $P = 0.0158^*$) affected microbial

Table 1
pH values, available water content (AWC), and available and organic P concentration of rhizosphere and bulk soil of *Helianthemum nummularium* subsp. *grandiflorum*, *Dryas octopetala* and *Silene acaulis* subsp. *cenisia*, and bare soil. Cannella valley, Majella massif (Italy). Numbers in parentheses are the standard errors (n = 3). For each line, mean values with different letters significantly differ for $P < 0.05$.

	Helianthemum		*Dryas*		*Silene*		Bare soil
	Rhizosphere	Bulk	Rhizosphere	Bulk	Rhizosphere	Bulk	
AWC (%)	23.25 (0.86)^{a}	13.96 (3.67)^{bcd}	20.62 (1.56)^{ab}	19.20 (0.48)^{abc}	12.90 (1.96)^{cd}	19.83 (3.68)^{ab}	11.59 (1.20)^{d}
pH	7.29 (0.06)^{d}	7.51 (0.05)^{bc}	7.47 (0.09)^{cd}	7.79 (0.06)^{a}	7.59 (0.04)^{abc}	7.66 (0.10)^{abc}	7.71 (0.08)^{ab}
Available P (mg kg^{-1})	51.27 (14.14)^{a}	17.00 (1.65)^{c}	29.85 (5.88)^{abc}	32.25 (7.25)^{abc}	48.50 (2.80)^{ab}	50.43 (12.79)^{a}	19.13 (2.24)^{c}
Organic P (mg kg^{-1})	1276.06 (46.53)^{ab}	1302.86 (11.60)^{a}	1307.67 (124.43)^{a}	1016.24 (69.79)^{bc}	1283.51 (79.35)^{ab}	882.14 (157.87)^{c}	809.72 (57.56)^{c}

Table 2
Content of exchangeable basic cations and Fe forms [carbonate-bound Fe (CB-Fe), Fe forming the easily reducible Fe–Mn oxy-hydroxides, namely the labile Fe (L-Fe), Fe of the non-crystalline Fe oxy-hydroxides plus that bound to organic matter (NC-Fe), and Fe of the crystalline Fe oxy-hydroxides (C-Fe)] of rhizosphere and bulk soil of *Helianthemum nummularium* subsp. *grandiflorum*, *Dryas octopetala* and *Silene acaulis* subsp. *cenisia*, and bare soil. Cannella valley, Majella massif (Italy). Numbers in parentheses are the standard errors (n = 3). For each line, mean values with different letters significantly differ for $P < 0.05$.

	Helianthemum		*Dryas*		*Silene*		Bare soil
	Rhizosphere	Bulk	Rhizosphere	Bulk	Rhizosphere	Bulk	
Exchangeable Ca ($cmol_{(+)}$ kg^{-1})	60.6 (0.7)^{a}	53.3 (1.1)^{a}	59.1 (10.2)^{a}	43.1 (4.8)^{ab}	55.5 (9.0)^{a}	33.1 (5.6)^{b}	27.2 (5.8)^{b}
Exchangeable Mg ($cmol_{(+)}$ kg^{-1})	1.2 (0.1)^{ab}	0.9 (0.1)^{abc}	1.3 (0.5)^{a}	0.6 (0.1)^{bc}	0.8 (0.2)^{abc}	0.4 (0.1)^{c}	0.3 (0.1)^{c}
Exchangeable K ($cmol_{(+)}$ kg^{-1})	2.0 (0.0)^{ab}	2.0 (0.1)^{ab}	2.3 (0.3)^{a}	1.7 (0.1)^{b}	1.8 (0.0)^{b}	1.8 (0.1)^{b}	1.8 (0.1)^{b}
Exchangeable Na ($cmol_{(+)}$ kg^{-1})	1.1 (0.1)^{bc}	1.4 (0.1)^{a}	1.2 (0.1)^{ab}	1.0 (0.0)^{c}	1.2 (0.04)^{ab}	1.3 (0.0)^{a}	1.2 (0.1)^{ab}
CB-Fe (mg kg^{-1})	3.8 (0.4)^{abc}	5.4 (0.4)^{a}	4.6 (0.7)^{ab}	3.7 (0.8)^{abc}	3.8 (0.4)^{abc}	1.8 (0.3)^{c}	3.2 (1.1)^{bc}
L-Fe (mg kg^{-1})	11.3 (2.9)^{b}	21.9 (4.8)^{a}	10.9 (1.5)^{b}	10.1 (1.1)^{b}	6.9 (1.4)^{b}	6.1 (0.4)^{b}	11.7 (1.2)^{b}
NC-Fe (mg kg^{-1})	6814.3 (81.6)^{a}	7303.6 (358.8)^{a}	3779.0 (1163.1)^{b}	3032.0 (632.7)^{b}	3245.4 (376.2)^{a}	2003.4 (151.3)^{b}	2787.4 (829.0)^{b}
C-Fe (mg kg^{-1})	1338.9 (153.2)^{a}	1454.4 (112.7)^{a}	1197.4 (165.0)^{ab}	433.9 (135.4)^{cd}	796.8 (206.4)^{bc}	189.7 (62.0)^{d}	404.1 (141.7)^{cd}

Table 3
Content of total N, water extractable N (WEN), ammonium (NH_4^+–N), nitrate (NO_3^-–N), and organic N of rhizosphere and bulk soil of *Helianthemum nummularium* subsp. *grandiflorum*, *Dryas octopetala* and *Silene acaulis* subsp. *cenisia*, and bare soil. Cannella valley, Majella massif (Italy). Numbers in parentheses are the standard errors (n = 3). For each line, mean values with different letters significantly differ for $P < 0.05$.

	Helianthemum		*Dryas*		*Silene*		Bare soil
	Rhizosphere	Bulk	Rhizosphere	Bulk	Rhizosphere	Bulk	
Total N (g kg^{-1})	13.71 (0.29)^{a}	10.10 (0.96)^{bc}	14.16 (1.73)^{a}	9.23 (1.02)^{bc}	13.00 (1.58)^{ab}	7.66 (1.11)^{c}	5.60 (0.51)^{c}
WEN (g kg^{-1})	0.20 (0.05)^{a}	0.20 (0.05)^{a}	0.13 (0.02)^{ab}	0.13 (0.02)^{ab}	0.12 (0.03)^{ab}	0.12 (0.03)^{ab}	0.07 (0.00)^{b}
NH_4^+–N (g kg^{-1})	0.04 (0.00)^{a}	0.03 (0.00)^{bcd}	0.02 (0.00)^{d}	0.02 (0.00)^{cd}	0.03 (0.00)^{ab}	0.02 (0.00)^{d}	0.03 (0.00)^{abc}
NO_3^-–N (g kg^{-1})	0.01 (0.00)^{c}	0.01 (0.00)^{c}	0.01 (0.00)^{bc}	0.01 (0.00)^{b}	0.02 (0.00)^{ab}	0.01 (0.00)^{b}	0.02 (0.00)^{a}
Organic N (g kg^{-1})	13.66 (0.28)^{a}	10.06 (0.96)^{bc}	14.13 (1.74)^{a}	9.20 (1.02)^{bc}	12.95 (1.58)^{abc}	7.63 (1.11)^{bc}	5.55 (0.51)^{c}

Table 4
Content of total organic C (TOC), water extractable organic C (WEOC) and microbial biomass C (C_{mic}), and amount of CO_2 evolved during basal respiration experiments ($\sum CO_2$–C) for rhizosphere and bulk soil of *Helianthemum nummularium* subsp. *grandiflorum*, *Dryas octopetala* and *Silene acaulis* subsp. *cenisia*, and bare soil. Cannella valley, Majella massif (Italy). Numbers in parentheses are the standard errors (n = 3). For each line, mean values with different letters significantly differ for $P < 0.05$.

	Helianthemum		*Dryas*		*Silene*		Bare soil
	Rhizosphere	Bulk	Rhizosphere	Bulk	Rhizosphere	Bulk	
TOC (g kg^{-1})	155.14 (4.41)^{a}	133.92 (12.85)^{ab}	118.89 (2.07)^{abc}	93.78 (8.04)^{abc}	137.41 (17.01)^{ab}	66.69 (9.25)^{bc}	50.09 (4.82)^{c}
WEOC (g kg^{-1})	1.14 (0.25)^{a}	0.24 (0.03)^{bc}	0.31 (0.02)^{b}	0.14 (0.00)^{c}	0.34 (0.03)^{b}	0.17 (0.00)^{c}	0.17 (0.01)^{c}
C_{mic} (mg kg^{-1})	1274.66 (7.81)^{a}	173.08 (20.83)^{d}	122.27 (17.71)^{e}	96.70 (1.95)^{f}	396.84 (1.97)^{b}	210.61 (9.34)^{c}	126.16 (17.95)^{e}
$\sum CO_2$–C (μg kg^{-1})	4106.06 (58.67)^{b}	2838.46 (95.09)^{c}	6740.27 (156.29)^{a}	1693.74 (64.68)^{d}	1764.38 (136.8)^{d}	1186.24 (149.67)^{e}	1278.95 (212.12)^{e}

community structure as expressed by the relative abundance of all identified PLFA peaks. NMDS plot indicated that the greater diversity between rhizosphere and bulk soil in the microbial community structure occurred for *Helianthemum* (Fig. 5a), and that the synergistic effect of plant species and soil fractions appeared mostly due to bacteria. In fact, as shown in Fig. 5b, axis 1 was mainly driven by the relative abundance of i15:0 and a15:0 fatty acids, which represent Gram-positive bacteria, and 10Me18:0, which represents actinomycetes. In contrast, axis 2 was driven by the relative abundance of 17:1ω9c fatty acid, which represents Gram-negative bacteria.

In all the samples, although the non-specific PLFA were 18–25% of the total, the most represented microbial group identified was that of bacteria, which ranged from 61% (bare soil) to 69% (bulk soil of *Helianthemum*) of the entire microbial community (Table 5). In order of abundance, bacteria were followed by actinomycetes, AMF, saprophytic fungi and protozoa. Among the bacteria, Gram-negative were the most abundant. Both bulk soil and rhizosphere of the three plants had a greater amount of bacteria than the bare soil, whereas *Helianthemum* and *Dryas* had both fractions richer in fungi than bare soil. Bacteria (Gram-positive and Gram-negative), saprophytic fungi and AMF were the highest in the rhizosphere of *Helianthemum*, whereas no significant difference was detected between rhizosphere and bulk soil of *Dryas* and *Silene*. The fungal to bacterial PLFAs ratio was always much <1, and showed similar values for all the samples.

4. Discussion

4.1. Difference in the soil properties of rhizosphere versus bulk soil for the three plant species

Our results indicated that the plant species effect was more significant than the soil fraction effect, suggesting that both above- and below-ground plant systems play the major role in driving the changes in soil properties, with significant influences on most measured variables. However, also the rhizosphere effect, which is closely related to the plant species, plays a decisive role in soil changes. Among the three species considered in this study, *Helianthemum*, showed the major differences between rhizosphere and bulk soil, followed by *Dryas* and then *Silene*. As a matter of fact, *Helianthemum* exerted a relevant "rhizosphere effect" as most of the measured parameters differed between rhizosphere and bulk: pH, labile Fe, AWC, available P, total and organic N, NH_4^+–N, WEOC, C_{mic}, CO_2–C evolved during the basal respiration, total PLFA, total bacteria, Gram-positive and Gram-negative bacteria, saprophytic fungi, AMF, and non-specific PLFA.

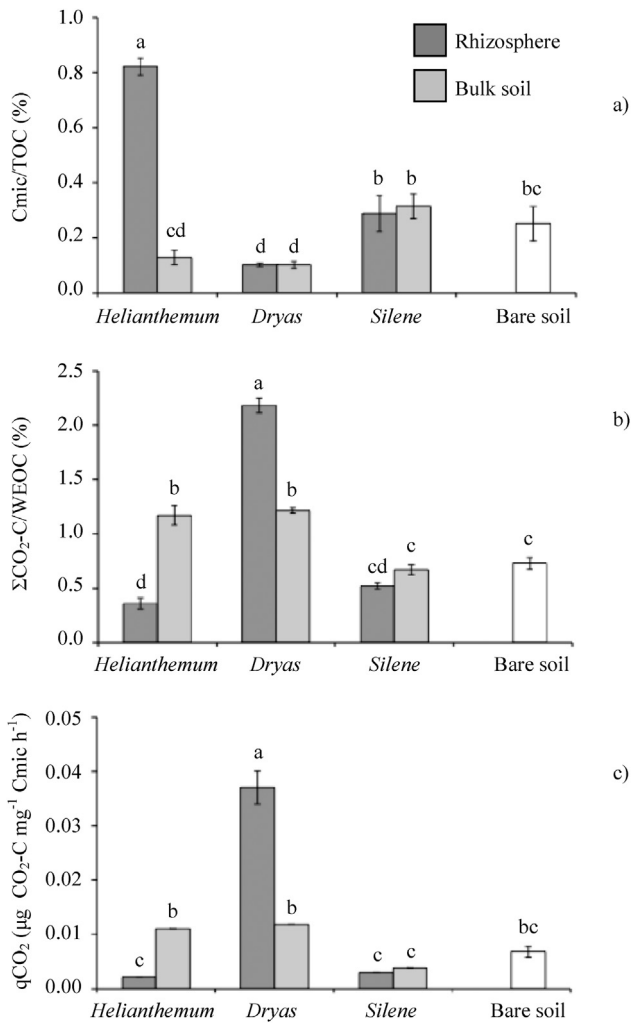

Fig. 4. a) Percentage of organic C present as microbial biomass (C_{mic}/TOC), b) percentage of water soluble organic C developed as CO_2–C ($\sum CO_2$–C/WEOC), and c) metabolic quotient (qCO_2) for rhizosphere and bulk soil of *Helianthemum nummularium* subsp. *grandiflorum*, *Dryas octopetala* and *Silene acaulis* subsp. *cenisia*, and bare soil. Cannella valley, Majella massif (Italy). Error bars are the standard errors. For each graph, columns with different letters significantly differ for $P < 0.05$.

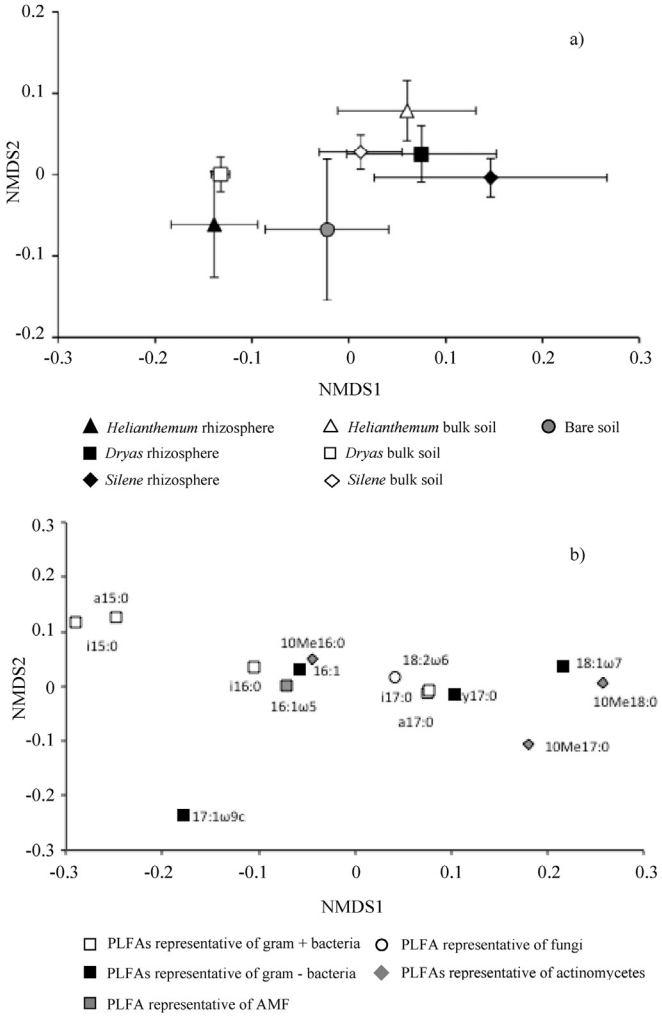

Fig. 5. a) Non-metric multidimensional scaling (NMDS) plot shows synergistic effect of plant species and soil fraction (rhizosphere and bulk soil) on soil microbial community structure (stress = 0.087). Cannella valley, Majella massif (Italy). Error bars indicate the standard errors of the centroids along each NMDS axis. b) NMDS scores for PLFAs.

Helianthemum and *Dryas* had lower pH values in the rhizosphere than in the bulk, so confirming the acidifying action that the roots exert on the soil in contact with them (e.g., Hinsinger et al., 2003). The acidification of the rhizosphere can occur by different processes, other than the CO_2 produced by the root respiration (Richter et al., 2007): 1) excretion of H^+ following the root absorption of cations in excess of anions (Haynes, 1990), and 2) release of organic acids to overcome nutrient deficiency (Rengel and Romheld, 2000; Hinsinger et al., 2003; Sandnes et al., 2005). For example, in P deficient soils, roots of natural and cultivated plants exude large amounts of low-molecular weight carboxylates that mobilize P by competing for the same adsorption sites in the soil matrix (Gerke et al., 2000; Fernández Sanjurjo et al., 2003; Wouterlood et al., 2005). The higher concentration of available P in the rhizosphere than in the bulk soil of *Helianthemum* may be the result of the release of organic acids and specific enzymes such as phosphatases, which may significantly increase P availability by promoting the hydrolysis of organic P in forms more accessible to the plant (Gerke et al., 2000; Wouterlood et al., 2005).

In the case of *Dryas*, the rhizosphere effect was exhibited by the lower pH and the greater amount of organic P, exchangeable K, Mg and Na, crystalline Fe oxy-hydroxides, total and organic N, WEOC, C_{mic} and CO_2–C evolved during the basal respiration in the root-affected soil than in the bulk. The higher WEOC content in the rhizosphere than in the bulk soil was mainly attributed to exudation of labile C compounds such as carbohydrates, aminoacids, aliphatic or aromatic organic acids, phenols, and fatty acids (Colin-Belgrand et al., 2003; Farrar et al., 2003), but also to an enhanced organic matter cycling occurring in the rhizosphere (Dijkstra et al., 2013), This C input into the rhizosphere represents an investment made by the plant to modify soil conditions and establish an appropriate environment for its development (Boddy et al., 2008).

The few differences between rhizosphere and bulk soil of *Silene* indicated that this species modifies the soil properties less than *Helianthemum* and *Dryas*. Interestingly, the estimated age of the *Silene* plants was greater than that of the other two species. *Silene* has apparently a lower rhizosphere effect than *Helianthemum* and *Dryas*, but is one of the best adapted plant to alpine environment, as it is able to colonize bare or recently deglaciated soils (e.g., Pysek and Liska, 1991; Chapin and Körner, 1995; Körner, 2003). This ability is mainly attributed to the domed shape of the canopy that mitigates temperature, stores moisture, and increases the quantity of nutrients underneath the cushion (Körner, 2003; Reid et al., 2010 and references herein). Indeed, in arctic and alpine environments, cushion plants as *Silene* are considered as nurse-plants that are able to facilitate the settlement of less tolerant plant species (Brooker

Table 5
Content of total PLFAs and of specific PLFAs used to quantify the relative abundance of the individual cell types comprising the soil microbial community in the rhizosphere and bulk soil of *Helianthemum nummularium* subsp. *grandiflorum*, *Dryas octopetala* and *Silene acaulis* subsp. *cenisia*, and bare soil. Cannella valley, Majella massif (Italy). Numbers in parentheses are the standard errors (n = 3). For each line, mean values with different letters significantly differ for $P < 0.05$.

	Helianthemum		*Dryas*		*Silene*		Bare soil
	Rhizosphere	Bulk	Rhizosphere	Bulk	Rhizosphere	Bulk	
Total PLFAs (nmol C g^{-1})	494.95 (42.55)^{a}	244.28 (66.70)^{b}	272.47 (46.38)^{b}	303.90 (28.87)^{b}	237.65 (55.03)^{bc}	229.91 (47.60)^{bc}	88.00 (4.11)^{c}
Bacterial PLFAs (nmol C g^{-1})	325.40 (41.94)^{a}	169.12 (48.39)^{b}	185.07 (35.76)^{b}	201.92 (22.07)^{b}	161.32 (37.78)^{b}	156.37 (33.61)^{b}	53.74 (2.36)^{c}
Gram+ bacteria PLFAs (nmol C g^{-1})	144.11 (15.21)^{a}	62.60 (22.79)^{bc}	69.71 (17.20)^{bc}	84.38 (9.60)^{b}	62.85 (17.42)^{bc}	60.00 (16.50)^{bc}	20.45 (2.13)^{c}
Gram− bacteria PLFAs (nmol C g^{-1})	181.29 (26.99)^{a}	106.52 (25.86)^{b}	115.36 (18.61)^{b}	117.54 (12.67)^{b}	98.47 (20.79)^{b}	96.37 (17.12)^{b}	33.29 (0.58)^{c}
Fungal PLFA (nmol C g^{-1})	15.44 (1.34)^{a}	8.99 (2.09)^{b}	8.57 (1.50)^{bc}	7.961 (1.26)^{bc}	5.15 (0.93)^{cd}	4.37 (0.45)^{d}	2.08 (0.24)^{d}
Fungal/bacterial PLFAs ratio	0.05 (0.00)^{ab}	0.05 (0.00)^{a}	0.05 (0.00)^{abc}	0.04 (0.00)^{bcd}	0.03 (0.00)^{cd}	0.03 (0.00)^{d}	0.04 (0.00)^{bcd}
AMF PLFAs (nmol C g^{-1})	17.15 (2.09)^{a}	7.82 (2.56)^{bc}	9.14 (2.25)^{bc}	13.22 (2.57)^{ab}	8.65 (2.12)^{bc}	8.22 (2.41)^{bc}	2.89 (0.33)^{c}
Protozoa PLFAs (nmol C g^{-1})	0.95 (0.18)^{a}	1.16 (0.24)^{a}	0.88 (0.48)^{a}	0.45 (0.06)^{a}	0.83 (0.23)^{a}	1.19 (0.33)^{a}	1.17 (0.05)^{a}
Actinomycetes PLFAs (nmol C g^{-1})	18.19 (1.44)^{a}	13.23 (2.80)^{ab}	16.53 (3.45)^{a}	12.33 (0.80)^{ab}	17.07 (3.02)^{a}	14.89 (3.26)^{a}	6.48 (0.28)^{b}
Not specific PLFAs (nmol C g^{-1})	121.00 (16.50)^{a}	44.63 (24.44)^{bc}	50.09 (5.92)^{bc}	70.19 (3.79)^{b}	44.63 (12.00)^{bc}	44.87 (8.10)^{bc}	21.65 (1.72)^{c}

et al., 2008; Antonsson et al., 2009; Molenda et al., 2012) and protect invertebrates from climate rigours (Molenda et al., 2012). As it may benefit of external resources because of the ecological function exerted by its canopy, *Silene* probably needs to invest lesser energy in the rhizosphere than *Helianthemum* and *Dryas*.

4.2. Microbial community structure and abundance, and microbial respiration in rhizosphere versus bulk soil within and among the three plant species

Our findings suggested that structure and abundance of the root-associated microbial community, as measured by PLFAs, were mainly driven by the combined effect of plant species and soil fraction. The most marked differences in the microbial community structure between rhizosphere and bulk soil were observed under *Helianthemum*. The large colonization of the *Helianthemum* rhizosphere by saprophytic fungi and AMF could be due to the ability of this plant species to form mycorrhizal association with both ectomycorrhizal fungi and AMF (Cornelissen et al., 2001). Although by the analysis of PLFAs it was not possible to distinguish the ectomycorrhizal by the saprophytic fungi as they are identified by the same PLFA (Karliński et al., 2007), we recognized a diffuse ectomycorrhizal infection in the roots of *Helianthemum* by optical microscope observation (Fig. 6a). The presence of mycorrhizal fungi could be partly responsible of the more abundant bacterial community present in the rhizosphere. Indeed, as reported by Marschner et al. (2005), changes in amount and/or composition of root and fungal exudates due to AMF colonization determine diversity and abundance of the bacterial community in the rhizosphere. The same authors also suggested that the influence of AMF on the bacterial population harbouring the rhizosphere can occur directly through the supply of easily available organic substances due to the growth and degeneration of the hyphal network or, indirectly, through the rhizodeposition stimulated by root and shoot growth. Hence, direct or indirect effects induced by the larger presence of the mycorrhizal fungi, together with rhizodeposition processes and bacterial activity (Buée et al., 2009) could be responsible of the significantly higher WEOC concentration in the rhizosphere than in the bulk soil. We also suggest that the larger amount of labile and hydrophilic organic molecules, partly made of gums and mucilages (Dakora and Phillips, 2002), produced by roots and rhizospheric microorganisms, together with the fine roots and the mycorrhiza hyphal network, fostered the higher AWC in the rhizosphere through the formation of stable aggregates (Goss and Kay, 2005; Fageria and Stone, 2006; Cocco et al., 2013). This would help *Helianthemum* to resist the summer drought that affects these well-drained soils.

The higher C_{mic} in the rhizosphere than in the bulk soil of *Helianthemum* may suggest that this plant species stimulates soil

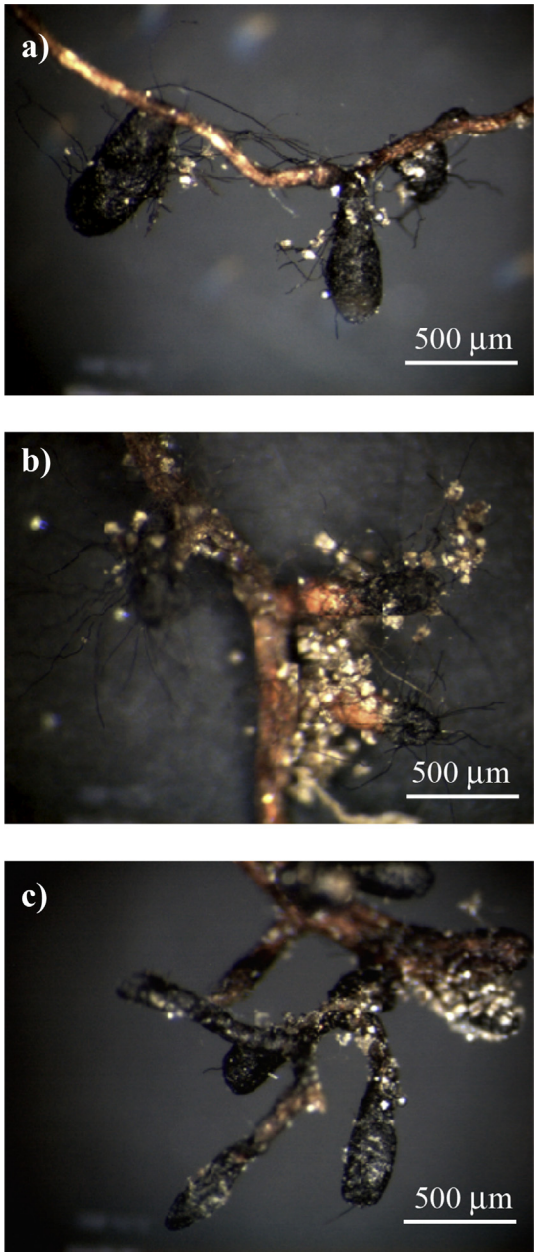

Fig. 6. Optical microscope micrographs showing ectomycorrhizal morphotypes detected in the fine roots of a) *Helianthemum nummularium* subsp. *grandiflorum*, b) *Dryas octopetala*, and c) *Silene acaulis* subsp. *cenisia*. Cannella valley, Majella massif (Italy).

microbes to benefit its own growth. The large extent of microbial biomass C, together with the high C_{mic}/TOC ratio and WEOC, indicated that the rhizosphere of *Helianthemum* was likely not limited by the availability of the energetic substrates and, in particular, of those easily degradable compounds comprising the WEOC. Further, the in parallel high amount of carbon consumed during the basal respiration experiment and the low $\sum CO_2$–C/WEOC ratio suggest a good adaptation of the microbial community to the rhizosphere environment. The low qCO_2 of the rhizosphere confirmed the high substrate-use efficiency of the microbial community (Anderson and Domsch, 1989), which means a prevalence of anabolic over catabolic processes (Chander and Brookes, 1991).

Conversely to *Helianthemum*, *Dryas* showed no significant difference in the microbial community structure between rhizosphere and bulk, as resulted by PLFA analysis. Although it has been reported that some species belonging to the genus *Dryas* may occasionally form symbiotic relationships with actinobacteria of the genus *Frankia* (Eskelinen et al., 2009), no evidence of root symbiosis with N-fixing organisms was detected in the studied *D. octopetala*. Because of this, we suggest that the greater amount of total and organic N found in the rhizosphere than in bulk soil might be due to the presence of a mutualistic association with ectomycorrhizal fungi (Fig. 6b), because they are important N supplier in cold and N-limited environments (Cornelissen et al., 2001; Hobbie and Hobbie, 2006). *Dryas* has been found to be associated in alpine and arctic environments with many different ectomycorrhizal fungi (e.g., Høiland, 1998; Cornelissen et al., 2001; Bjorbækmo et al., 2010), which represent in most cases a high proportion of the rhizospheric fungal community (Taylor, 2002; Bjorbækmo et al., 2010). The dominance of ectomycorrhizal fungi in the rhizosphere has been found to produce a positive feedback between plant growth rate, leaf and litter quality, and decomposition rate (e.g., Berendse, 1994; Cornelissen et al., 1999, 2001; Aerts and Chapin, 2000) as they hasten organic matter cycling. However, the relatively low C_{mic} concentration, and the highest CO_2–C evolved during the basal respiration (which was three-fold higher than that of the bulk, and 64% more than that of the *Helianthemum* rhizosphere), $\sum CO_2$–C/WEOC ratio, and qCO_2 suggested a low efficiency of the microbial community harbouring the *Dryas* rhizosphere in the use of energetic substrates (Chander and Brookes, 1991). The intense organic matter cycling, although with high energy expending, should have further favoured the release and accumulation in the rhizosphere of available nutrient such as Mg and K, other than Ca; the uptake of these cations would have promoted the excretion of protons, so contributing to rhizosphere acidification.

For *Silene*, no difference was detected between rhizosphere and bulk for the abundance of the different microbial groups evaluated by PLFA analysis and for the microbial community structure as indicated by NMDS analysis. However, the higher amount of C_{mic} and respired CO_2–C, and the lower qCO_2 value in the rhizosphere than in the bulk soil indicated that the rhizosphere of *Silene* hosted a relatively well adapted microbial community. As seen for *Helianthemum* and *Dryas*, *Silene* showed a relative abundance of ectomycorrhizal association (Fig. 6c), although it has been reported as a weakly or non-mycorrhizable species (Väre et al., 1992; Derelle

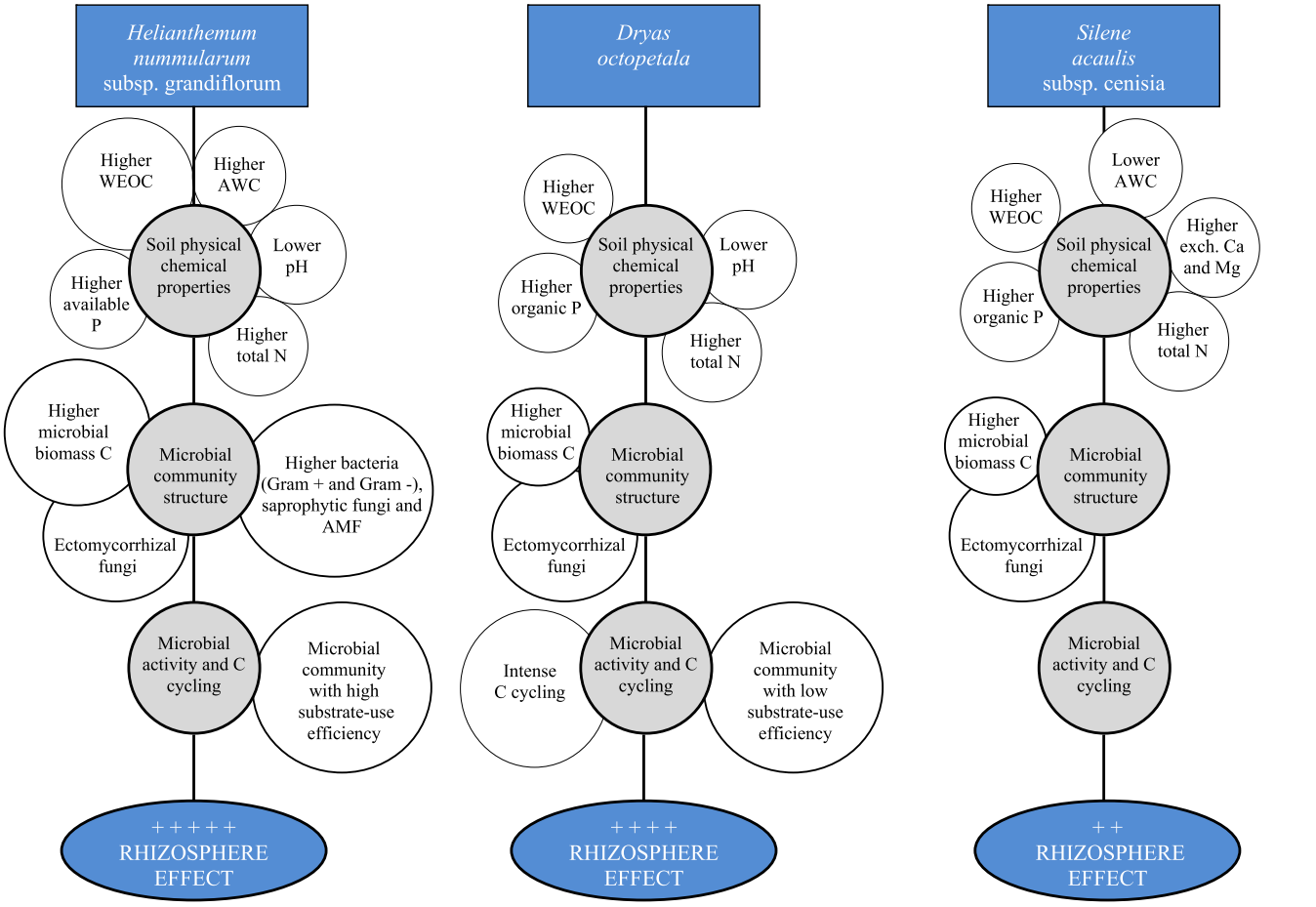

Fig. 7. Schematic representation of the rhizosphere effect induced by *Helianthemum nummularium* subsp. *grandiflorum*, *Dryas octopetala* and *Silene acaulis* subsp. *cenisia*. Cannella valley, Majella massif (Italy). The rhizosphere effect of each species is evaluated by contrasting the properties of the rhizosphere with those of the bulk. The absence of circles means no difference occurring between rhizosphere and bulk, while the dimension of the circle is indicative of the extent of the difference (not in scale).

et al., 2012) that, especially in arctic and alpine environments, is often colonized by dark septate fungi that are characterized by the formation of intracellular microsclerotia (Väre et al., 1992; Treu et al., 1996). The ecological role of dark septate fungi is not clear but some authors reported that they aid alpine plants to uptake P and N (Haselwandter, 1987; Mullen et al., 1998). Although we did not investigate on the presence of dark septate fungi in the *Silene* roots, the higher contents of organic P and total N in the rhizosphere than in the bulk soil might be ascribed to the combined effect of ecto- and/or endomycorrhizal symbioses. Further, the symbiotic association with mycorrhizal fungi may represent for *Silene*, which is characterized by a taproot system, a way to increase considerably the soil volume explored by the roots (Li et al., 1991; Jakobsen et al., 1992; Smith and Read, 1997).

4.3. Conclusions

In this work we evaluated the rhizosphere effect of three plant species that typically colonize poorly developed soils of deglaciated areas under periglacial conditions. The results showed that, even under a hostile climate, the changes in soil physical and chemical properties are mainly driven by the plant species effect, whereas the changes in the structure of root-associated microbial community are driven by the combined effect of plant species and soil fraction (rhizosphere or bulk soil). Indeed, the three plant species considered in this study modified the soil properties and the microbial community structure differently, so to create a soil environment suitable for their needs. In the case of *Helianthemum*, a synergistic effect occurred between the root activity (i.e., exudation processes, root turnover) and rhizosphere microbial community. Conversely, when the root activity does not foster a microbial community structure specifically designed for the rhizosphere, as in the case of *Dryas*, an intense consumption of the energetic resources supplied by the plant occurred to make the nutrients available. However, even though we cannot exclude any minimum effect due to spatial variability, since the *Dryas* plants were younger than the *Helianthemum* ones, it is possible that *Dryas* rhizosphere had still not produced so many differences as the older *Helianthemum*. Conversely to *Helianthemum* and *Dryas*, *Silene* induced a very slight rhizosphere effect notwithstanding its age greater than the other two species, and its ability to colonize harsh environments was likely linked mostly to the shape and functions of its canopy rather than to a functional rhizosphere effect. Fig. 7 schematically resumes the intensity of the rhizosphere effect for the three plant species.

Acknowledgements

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Appendix

Morphological description of the soils under *Helianthemum nummularium* subsp. *grandiflorum*, *Dryas octopetala* and *Silene acaulis* subsp. *cenisia*, and of the bare area. Cannella valley, Majella massif (Italy). For symbols see legend.

Landform: moderately steep (10–12°) – exposure: E–SE – altitude: 2440–2443 m – mean annual air temperature: 2.3 °C – mean annual precipitation: 2100 mm – parent material: thick morainic deposits (till) made of coralline and nummulitic limestone, arenaceous limestone, flintstone.

	Depth (cm)	Colour[a]	Structure[b]	Roots[c]	Boundary[d]	Other observations
Soil under *Helianthemum nummularium* subsp. *grandiflorum* mat: Oxyaquic Haplocryoll, loamy-skeletal, mixed, frigid (SSS, 2010)						
Oi	2–0	–	–	0	aw	Skeleton (by volume): 10%, mainly pebbles; few mesofauna
A1	0–6	7.5YR 2.5/1	2m cr	3mi, vf, f, m, co	cs	Skeleton (by volume): 15%, mainly pebbles
A2	6–19	10YR 2/1	2m sbk	3mi, vf, f, m, co	cs	Skeleton (by volume): 15%; mainly pebbles
C&A	19–29	5YR 2.5/2	2f sbk	3mi, vf, f, m, co	cs	Skeleton (by volume): 75%; silt caps
Bw	29–34	7.5YR 4/4	1f-m abk	2mi, vf, f; 3m, co	cw	Skeleton (by volume): 70%; silt caps
BC	34–67	7.5YR 4/6	1f-m abk	2mi, vf, f, m, co	cw	Skeleton (by volume): 80%; open work; silt caps
C	67–79+	2.5YR 5/6	Fragmental	1mi, vf, f; v_1m, co	–	Skeleton (by volume): 80%; open work
Soil under *Dryas octopetala* mat: Oxyaquic Haplocryoll, loamy-skeletal, mixed, frigid (SSS, 2010)						
Oi	2–0	–	–	0	aw	Skeleton (by volume): 15%, mainly pebbles; few mesofauna
A1	0–5	5YR 2.5/1	2m cr	3mi, vf, f, m, co	cw	Skeleton (by volume): 25%, mainly pebbles
A2	5–15	7.5YR 2.5/1	1m sbk	3mi, vf, f, m, co	cs	Skeleton (by volume): 20%, mainly pebbles
C&A	15–27	7.5YR 3/3	1m sbk	3mi, vf, f, m, co	cs	Skeleton (by volume): 80%
BC	27–66	7.5YR 4/6	1m abk	2mi, vf, f, m, co	cw	Skeleton (by volume): 80%; open work; silt caps
C	66–80+	2.5YR 5/6	Fragmental	2mi, vf; 1f, m, co	–	Skeleton (by volume): 85%; open work; silt caps
Soil at the edge of *Silene acaulis* subsp. *cenisia* cushion: Oxyaquic Haplocryoll, loamy-skeletal, mixed, frigid (SSS, 2010)						
A1	0–7	10YR 2.2	2m sbk	2mi, vf, f; 1m, co	cw	Skeleton (by volume): 40%, mainly pebbles
A2	7–12	7.5YR 2.5/3	1f-m sbk-abk	2mi, vf; 1f, m, co	cw	Skeleton (by volume): 60%, mainly pebbles
C&A	12–21	5YR 3/3	1m sbk	1mi, vf, f, m, co	cs	Skeleton (by volume): 70%; open work
BC	21–63	5YR 5/6	1f abk	$v_1$mi, vf, f; 1m, co	cw	Skeleton (by volume): 85%; open work
C	63–80+	2.5YR 5/6	Fragmental	v_1m, co	–	Skeleton (by volume): 80%; open work
Soil of the bare area: Oxyaquic Cryorthent, loamy-skeletal, mixed, frigid (SSS, 2010)						
C	0–10	5YR 5/4	Fragmental	0	aw	Skeleton (by volume): 50%, half are pebbles
A	10–14	5YR 3/4	1f-m sbk	1mi, vf, f	cw	Skeleton (by volume): 70%; mainly pebbles
C&A	14–20	5YR 4/4	1f-m abk	$v_1$mi, vf, f	cw	Skeleton (by volume): 80%; open work; silt caps
C	20–77+	2.5YR 4/6	Fragmental	0	–	Skeleton (by volume): 80%; open work; silt caps

[a] Moist and crushed, according to the Munsell Soil Color Charts.
[b] 1 = Weak, 2 = moderate, 3 = strong; f = fine, m = medium, c = coarse; cr = crumb, abk = angular blocky, sbk = subangular blocky.
[c] 0 = Absent, v_1 = very few, 1 = few, 2 = plentiful, 3 = abundant; mi = micro, vf = very fine, f = fine, m = medium, co = coarse.
[d] a = Abrupt, c = clear; w = wavy, s = smooth.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2015.07.010.

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