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

Elsevier Editorial System(tm) for Soil Biology and Biochemistry
Manuscript Draft

Manuscript Number: SBB9944R3

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

Article Type: Research Paper

Keywords: High-mountain soils; soil organic C; phospholipid fatty acids; Helianthemum nummularium subsp. grandiflorum; Dryas octopetala; Silene acaulis subsp. cenisia

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Manuscript Region of Origin: ITALY

Università degli Studi di Perugia

DIPARTIMENTO DI SCIENZE AGRARIE, ALIMENTARI E AMBIENTALI

Borgo XX Giugno 72, 06121 Perugia (Italy)

Perugia, June 21st, 2015

The Editor in Chief of Soil Biology and Biochemistry

Dear Prof. Burns,

We submit the revised version of the manuscript (SBB 9944R2) *"Rhizosphere effect of three plant species of environment under periglacial conditions (Majella Massif, central Italy)"* by L. Massaccesi*, G.M.N. Benucci, G. Gigliotti, S. Cocco, G. Corti, A. Agnelli.

The manuscript was carefully revised according to the Reviewer 1's suggestions. Here below, please, find the Revision notes, where is reported how and where the comments have been incorporated in the text .

Sincerely yours

Luisa Massaccesi

Revision notes

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Figure captions: Check 'Figure 3' format.

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Highlights

- Rhizosphere and bulk soil of three plants species from a periglacial environment were sampled
- Different plants produced distinct rhizosphere effects even in extreme ecosystems
- Microbial community was decisive in modifying soil properties
- Among the plants species tested, *Helianthemum* had the strongest rhizosphere effect

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

Massaccesi, L.[1*], Benucci G.M.N.[1], Gigliotti G.[2], Cocco, S.[3], Corti, G.[3], Agnelli, A.[1]

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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.

Keywords: High-mountain soils; soil organic C; phospholipid fatty acids; *Helianthemum nummularium* subsp. *grandiflorum*; *Dryas octopetala*; *Silene acaulis* subsp. *cenisia*

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; Richter at al., 2007). 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 vascular plants strongly modifies the soil properties and the structure and function of the soil microbial community (Yergeau et al., 2007; Taixeira et al., 2010). 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; Jorquera et al., 2014; Ciccazzo et al., 2014). In fact, most arctic and alpine vascular plants allocate 10-30% of net carbon fixation to establish mycorrhyzal associations (Read et al., 2004; Cripps and

79 Eddington, 2005), although allocation patterns of these energetic resources depend on plant species
80 and soil nutrient availability (Högberg et al., 2003; Wardle et al., 2004; Wookey et al., 2009).
81 Hence, different plants colonizing the same soil might differently shape a specific rhizosphere
82 microbial community depending on the quantity and quality of their root exudates (Haichar et al.,
83 2008; Huang et al., 2014).
84 Our research focuses on the rhizosphere effect of three plant species [*Helianthemum nummularium*
85 (L.) Mill. subsp. *grandiflorum* (Scop.) Sch. and Th., *Dryas octopetala* (L.), and *Silene acaulis* (L.)
86 Jacq. subsp. *cenisia* (Vierh.) P. Fourn.] that sparsely occupy soils of deglaciated areas actually
87 submitted to periglacial conditions (central Apennines, Italy). These soils are characterized by
88 environmental constraints such as harsh climatic and nutritional conditions. Specifically, we tested
89 the following hypotheses:
90 (i) the physical and chemical soil properties differ in rhizosphere versus bulk soil for the three plant
91 species;
92 (ii) the microbial community structure and abundance, and microbial respiration differ in
93 rhizosphere versus bulk soil within and among the three plant species.
94 To this aim, we investigated physical, chemical and microbiological properties of both rhizosphere
95 and bulk soil, and the results were compared with those of the adjacent bare soil.
96
97 **2. Materials and methods**
98 *2.1. Site description*
99 The study site is located in one of the highest mountains of central Apennines (Italy), the Majella
100 massif (Figure 1) and, in particular, in the Cannella Valley, whose altitude ranges from 1900 to
101 2750 m, and has a southeast orientation. The mean annual precipitation is about 2100 mm (mostly
102 snow) and the mean annual air temperature is 2.3 °C. January is the coldest month, with an average
103 temperature of -4.3 °C, whereas August is the warmest month, with an average temperature of 11.4
104 °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 *Helianthemum nummularium* (L.) Mill. subsp. *grandiflorum* (Scop.) Sch. and Th., *Dryas octopetala* L., *Silene 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. *Helianthemum 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. *Dryas 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, *Silene 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 x 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 *Silene 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

156 The available water content (AWC) was calculated by difference between the amount of water
157 retained by the soil at 33 kPa and at 1500 kPa, which was determined by pressure plate extractor
158 (Soilmoisture Equipment Corp., Santa Barbara, CA). The soil pH was determined
159 potentiometrically in water (solid:liquid ratio of 1:2.5) after one night of equilibration using a
160 Thermo Scientific™ Orion™ 2-Star Benchtop pH-meter. Potentially plant-available P and organic
161 P was determined by the Olsen method (Olsen et al., 1954) and the ignition method (Kuo, 1996),
162 respectively. To determine the exchangeable Ca, Mg, K and Na, 2 g of each sample were placed
163 into a centrifuge tube, submerged with 0.2 M $BaCl_2$ solution (solid/liquid ratio of 1:10) and shaken
164 for about 10 min (Corti et al., 1997). The mixture was left to rest for a while and then gently shaken
165 for few seconds to re-suspend the sediments and then centrifuged. The extracted solution was
166 filtered through Whatman 42 filter paper, and analysed by atomic absorption with a Shimadzu AA-
167 6300 (Tokyo, Japan) spectrophotometer.
168 Iron was sequentially extracted from the samples with 1) 0.1 M Na-acetate at pH 5 to extract the Fe
169 bound to carbonates (Loeppert and Suarez, 1996), 2) 0.1 M hydroxylamine hydrochloride in 0.01 M
170 HNO_3 to estimate the "labile Fe", namely the Fe forming the easily reducible Fe-Mn oxy-
171 hydroxydes (Berna et al., 2000), 3) NH_4-oxalate/oxalic acid at pH 3.0 in the dark to recover the Fe
172 of the non-crystalline Fe oxy-hydroxydes plus that bound to organic matter (Blakemore et al.,
173 1981), and 4) 0.25 M hydroxylamine hydrochloride in 0.25 M HCl to obtain the Fe of the
174 crystalline Fe oxy-hydroxydes (Berna et al., 2000). The Fe in the extracts was determined by a
175 Shimadzu AA-6300 atomic absorption spectrophotometer.
176 The total organic C content (TOC) and total N were determined by a dry combustion analyser (EA-
177 1110, Carlo Erba Instruments, Milan, Italy). Prior to analysis, the specimens were treated with 0.1
178 M HCl to eliminate inorganic C. Water extractable organic C (WEOC) was obtained according to
179 Agnelli et al. (2014) with the following procedure: 1 g of sample was placed into a plastic
180 container, submerged with distilled water (solid:liquid ratio 1:10) and shaken overnight with an
181 orbital shaker (140 rpm). The suspension was left to rest for a while, centrifuged at 1400 *g* for 10

182 minutes, and then filtered through a 0.45 μm membrane filter. The obtained supernatant solution
183 received a few drops of concentrated H_3PO_4 to eliminate carbonates and was analysed for organic C
184 by a TOC-5000A analyser (Shimadzu Corp., Tokyo, Japan). On the same solution, the amount of
185 water extractable N (WEN), that comprises part of the inorganic N forms (NH_4^+-N and NO_3^--N) and
186 the most soluble organic N forms, was determined by dry combustion analyser.
187 The inorganic N was extracted by treating the samples with 2 M KCl solution (solid:liquid ratio
188 1:10); the suspension was shaken for 1 h with an orbital shaker (140 rpm), and filtered through
189 Whatman 42 filter paper. The different forms of inorganic N (NH_4^+-N and NO_3^--N) were measured
190 on soil extracts by a FOSS Fiastar^{TM} 5000 system (Hillerod, Denmark). The organic N content was
191 obtained by the difference between the total N and inorganic N content.
192
193 *2.4. Microbial biomass C, basal respiration*
194 The amount of microbial biomass-C (C_{mic}) was determined by the fumigation-extraction method
195 (Vance et al., 1987), after 62 days of conditioning at 25°C and at 50% of their total water holding
196 capacity. During this incubation period, basal respiration was measured by alkali (1 M NaOH
197 solution) absorption of the CO_2 developed and titration of the residual OH^- with a standardised HCl
198 solution. Basal respiration was expressed as the cumulative amount of CO_2-C evolved during the
199 experiment.
200
201 *2.5. Soil microbial community structure*
202 The abundance and structure of soil microbial community in the rhizosphere, bulk and bare soil
203 were assessed by analysing the ester-linked phospholipid fatty-acids (PLFAs), which are specific
204 membrane components of living cells and are not found in storage products or in dead cells.
205 Specifically, the technique was used to measure the relative abundance of active fungi and bacteria
206 (Bardgett et al., 1996), which are responsible for 90–95% of total heterotrophic metabolism in most
207 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 arbuscolar 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 analysed 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 (Figure 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 (Figure 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,

259 respectively. The PCA indicated that differences between rhizosphere and bulk soil of the three
260 species occurred, although to a different extent: *Helianthemum* was the species with the greatest
261 differences between rhizosphere and bulk soil, followed by *Dryas* and, then, *Silene*. Further, PCA
262 showed that bulk soil and rhizosphere of *Silene* and the bulk soil of *Dryas* were closer and more
263 similar to the bare soil than the others.
264 PCA-axis 1 appeared to be positively driven by microbial community, total N, TOC content and
265 exchangeable Ca and Mg, whereas it was negatively driven by pH and NO_3^--N (Figure Ia of the
266 Supplementary Data). PCA-axis 2 was mainly associated with positive relationships to CO_2-
267 C/WEOC ratio and qCO_2, and with negative relationships to C_{mic}/TOC ratio, available P and C_{mic}
268 contents (Figure Ib of the Supplementary Data). The PCA scores of the *Helianthemum* rhizosphere
269 were placed on the right side of PCA-axis 1, indicating a strong positive relationship with the soil
270 properties driving axis 1. Conversely, the bare soil and the bulk soil of *Silene* were the most
271 negatively associated with axis 1. A relationship with the soil properties that positively drove axis 2
272 was indicated for the rhizosphere of *Dryas* and the bulk soil of *Helianthemum*.
273
274 *3.2. Available water content (AWC), pH, and available and organic P*
275 The *Helianthemum* rhizosphere had an AWC higher than that of the bulk soil (Table 1), while
276 rhizosphere and bulk soil of *Dryas* showed similar values. Conversely, *Silene* rhizosphere had an
277 AWC lower than that of the bulk soil, analogous to that of bare soil.
278 The pH values of the rhizosphere of *Helianthemum* and *Dryas* were lower than those of the
279 respective bulk and bare soil (Table 1), while *Silene* showed no difference among rhizosphere, bulk
280 and bare soil. Only for *Helianthemum*, the available P content showed a significant difference
281 between rhizosphere and bulk soil, with the lowest value in the latter (Table 1). *Dryas* and *Silene*
282 exhibited a higher amount of organic P in the rhizosphere than in the bulk and bare soil, while under
283 *Helianthemum* organic P was similar in both soil fractions.
284

285 *3.3. Exchangeable cations and extractable forms of Fe*

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

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

295

296 *3.4. Nitrogen, total and water soluble organic C, microbial biomass C and basal respiration.*

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

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

307 The microbial biomass C (C_{mic}) had a greater concentration in the rhizosphere than in the bulk soil
308 of the three plants (Table 4), with the highest value in the rhizosphere of *Helianthemum* (more than
309 7 fold-higher than the respective bulk soil). Surprisingly, the C_{mic} content of the bare soil was
310 similar to that of the rhizosphere and higher than the bulk soil of *Dryas*. The CO_2 evolved during

311 the basal respiration experiment (ΣCO_2-C) was higher in the rhizosphere than in the bulk soil for the
312 three plants, with the highest values recorded in the rhizosphere of *Dryas*. The bare soil and the
313 bulk soil of *Silene* showed the lowest amount of ΣCO_2-C. The rhizosphere of *Helianthemum*
314 showed the largest percentage of C_{mic}/TOC (Figure 4a) and the lowest percentage of organic C
315 consumed during the incubation experiment with respect to WEOC (ΣCO_2-C/WEOC) (Figure 4b).
316 For *Dryas*, the percentage of C_{mic}/TOC was similar in both rhizosphere and bulk soil, but the
317 rhizosphere showed the largest ΣCO_2-C/WEOC percentage and qCO_2 value.
318
319 *3.5. Microbial community abundance and structure*
320 Interactions between plant species and soil fractions (PERMANOVA, $F_{2,20}$=3.35, R^2=0.246,
321 P=0.0158*) affected microbial community structure as expressed by the relative abundance of all
322 identified PLFA peaks. NMDS plot indicated that the greater diversity between rhizosphere and
323 bulk soil in the microbial community structure occurred for *Helianthemum* (Figure 5a), and that the
324 synergistic effect of plant species and soil fractions appeared mostly due to bacteria. In fact, as
325 shown in Figure 5b, axis 1 was mainly driven by the relative abundance of i15:0 and a15:0 fatty
326 acids, which represent Gram-positive bacteria, and 10Me18:0, which represents actinomycetes. In
327 contrast, axis 2 was driven by the relative abundance of 17:1ω9c fatty acid, which represents Gram-
328 negative bacteria.
329 In all the samples, although the non-specific PLFA were 18-25% of the total, the most represented
330 microbial group identified was that of bacteria, which ranged from 61% (bare soil) to 69% (bulk
331 soil of *Helianthemum*) of the entire microbial community (Table 5). In order of abundance, bacteria
332 were followed by actinomycetes, AMF, saprophytic fungi and protozoa. Among the bacteria, Gram-
333 negative were the most abundant. Both bulk soil and rhizosphere of the three plants had a greater
334 amount of bacteria than the bare soil, whereas *Helianthemum* and *Dryas* had both fractions richer in
335 fungi than bare soil. Bacteria (Gram-positive and Gram-negative), saprophytic fungi and AMF were
336 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.

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

363 significantly increase P availability by promoting the hydrolysis of organic P in forms more
364 accessible to the plant (Gerke et al., 2000, Wouterlood et al., 2005).
365 In the case of *Dryas*, the rhizosphere effect was exhibited by the lower pH and the greater amount
366 of organic P, exchangeable K, Mg and Na, crystalline Fe oxy-hydroxydes, total and organic N,
367 WEOC, C_{mic} and CO_2-C evolved during the basal respiration in the root-affected soil than in the
368 bulk. The higher WEOC content in the rhizosphere than in the bulk soil was mainly attributed to
369 exudation of labile C compounds such as carbohydrates, aminoacids, aliphatic or aromatic organic
370 acids, phenols, and fatty acids (Colin-Belgrand et al., 2003; Farrar et al., 2003), but also to an
371 enhanced organic matter cycling occurring in the rhizosphere (Dijkstra et al., 2013), This C input
372 into the rhizosphere represents an investment made by the plant to modify soil conditions and
373 establish an appropriate environment for its development (Boddy et al., 2008).
374 The few differences between rhizosphere and bulk soil of *Silene* indicated that this species modifies
375 the soil properties less than *Helianthemum* and *Dryas.* Interestingly, the estimated age of the *Silene*
376 plants was greater than that of the other two species. *Silene* has apparently a lower rhizosphere
377 effect than *Helianthemum* and *Dryas*, but is one of the best adapted plant to alpine environment, as
378 it is able to colonize bare or recently deglaciated soils (e.g., Pysek and Liska, 1991; Chapin and
379 Körner, 1995; Körner, 2003). This ability is mainly attributed to the domed shape of the canopy that
380 mitigates temperature, stores moisture, and increases the quantity of nutrients underneath the
381 cushion (Körner, 2003; Reid et al., 2010 and references herein). Indeed, in arctic and alpine
382 environments, cushion plants as *Silene* are considered as nurse-plants that are able to facilitate the
383 settlement of less tolerant plant species (Broker et al., 2009; Antonsson et al., 2009; Molenda et al.,
384 2012) and protect invertebrates from climate rigors (Molenda et al., 2012). As it may benefit of
385 external resources because of the ecological function exerted by its canopy, *Silene* probably needs
386 to invest lesser energy in the rhizosphere than *Helianthemum* and *Dryas*.
387

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., 2006), we recognized a diffuse ectomycorrhizal infection in the roots of *Helianthemum* by optical microscope observation (Figure 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;

413 Fageria and Stone, 2006; Cocco et al., 2013). This would help *Helianthemum* to resist the summer
414 drought that affects these well-drained soils.
415 The higher C_{mic} in the rhizosphere than in the bulk soil of *Helianthemum* may suggest that this plant
416 species stimulates soil microbes to benefit its own growth. The large extent of microbial biomass C,
417 together with the high C_{mic}/TOC ratio and WEOC, indicated that the rhizosphere of *Helianthemum*
418 was likely not limited by the availability of the energetic substrates and, in particular, of those
419 easily degradable compounds comprising the WEOC. Further, the in parallel high amount of carbon
420 consumed during the basal respiration experiment and the low ΣCO_2-C/WEOC ratio suggest a good
421 adaptation of the microbial community to the rhizosphere environment. The low qCO_2 of the
422 rhizosphere confirmed the high substrate-use efficiency of the microbial community (Anderson and
423 Domsch, 1989), which means a prevalence of anabolic over catabolic processes (Chander and
424 Brookes, 1991).
425 Conversely to *Helianthemum*, *Dryas* showed no significant difference in the microbial community
426 structure between rhizosphere and bulk, as resulted by PLFA analysis. Although it has been
427 reported that some species belonging to the genus *Dryas* may occasionally form symbiotic
428 relationships with actinobacteria of the genus *Frankia* (Eskelinen et al., 2009), no evidence of root
429 symbiosis with N-fixing organisms was detected in the studied *Dryas octopetala*. Because of this,
430 we suggest that the greater amount of total and organic N found in the rhizosphere than in bulk soil
431 might be due to the presence of a mutualistic association with ectomycorrhizal fungi (Figure 6b),
432 because they are important N supplier in cold and N-limited environments (Cornelissen et al., 2001;
433 Hobbie and Hobbie, 2006). *Dryas* has been found to be associated in alpine and arctic environments
434 with many different ectomycorrhizal fungi (e.g., Høiland, 1998; Cornelissen et al., 2001;
435 Bjorbækmo et al., 2010), which represent in most cases a high proportion of the rhizospheric fungal
436 community (Taylor, 2002; Bjorbækmo et al., 2010). The dominance of ectomycorrhizal fungi in the
437 rhizosphere has been found to produce a positive feedback between plant growth rate, leaf and litter
438 quality, and decomposition rate (e.g., Berendse, 1994; Cornelissen et al., 1999, 2001; Aerts and

439 Chapin, 2000) as they hasten organic matter cycling. However, the relatively low C_{mic}
440 concentration, and the highest CO_2-C evolved during the basal respiration (which was three-fold
441 higher than that of the bulk, and 64% more than that of the *Helianthemum* rhizosphere), ΣCO_2-
442 C/WEOC ratio, and qCO_2 suggested a low efficiency of the microbial community harbouring the
443 *Dryas* rhizosphere in the use of energetic substrates (Chander and Brookes, 1991). The intense
444 organic matter cycling, although with high energy expending, should have further favoured the
445 release and accumulation in the rhizosphere of available nutrient such as Mg and K, other than Ca;
446 the uptake of these cations would have promoted the excretion of protons, so contributing to
447 rhizosphere acidification.
448 For *Silene*, no difference was detected between rhizosphere and bulk for the abundance of the
449 different microbial groups evaluated by PLFA analysis and for the microbial community structure
450 as indicated by NMDS analysis. However, the higher amount of C_{mic} and respired CO_2-C, and the
451 lower qCO_2 value in the rhizosphere than in the bulk soil indicated that the rhizosphere of *Silene*
452 hosted a relatively well adapted microbial community. As seen for *Helianthemum* and *Dryas*, *Silene*
453 showed a relative abundance of ectomycorrhizal association (Figure 6c), although it has been
454 reported as a weakly or non-mycorrhizable species (Väre et al., 1992; Derelle et al., 2012) that,
455 especially in arctic and alpine environments, is often colonized by dark septate fungi that are
456 characterized by the formation of intracellular microsclerotia (Väre et al., 1992; Treu et al., 1996).
457 The ecological role of dark septate fungi is not clear but some authors reported that they aid alpine
458 plants to uptake P and N (Haselwandter et al., 1987; Mullen et al., 1998). Although we did not
459 investigate on the presence of dark septate fungi in the *Silene* roots, the higher contents of organic P
460 and total N in the rhizosphere than in the bulk soil might be ascribed to the combined effect of ecto-
461 and/or endomycorrhizal symbioses. Further, the symbiotic association with mycorrhizal fungi may
462 represent for *Silene*, which is characterized by a taproot system, a way to increase considerably the
463 soil volume explored by the roots (Li et al., 1991; Jakobsen et al., 1992; Smith and Read, 1997).
464

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. Figure 7 schematically resumes the intensity of the rhizosphere effect for the three plant species.

Acknowledgments

This research benefited of funds from the Majella National Park. The authors are indebted with Luca Calamai (CISM–UNIFI) for his help in the PLFA analysis.

References

491 Aerts, R., Chapin, F.S. III, 2000. The mineral nutrition of wild plants revisited: a re-evaluation of
492 processes and patterns. Advances in Ecological Research 30, 1–67.
493 Agnelli, A., Bol, R., Trumbore, S.E., Dixon, L., Cocco, S., Corti, G., 2014. Carbon and nitrogen in
494 soil and vine roots in harrowed and grass-covered vineyards. Agriculture, Ecosystems and
495 Environment 193, 70–82.
496 Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance.
497 Australian Journal of Ecology 26, 32-46.
498 Anderson, T-H., Domsch, K.H., 1989. Ratios of microbial biomass carbon to total organic carbon in
499 arable soils. Soil Biology and Biochemistry 21, 471–479.
500 Antonsson, H., Björk, R.G., Molau, U., 2009. Nurse plant effect of the cushion plant *Silene acaulis*
501 (L.) Jacq. in an alpine environment in the subarctic Scandes, Sweden. Plant Ecology and
502 Diversity 2, 17–25.
503 Bardgett, R.D., Hobbs, P.J., Frostegård, Å., 1996. Changes in soil fungal:bacterial biomass
504 following reductions in the intensity of management of an upland grassland. Biology and
505 Fertility of Soil 22, 261–264.
506 Benedict, J.B., 1989. Use of *Silene acaulis* for dating: the relationship of cushion diameter to age.
507 Arctic and Alpine Research 21, 91–96.
508 Berendse, F., 1994. Litter decomposability – a neglected component of plant fitness. Journal of
509 Ecology 82, 87–190.
510 Berna, F., Corti, G., Ugolini, F.C., Agnelli, A., 2000. Assessment of the role of rock fragments in
511 the retention of cadmium and lead in irrigated arid stony soils. Annali di Chimica 90, 209-217.
512 Beschel, R.E., 1958. Ricerche lichenometriche sulle morene del gruppo del Gran Paradiso. Nuovo
513 Giornale Botanico Italiano 65, 538–591.
514 Bjorbækmo, M.F.M., Carlsen, T., Brysting, A., Vrålstad, T., Hiland, K., Ugland, K.I., Geml, J.,
515 Schumacher, T., Kauserud, H., 2010. High diversity of root associated fungi in both alpine and
516 arctic *Dryas octopetala*. BMC Plant Biology 10, 244-256.

517 Blakemore, L.C., Searle, P.L., Daly, B.K., 1981. Methods for chemical analysis of soils. New
518 Zealand Soil Bureau Scientific, Report 10a. Department Scientific and Industrial Research, New
519 Zealand.
520 Blaschke, H., 1991. Multiple mycorrhizal associations of individual calcicole host plants in the
521 alpine grass-heath zone. Mycorrhiza 1, 31–34.
522 Boddy, E., Roberts, P., Hill, P.W., Farrar, J., Jones, D.L., 2008. Turnover of low molecular weight
523 dissolved organic C (DOC) and microbial C exhibit different temperature sensitivities in Arctic
524 tundra soils. Soil Biology and Biochemistry 40, 1557-1566.
525 Brooker, R.W., Maestre, F.T., Callaway, R.M., Lortie, C.L., Cavieres, L.A., Kunstler, G.,
526 Liancourt, P., Tielbörger, K., Travis, J.M.J., Anthelme, F., Armas, C., Coll, K., Corcket, E.,
527 Delzon, S., Forey, E., Kikvidze, Z., Olofsson, J., Pugnaire, F., Quiroz, C.L., Saccone, P.,
528 Schiffers, K., Seifan, M., Touzard, B., Michalet, R., 2008. Facilitation in plant communities: the
529 past, the present, and the future. Journal of Ecology 96, 8−34.
530 Buée, M., De Boer, W., Martin, F., van Overbeek, L., Jurkevitch, E., 2009. The rhizosphere zoo:
531 An overview of plant-associated communities of microorganisms, including phages, bacteria,
532 archaea, and fungi, and of some of their structuring factors. Plant and Soil 231, 189-212.
533 Chander, K., Brookes, P.C., 1991. Effects of heavy metals from past applications of sewage sludge
534 on microbial biomass and organic matter accumulation in a sandy loam and silty loam U.K. soil.
535 Soil Biology and Biochemistry 23, 927–932.
536 Chapin, F.S., Körner, C.H., 1995. Patterns, causes, changes, and consequences of biodiversity in
537 arctic and alpine ecosystems. In: Chapin, F.S., Körner, C.H. (Eds.), Arctic and Alpine
538 Biodiversity. Springer-Verlag Berlin Heidelberg, pp. 313-320.
539 Chung, H.G., Zak, D.R., Reich, P.B., Ellsworth, D.S., 2007. Plant species richness, elevated CO_2,
540 and atmospheric nitrogen deposition alter soil microbial community composition and function.
541 Global Change Biology 13, 980 – 989.

542 Ciccazzo, S., Esposito, A., Rolli, E., Zerbe, S., Daffonchio, D., Brusetti, L., 2014. Different pioneer
543 plant species select specific rhizosphere bacterial communities in a high mountain environment.
544 SpringerPlus 3, 391.
545 Cocco, S., Agnelli, A., Gobran, G., Corti, G., 2013. Modifications induced by the roots of *Erica*
546 *arborea* L. to create a suitable environment in soils developed from alkaline and fine-textured
547 marine sediments. Plant and Soil 368, 297–313.
548 Colin-Belgrand, M., Dambrine, E., Bienaimé, S., Nys, C., Turpault, M.P., 2003. Influence of tree
549 roots on nitrogen mineralization. Scandinavian Journal of Forest Research 18, 260–268.
550 Cornelissen, J.H.C., Aerts, R., Cerabolini, B., Werger, M.J.A., van der Heijden, M.G.A., 2001.
551 Carbon cycling traits of plant species are linked with mycorrhizal strategy. Oecologia 129, 611–
552 619.
553 Cornelissen, J.H.C., Pérez-Harguindeguy, N., Díaz, S., Grime, J.P., Marzano, B., Cabido, M.,
554 Vendramini, F., Cerabolini, B., 1999. Leaf structure and defence control litter decomposition
555 rate across species and life forms in regional floras on two continents. New Phytologist 143,
556 191–200.
557 Corti, G., Agnelli, A., Cuniglio, R., Fernández Sanjurjo, M., Cocco, S., 2005. Characteristics of
558 rhizosphere soil from natural and agricultural environments. In: Huang, P.M., Gobran, G.R.
559 (Eds.), Biogeochemistry of the Trace Elements in the Rhizosphere. Elsevier Science,
560 Amsterdam, pp. 57–128.
561 Corti, G., Agnelli, A., Ugolini, F.C., 1997. Release of Al by hydroxy-interlayered vermiculite and
562 hydroxy-interlayered smectite during determination of cation exchange capacity in fine earth and
563 rock fragments fractions. European Journal of Soil Science 48, 249–262.
564 Corti, G., Cocco, S., Basili, M., Cioci, C., Warburton, J., Agnelli, A., 2012. Soil formation in kettle
565 holes from high altitudes in central Apennines, Italy. Geoderma 170, 280–294.

566 Cripps, C.L., Eddington, L.H., 2005. Distribution of mycorrhizal types among alpine vascular plant
567 families on the Beartooth Plateau, Rocky Mountains, USA, in reference to large-scale patterns in
568 arctic-alpine habitats. Arctic, Antarctic, and Alpine Research 37, 177–188.
569 Dakora, F.D., Phillips, D.A., 2002. Root exudates as mediators of mineral acquisition in low-
570 nutrient environments. Plant and Soil 245, 35–47.
571 De Deyn, G., Quirk, H., Bardgett, R., 2011. Plant species richness, identity and productivity
572 differentially influence key groups of microbes in grassland soils of contrasting fertility. Biology
573 Letters 7, 75–78.
574 Derelle, D., Declerck, S., Genet, P., Dajoz, I., van Aarle, I.M., 2012. Association of highly and
575 weakly mycorrhizal seedlings can promote the extra- and intraradical development of a common
576 mycorrhizal network. FEMS Microbiology Ecology 79, 251–259.
577 Dijkstra, F.A., Carrillo, Y., Pendall, E., Morgan, J.A., 2013. Rhizosphere priming: a nutrient
578 perspective. Frontiers in Microbiology 4, 216.
579 Edwards, I.P., Bürgmann, H., Miniaci, C., Zeyer, J., 2006. Variation in microbial community
580 composition and culturability in the rhizosphere of *Leucanthemopsis alpina* (L.) Heywood and
581 adjacent bare soil along an alpine chronosequence. Microbial Ecology 52, 679–692.
582 Eskelinen, A., Stark, S., Männistö, M., 2009. Links between plant community composition, soil
583 organic matter quality and microbial communities in contrasting tundra habitats. Oecologia 161,
584 113-123.
585 Fageria, N.K., Stone, L.F., 2006. Physical, chemical, and biological changes in the rhizosphere and
586 nutrient availability. Journal of Plant Nutrition 29, 1327–1356.
587 Farrar, J., Hawes, M., Jones, D., Lindow, S., 2003. How roots control the flux of carbon to the
588 rhizosphere. Ecology 84, 827–837.
589 Federle, T.W., 1986. Microbial distribution in soil—new techniques. In: Megusar, F., Gantar, M.
590 (Eds.), Perspectives in Microbial Ecology. Slovene Society for Microbiology, Ljubljana, pp.
591 493–498.

592 Fernández Sanjurjo, M.J., Corti, G., Certini, G., Ugolini, F.C., 2003. Pedogenesis induced by
593 *Genista aetnensis* (Biv.) DC. on basaltic pyroclastic deposits at different altitudes, Mt. Etna,
594 Italy. Geoderma 115, 223-243.
595 Fierer, N., Schimel, J.P., Holden, P.A., 2003. Variation in microbial community composition
596 through two soil depth profiles. Soil Biology and Biochemistry 35, 167–176.
597 Finzi, A.C., Abramoff, R.Z., Spiller, K.S., Brzosteck, E.R., Darby, B.A., Kramer, M.A., Phillips,
598 R.P., 2015. Rhizosphere processes are quantitatively important components of terrestrial carbon
599 and nutrient cycles. Global Change Biology 21, 2082–2094.
600 Fritze, H., Pietikainen, J., Pennanen, T., 2000. Distribution of microbial biomass and phospholipid
601 fatty acids in Podzol profiles under coniferous forest. European Journal of Soil Science 51, 565–
602 573.
603 Frostegård, Å., Bååth, E., Tunlid, A., 1993. Shifts in the structure of soil microbial communities in
604 limed forests as revealed by phospholipid fatty acid analysis. Soil Biology and Biochemistry 25,
605 723–730.
606 Gerke, J., Beissner, L., Römer, W., 2000. The quantitative effect of chemical phosphate
607 mobilization by carboxylate anions on P uptake by a single root. II. The importance of soil and
608 plant parameters for uptake of mobilized P. Journal of Plant Nutrition and Soil Science 163,
609 213–219.
610 Giraudi, C., 2004. The Apennine glaciations in Italy. In: Ehlers, J., Gibbard, P.L. (Eds.), Quaternary
611 Glaciations – Extent and Chronology. Part I: Europe. Development in Quaternary Science 2,
612 Series Editor: J. Rose. Elsevier, Amsterdam, pp. 215-223.
613 Goss, M.J., Kay, B.D., 2005. Soil aggregation. In: Zobel, R.W., Wright, S.F. (Eds.), Roots and Soil
614 Management: Interactions Between Roots and the Soil. ASA, CSSA, and SSSA, Madison, WI,
615 pp. 163–180.

Haichar, F.Z., Marol, C., Berge, O., Rangel-Castro, J.I., Prosser, J.I., Balesdent, J., Heulin, T., Achouak, W., 2008. Plant host habitat and root exudates shape soil bacterial community structure. The ISME Journal 2, 1221–1230.

Haselwandter, K., 1987. Mycorrhizal infection and its possible ecological significance in climatically and nutritionally stressed alpine plant communities. Angewandte Botanik 61, 107–114.

Haynes, R.J., 1990. Active ion uptake and maintenance of cation-anion balance: a critical examination of their role in regulating rhizosphere pH. Plant and Soil 126, 247–264.

Hinsinger, P., Gobran, G.R., Gregory, P.J., Wenzel, W.W., 2005. Rhizosphere geometry and heterogeneity arising from root-mediated physical and chemical processes. New Phytologist 168, 293–303.

Hinsinger, P., Plassard, C., Tang, C., Jaillard, B., 2003. Origins of root-induced pH changes in the rhizosphere and their responses to environmental constraints: a review. Plant and Soil 248, 43–59.

Hobbie, J.E., Hobbie, E.A., 2006. ^{15}N in symbiotic fungi and plants estimates nitrogen and carbon flux rates in arctic tundra. Ecology 87, 816-822.

Högberg, M.N., Bååth, E., Nordgren, A., Arnebrant, K., Högberg, P., 2003. Contrasting effects of nitrogen availability on plant carbon supply to mycorrhizal fungi and saprotrophs - a hypothesis based on field observations in boreal forest. New Phytologist, 160, 225-238.

Høiland, K., 1998. Studies of ectomycorrhiza on Svalbard. Agarica 15, 133-147.

Huang, X.F., Chaparro, J.M., Reardon, K.F., Zhang, R., Shen, Q., Vivanco, J.M., 2014. Rhizosphere interactions: root exudates, microbes, and microbial communities. Botany 92, 267–275.

Jackson, R.B., Canadell, J., Ehleringer, J.R., Mooney, H.A., Sala, O.E., Schulze, E.D., 1996. A global analysis of root distributions for terrestrial biomes. Oecologia 108, 389- 411.

641 Jakobsen, I., Abbott, L.K., Robson, A.D., 1992. External hyphae of vesicular-arbuscular
642 mycorrhizal fungi associated with *Trifolium subterraneum* L.1. Spread of hyphae and
643 phosphorus inflow into roots. New Phytologist 120, 371–380.
644 Jorquera, M.A., Martínez, O.A., Marileo, L.G., Acuña, J.J., Saggar, S., Mora, M.L., 2014. Effect of
645 nitrogen and phosphorus fertilization on the composition of rhizobacterial communities of two
646 Chilean Andisol pastures. World Journal of Microbial Biotechnology 30, 99–107.
647 Karliński, N., Ravnskov, S., Kieliszewska-Rokicka, B., Larsen, J., 2007. Fatty acid composition of
648 various ectomycorrhizal fungi and ectomycorrhizas of Norway spruce. Soil Biology and
649 Biochemistry 39, 854–866.
650 Körner, C., 2003. Alpine Plant Life, 2nd edition. Springer, Berlin.
651 Kroppenstedt, R.M., 1985. Fatty acid and menaquinone analysis of actinomycetes and related
652 organisms. In: Goodfellow, M., Minnikin, D.E. (Eds.), Chemical Methods in Bacterial
653 Systematics. Society for Applied Bacteriology (Technical Series no. 20), Academic Press,
654 London, pp. 173–199.
655 Kuo, S., 1996. Phosphorus. In: Sparks, D.L. (Ed.), Methods of Soil Analysis, Part 3. Chemical
656 Methods. SSSA and ASA, Madison, WI, pp. 869–919.
657 Kuzyakov, Y., 2002. Review: Factors affecting rhizosphere priming effects. Journal of Plant
658 Nutrition and Soil Science 165, 382–396.
659 Larcher, W., Kainmuller, C., Wagner, J., 2010. Survival types of high mountain plants under
660 extreme temperatures. Flora 205, 3–18.
661 Li, X-L., George, E., Marschner, H., 1991. Extension of the phosphorus depletion zone in VA-
662 mycorrhizal white clover in a calcareous soil. Plant and Soil 136, 41-48.
663 Loeppert, R.H., Suarez, D.L., 1996. Carbonate and Gypsum. In: Sparks, D.L. (Ed.), Methods of Soil
664 Analysis, Part 3. Chemical Methods. SSSA and ASA, Madison, WI, pp. 437-474.
665 Marschner, P., Solaiman, Z., Rengel, Z., 2005. Rhizosphere properties of *Poaceae* genotypes under
666 P-limiting conditions. Plant and Soil 283, 11-24.

667 Massaccesi, L., Agnelli, A., Bardgett, R.D., Ostle, N., Wilby, A., Orwin, K.H., 2015. Impact of
668 plant species evenness, dominant species identity and spatial arrangement on the structure and
669 functioning of soil microbial communities in a model grassland. Oecologia 177, 747–759.
670 McCarthy, D.P., 1992. Dating with cushion plants: establishment of a *Silene acaulis* growth curve
671 in the Canadian Rockies. Arctic and Alpine Research 24, 50–55.
672 Molenda, O., Reid, A., Lortie. C.J., 2012. The alpine cushion plant *Silene acaulis* as foundation
673 species: a bug's-eye view to facilitation and microclimate. PLoS ONE 7, e37223.
674 Mullen, R.B., Schmidt, S.K., Jaeger, C.H., 1998. Nitrogen uptake during snowmelt by the snow
675 buttercup, *Ranunculus adoneus*. Arctic and Alpine Research 30, 121-125.
676 Olsen, S.R., Cole, C.V., Watanabe, F.S., Dean, L.A., 1954. Estimation of available phosphorus in
677 soils by extraction with sodium bicarbonate. US Dept. Agric. Circ. 939, Washington.
678 Olsson, P.A., 1999. Signature fatty acids provide tools for determination of the distribution and
679 interactions of mycorrhizal fungi in soil. FEMS Microbiology Ecology 29, 303–310.
680 Petersen, H., Luxton, M., 1982. A comparative-analysis of soil fauna populations and their role in
681 decomposition processes. Oikos 39, 287-388.
682 Pysek, P., Liska, J., 1991. Colonisation of *Sibbaldia tetrandra* cushions on alpine scree in the
683 Pamiro-Alai Mountains, central Asia. Arctic and Alpine Research 23, 263-272.
684 R Core Team, 2014. R: a language and environment for statistical computing. R Foundation for
685 Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.
686 Read, D.J., Leake, J.R., Perez-Moreno, J., 2004. Mycorrhizal fungi as drivers of ecosystem
687 processes in heathland and boreal forest biomes. Canadian Journal of Botany 82, 1243-1263.
688 Reid, A.M., Lamarque, L.J., Lortie, C.J., 2010. A systematic review of the recent ecological
689 literature on cushion plants: champions of plant facilitation. Web Ecology 10, 44–49.
690 Rengel, Z., Romheld, V., 2000. Root exudation and Fe uptake and transport in wheat genotypes
691 differing in tolerance to Zn deficiency. Plant and Soil 222, 25–34.

692 Richter, D.B., Oh, N-H., Fimmen, R., Jackson, J., 2007. The rhizosphere and soil formation. In:
693 Cardon, Z.G., Whitbeck, J.L. (Eds.), The Rhizosphere: an Ecological Perspective. Elsevier,
694 Burlington, pp. 179–200.
695 Sandnes, A., Eldhuset, T., Wollebæk, G., 2005. Organic acids in root exudates and soil solution of
696 Norway spruce and silver birch. Soil Biology and Biochemistry 37, 259 – 269.
697 Schoeneberger, P.J., Wysoki, D.A., Benham, E.C., Broderson, W.D., 1998. Field book for
698 describing and sampling soils. Natural Resources Conservation Service, USDA, National Soil
699 Survey Center, Lincoln.
700 Schwintzer, C.R., Tjepkema, J.D., 1990. The Biology of *Frankia* and Actinorhizal Plants.
701 Academic Press, San Diego, CA.
702 Smith, S.E., Read, D.J., 1997. Mycorrhizal symbiosis, 2nd edition. Academic Press, San Diego, CA.
703 SSS (Soil Survey Staff). 2010. Keys to soil taxonomy, 11th edn. United States Department of
704 Agriculture and Natural Resources Conservation Service, Washington, DC.
705 Taylor, A.F.S., 2002. Fungal diversity in ectomycorrhizal communities: sampling effort and species
706 detection. Plant and Soil 244, 19–28.
707 Teixeira, L.C.R.S, Peixoto, R.S., Cury, J.C., Sul, W.J., Pellizari, V.H., Tiedje, J., Rosado, A.S.,
708 2010. Bacterial diversity in rhizosphere soil from Antarctic vascular plants of Admiralty Bay,
709 maritime Antarctica. The ISME Journal 4, 989–1001.
710 Treu, R., Laursen, G.A., Stephenson, S.L., Landolt, J.C., Densmore, R., 1996. Mycorrhizae from
711 Denali National Park and Preserve, Alaska. Mycorrhiza 6, 21–29.
712 Tscherko, D., Hammesfahr, U., Marx, M.C., Kandeler, E., 2004. Shifts in rhizosphere microbial
713 communities and enzyme activity of *Poa alpina* across an alpine chronosequence. Soil Biology
714 and Biochemistry 36, 1685–1698.
715 Tscherko, D., Hammesfahr, U., Zeltner, G., Kandeler, E., Böcker, R., 2005. Plant succession and
716 rhizosphere microbial communities in a recently deglaciated alpine terrain. Basic and Applied
717 Ecology 6, 367–383.

718 Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring microbial
719 biomass C. Soil Biology and Biochemistry 19, 703–707.
720 Väre, H., Vestberg, M., Eurola, S., 1992. Mycorrhiza and root-associated fungi in Spitsbergen.
721 Mycorrhiza 1, 93–104.
722 Wardle, D.A., Bardgett, R.D., Klironomos, J.N., Setälä, H., van der Putten, W.H., Wall, D.H., 2004.
723 Ecological linkages between aboveground and belowground biota. Science 304, 1629–1633.
724 Wookey, P.A., Aerts, R., Bardgett, R.D., Baptist, F., Bråthen, K.A., Cornelissen, J.H.C., Gough, L.,
725 Hartley, I.P., Hopkins, D.W., Lavorel, S., Shaver, G.R., 2009. Ecosystem feedbacks and cascade
726 processes: understanding their role in the responses of arctic and alpine ecosystems to
727 environmental change. Global Change Biology 15, 1153–1172.
728 Wouterlood, M., Lambers, H., Veneklaas, E.J., 2005. Plant phosphorus status has a limited
729 influence on the concentration of phosphorus-mobilising carboxylates in the rhizosphere of
730 chickpea. Functional Plant Biology 32, 153–159.
731 Yergeau, E., Bokhorst, S., Huiskes, A.H.L., Boschker, H.T.S., Aerts, R., Kowalchuk, G.A., 2007.
732 Size and structure of bacterial, fungal and nematode communities along an Antarctic
733 environmental gradient. FEMS Microbiology Ecology 59, 436–451.

Figure captions

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

Figure 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).

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

Figure 4. a) Percentage of organic C present as microbial biomass (Cmic/TOC), b) percentage of water soluble organic C developed as CO_2-C (ΣCO_2-C/WEOC), and c) metabolic quotient (qCO_2) for rhizosphere and bulk soil of *Heliantemum nummularium* subsp. *grandiflorum, Dryas octopetala and Silene acaulis* subsp. c*enisia,* and bare soil. Cannella valley, Majella massif (Italy). Error bars are the standard errors. For each graph, columns with different letters significantly differ for $P < 0.05$.

Figure 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.

Figure 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. c*enisia*. Cannella valley, Majella massif (Italy).

Figure 7. Schematic representation of the rhizosphere effect induced by *Heliantemum nummularium* subsp. *grandiflorum, Dryas octopetala* and *Silene acaulis* subsp. c*enisia.* 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).

Table 1

Table 1. pH values, available water content (AWC), and available and organic P concentration of rhizosphere and bulk soil of *Heliantemum 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

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-hydroxydes plus that bound to organic matter (NC-Fe), and Fe of the crystalline Fe oxy-hydroxydes (C-Fe)] of rhizosphere and bulk soil of *Heliantemum 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

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 *Heliantemum 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

Table 4. Content of total organic C (TOC), water extractable organic C (WEOC) and microbial biomass C (Cmic), and amount of CO_2 evolved during basal respiration experiments (ΣCO_2-C) for rhizosphere and bulk soil of *Heliantemum 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}
Cmic ($mg\ kg^{-1}$)	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}
ΣCO_2-C ($\mu g\ kg^{-1}$)	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}

Table 5

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 *Heliantemum 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 (nmoli-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}

Appendix. Morphological description of the soils under *Heliantemum 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	Colour[a]	Structure[b]	Roots[c]	Boundary[d]	Other observations
	cm					
Soil under *Heliantemum 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.

Figure 1

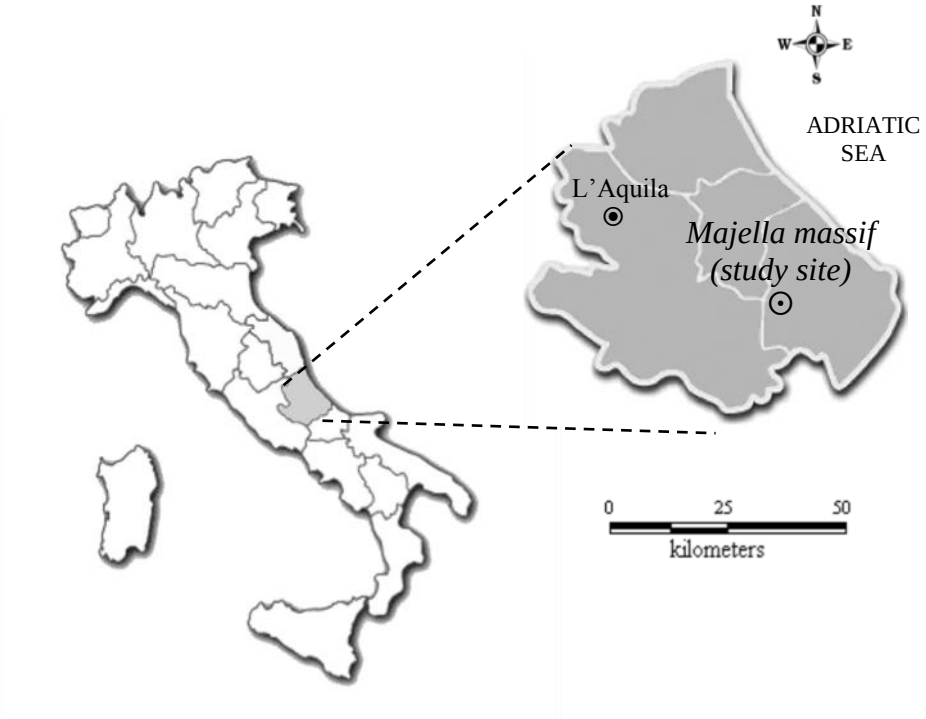

N
W
E
S
ADRIATIC
SEA
L'Aquila
Majella massif
(study site)
0
25
50
kilometers

Figure 2

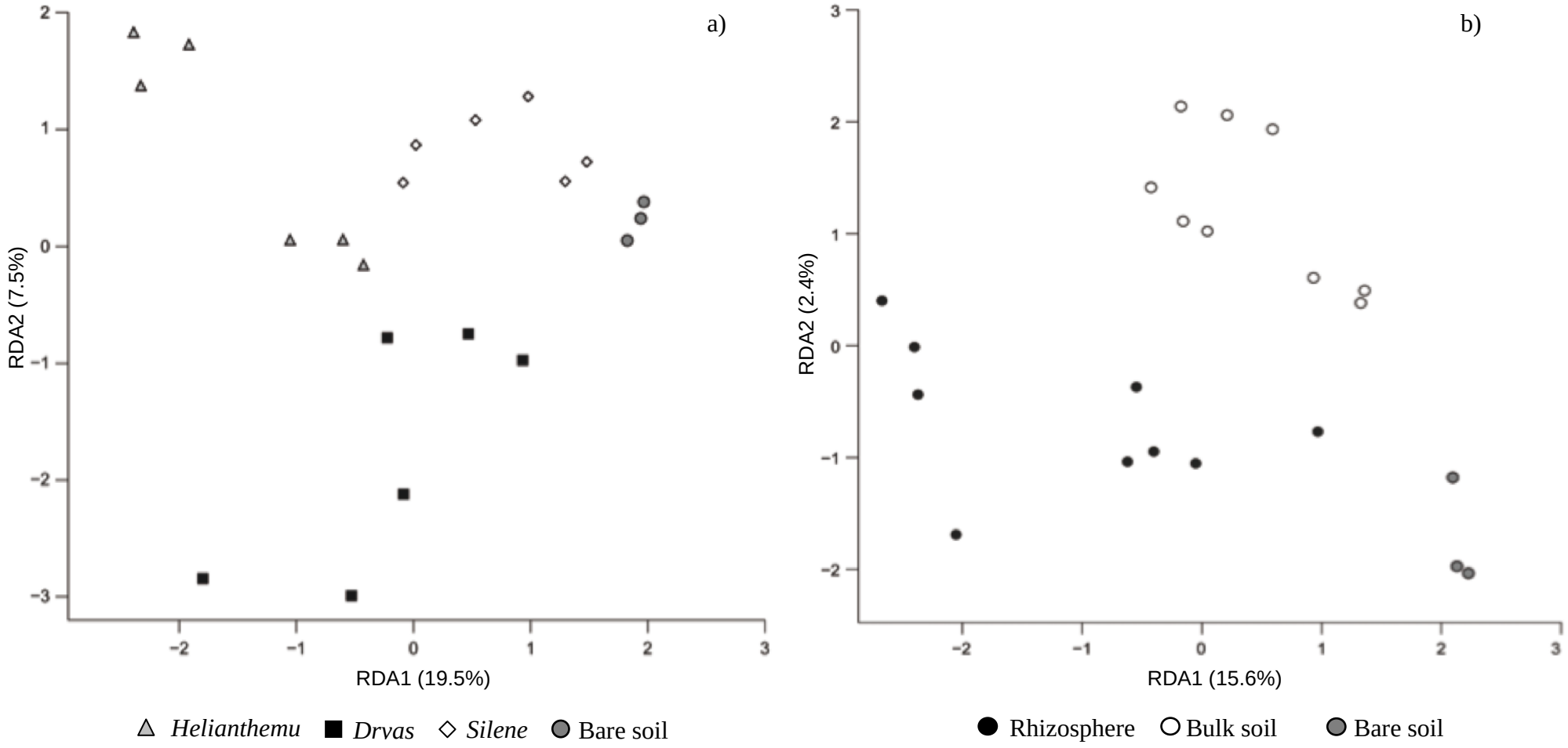

a)
RDA2 (7.5%)
RDA1 (19.5%)
Helianthemu
Dryas
Silene
Bare soil
b)
RDA2 (2.4%)
RDA1 (15.6%)
Rhizosphere
Bulk soil
Bare soil

Figure 3

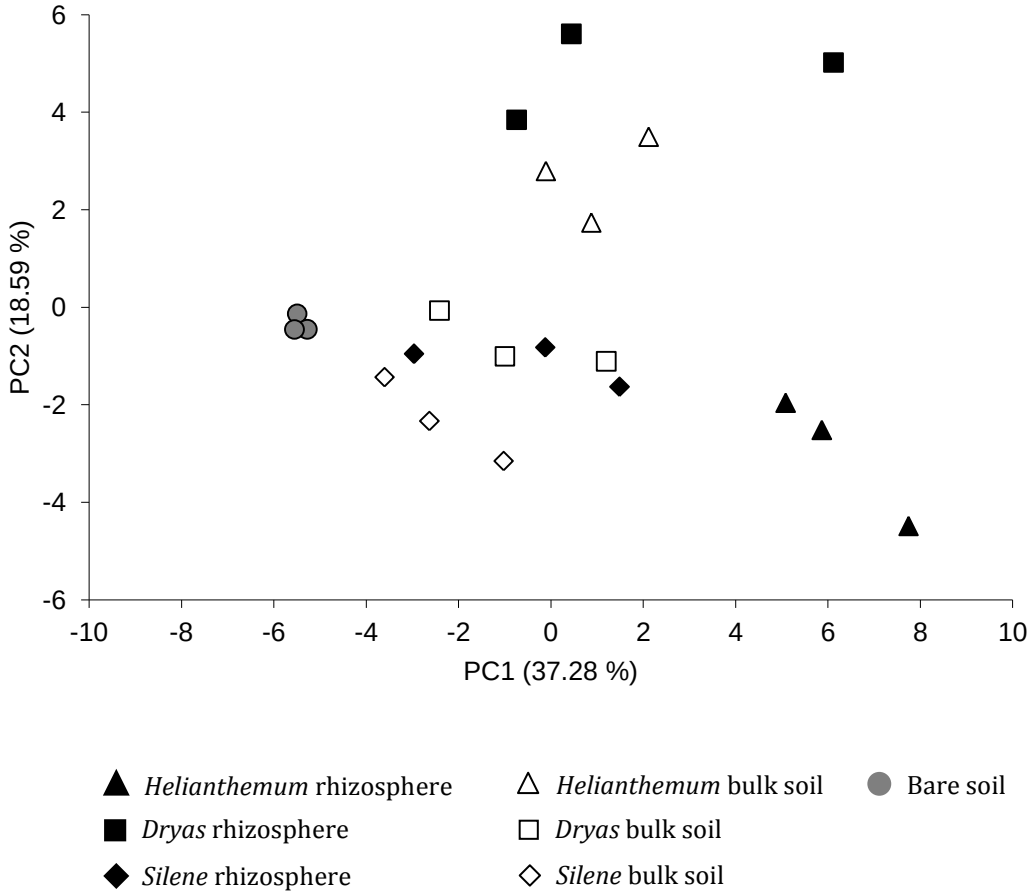

6
4
2
0
-2
-4
-6
PC2 (18.59 %)
-10
-8
-6
-4
-2
0
2
4
6
8
10
PC1 (37.28 %)
Helianthemum rhizosphere
Helianthemum bulk soil
Bare soil
Dryas rhizosphere
Dryas bulk soil
Silene rhizosphere
Silene bulk soil

Figure 4

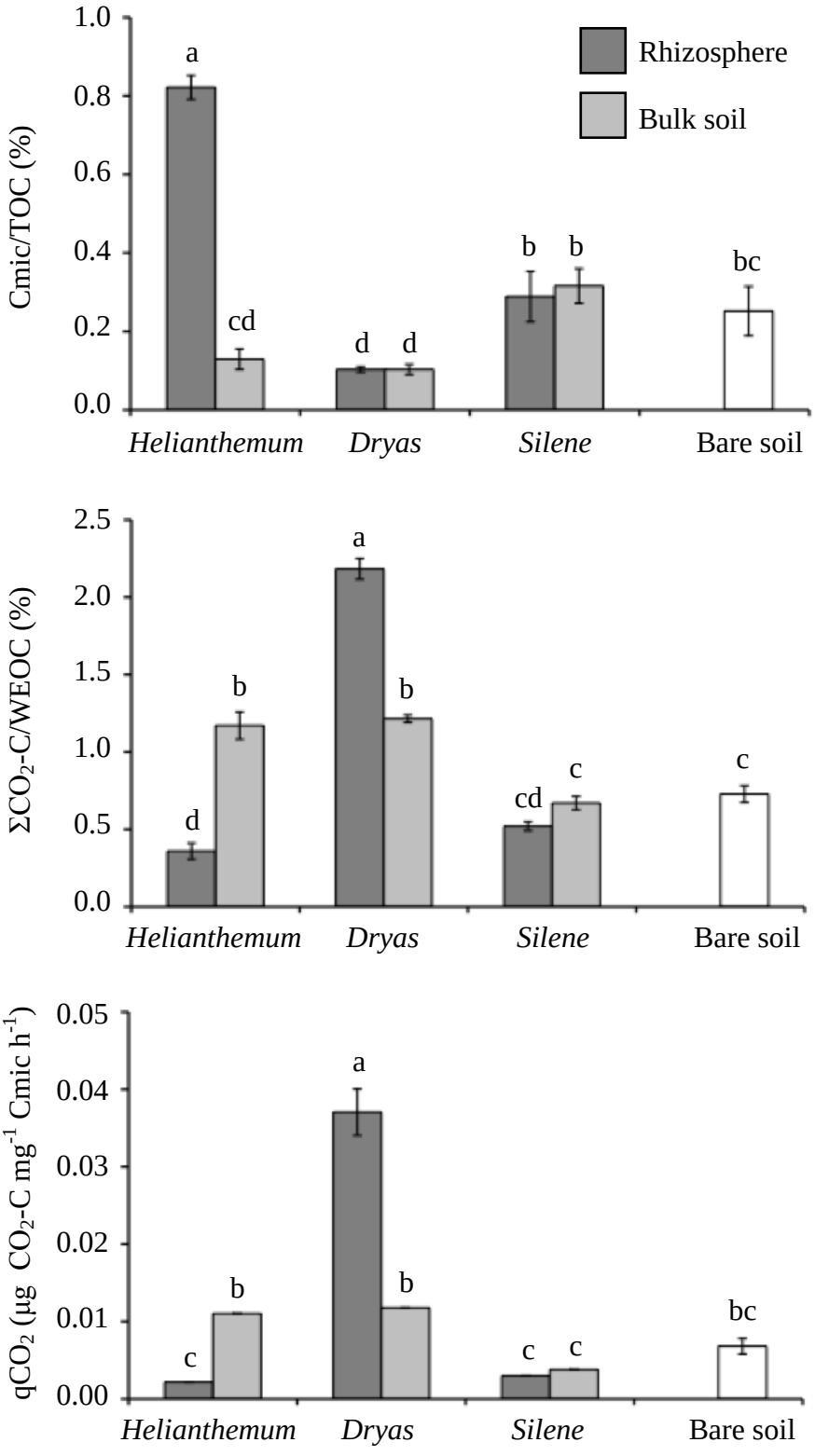
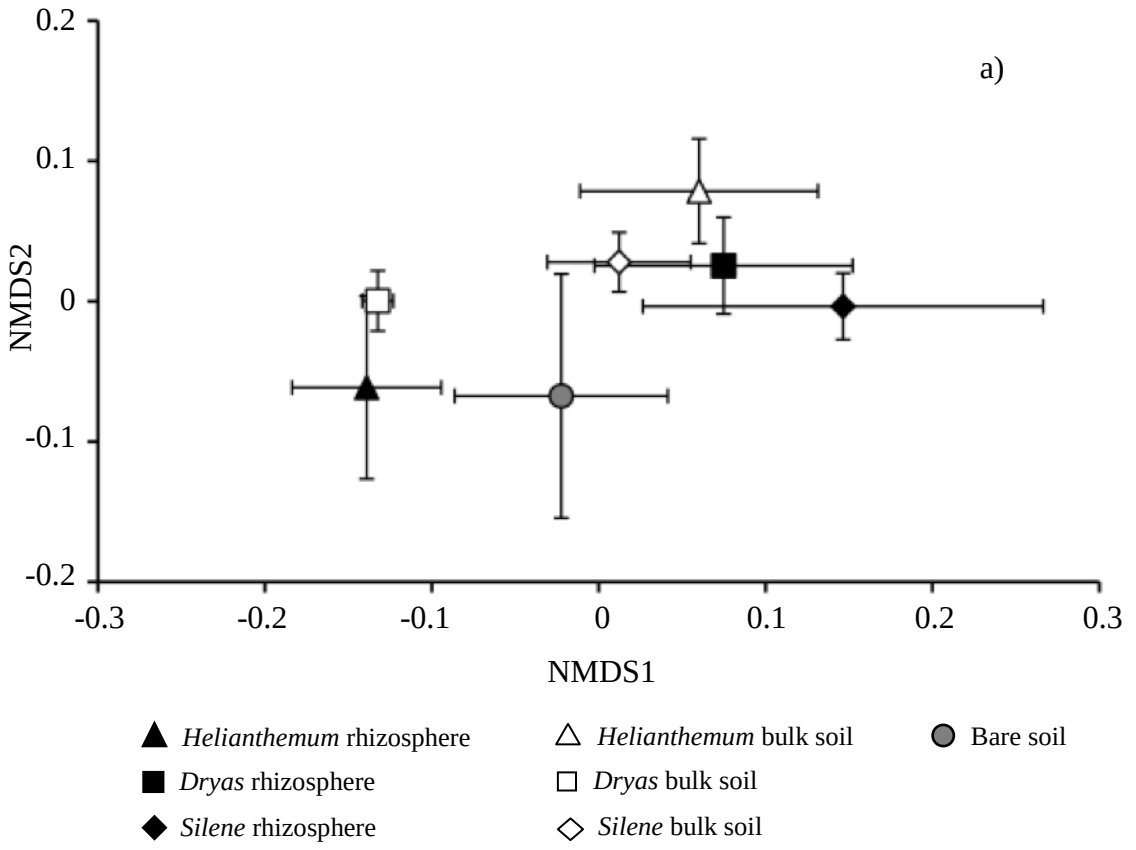

Rhizosphere
Bulk soil
Cmic/TOC (%)
1.0
0.8
0.6
0.4
0.2
0.0
a
cd
d d
b b
bc
Helianthemum
Dryas
Silene
Bare soil
a)
ΣCO2-C/WEOC (%)
2.5
2.0
1.5
1.0
0.5
0.0
d
b
a
b
cd
c
c
Helianthemum
Dryas
Silene
Bare soil
b)
qCO2 (µg CO2-C mg-1 Cmic h-1)
0.05
0.04
0.03
0.02
0.01
0.00
c
b
a
b
c c
bc
Helianthemum
Dryas
Silene
Bare soil
c)

Figure 5


a)
NMDS2
NMDS1
0.2
0.1
0
-0.1
-0.2
-0.3
-0.2
-0.1
0
0.1
0.2
0.3
Helianthemum rhizosphere
Helianthemum bulk soil
Bare soil
Dryas rhizosphere
Dryas bulk soil
Silene rhizosphere
Silene bulk soil

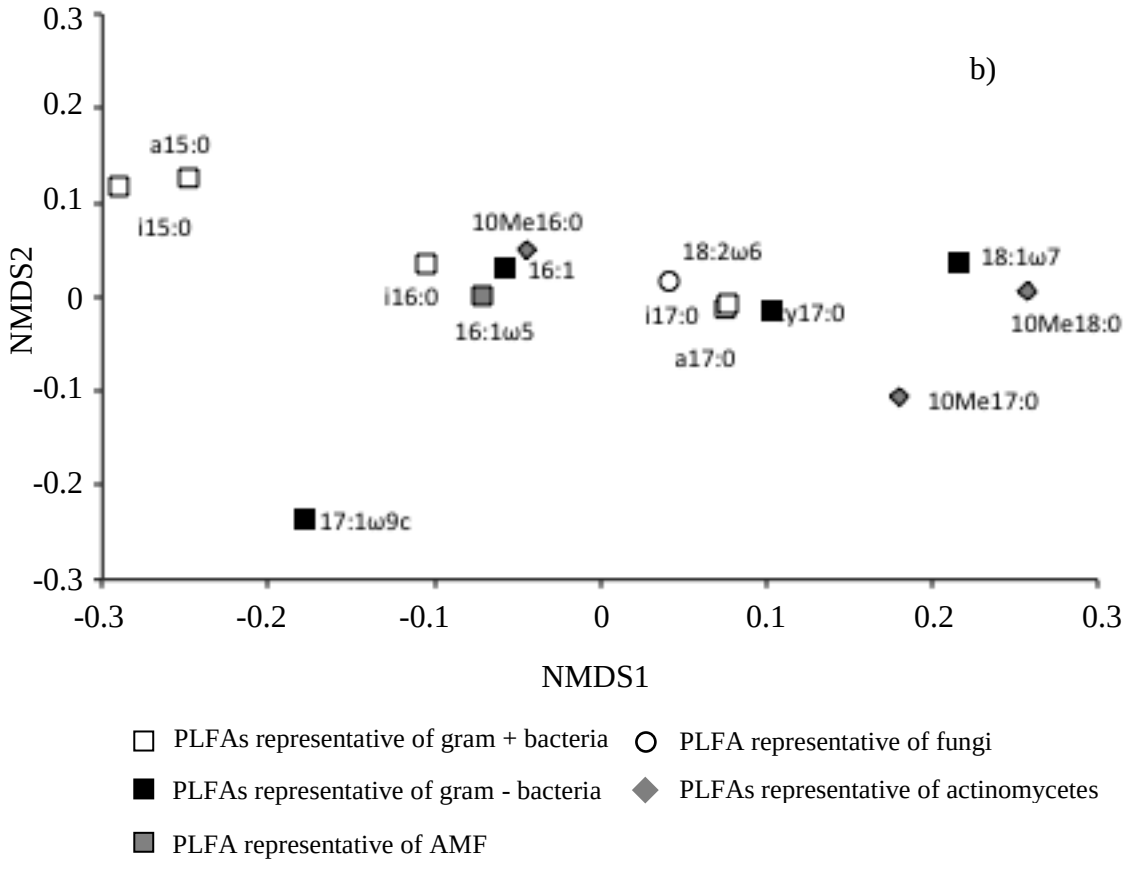

b)
NMDS2
NMDS1
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
-0.3
-0.2
-0.1
0
0.1
0.2
0.3
a15:0
i15:0
10Me16:0
16:1
i16:0
16:1ω5
18:2ω6
i17:0
a17:0
y17:0
18:1ω7
10Me18:0
10Me17:0
17:1ω9c
PLFAs representative of gram + bacteria
PLFA representative of fungi
PLFAs representative of gram - bacteria
PLFAs representative of actinomycetes
PLFA representative of AMF

Figure 6

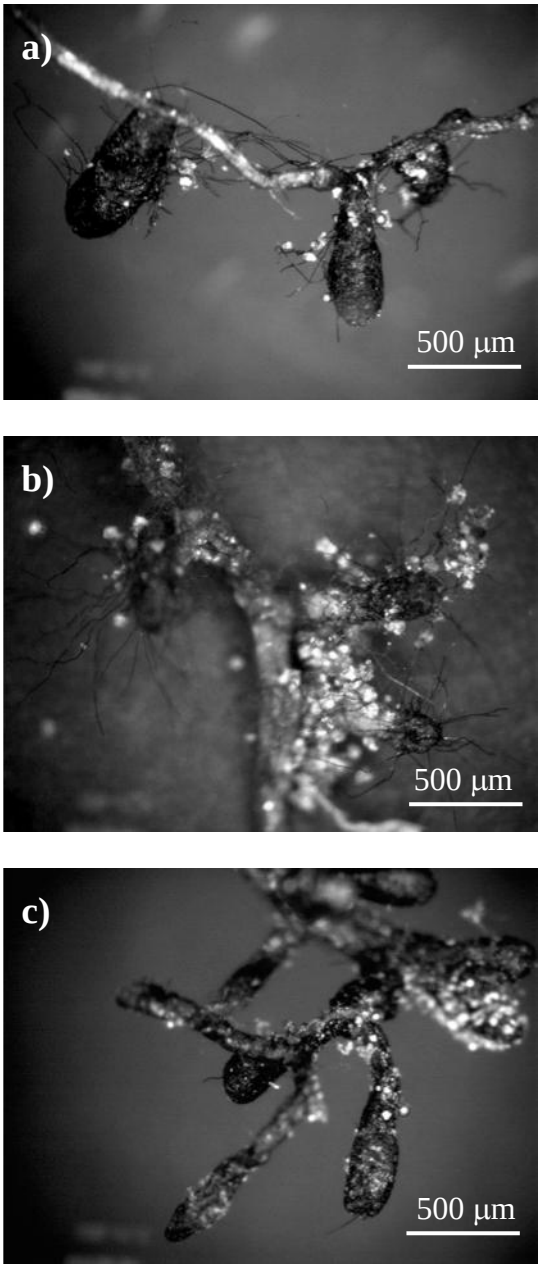
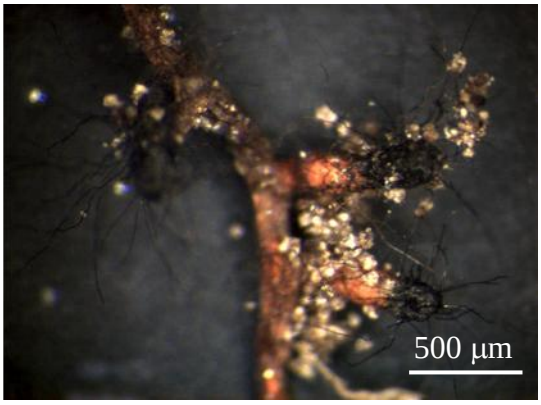


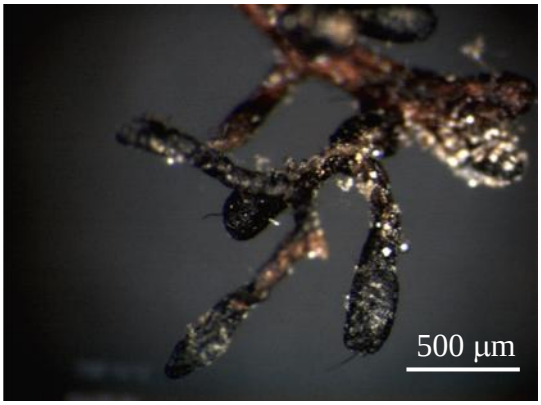
Figure 6 colour

(a)

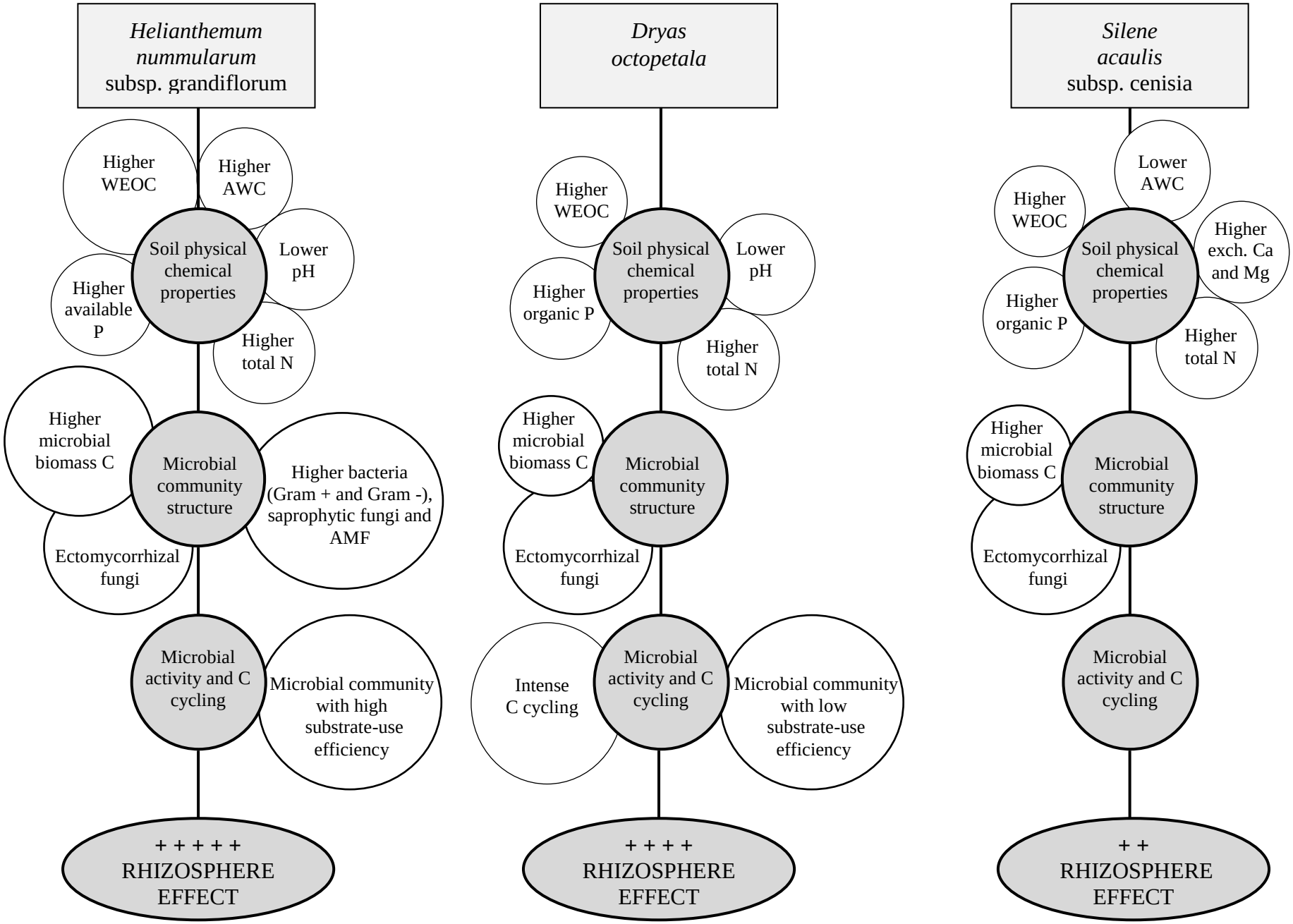
(b)



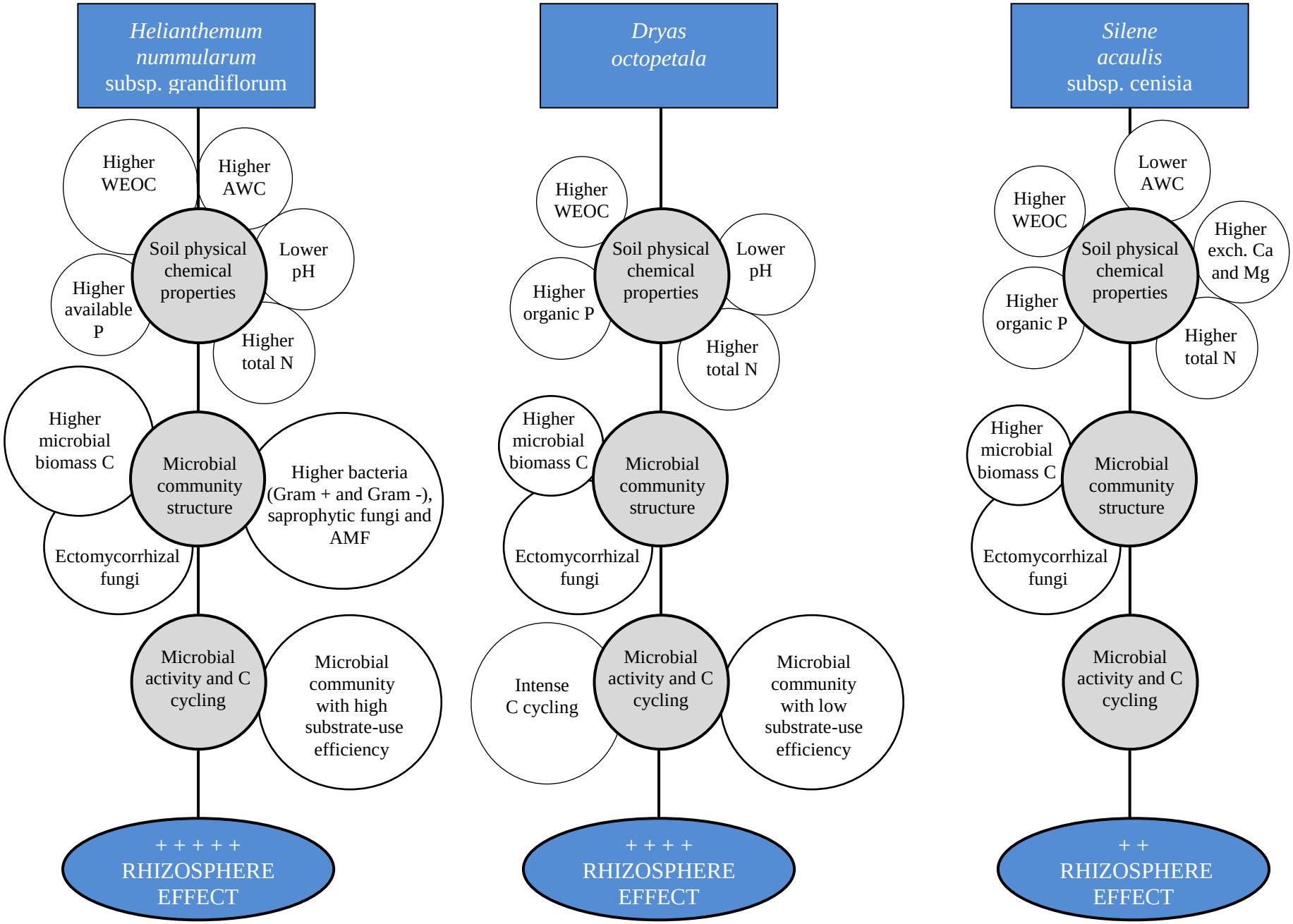
(c)



Figure


Helianthemum nummularum subsp. grandiflorum
Higher WEOC
Higher AWC
Soil physical chemical properties
Lower pH
Higher available P
Higher total N
Higher microbial biomass C
Microbial community structure
Higher bacteria (Gram + and Gram -), saprophytic fungi and AMF
Ectomycorrhizal fungi
Microbial activity and C cycling
Microbial community with high substrate-use efficiency
+ + + + +
RHIZOSPHERE EFFECT
Dryas octopetala
Higher WEOC
Soil physical chemical properties
Lower pH
Higher organic P
Higher total N
Higher microbial biomass C
Microbial community structure
Ectomycorrhizal fungi
Intense C cycling
Microbial activity and C cycling
Microbial community with low substrate-use efficiency
+ + + +
RHIZOSPHERE EFFECT
Silene acaulis subsp. cenisia
Lower AWC
Higher WEOC
Soil physical chemical properties
Higher exch. Ca and Mg
Higher organic P
Higher total N
Higher microbial biomass C
Microbial community structure
Ectomycorrhizal fungi
Microbial activity and C cycling
+ +
RHIZOSPHERE EFFECT

Figure


Helianthemum nummularum subsp. grandiflorum
Higher WEOC
Higher AWC
Soil physical chemical properties
Lower pH
Higher available P
Higher total N
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Microbial community structure
Higher bacteria (Gram + and Gram -), saprophytic fungi and AMF
Ectomycorrhizal fungi
Microbial activity and C cycling
Microbial community with high substrate-use efficiency
+ + + + +
RHIZOSPHERE EFFECT
Dryas octopetala
Higher WEOC
Soil physical chemical properties
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+ + + +
RHIZOSPHERE EFFECT
Silene acaulis subsp. cenisia
Lower AWC
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Soil physical chemical properties
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Higher microbial biomass C
Microbial community structure
Ectomycorrhizal fungi
Microbial activity and C cycling
+ +
RHIZOSPHERE EFFECT

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