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

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*Original*

Rhizosphere effect of three plant species of environment under periglacial conditions (Majella Massif, central Italy) / Massaccesi, L; Benucci, G. M. N.; Gigliotti, G.; Cocco, Stefania; Corti, Giuseppe; Agnelli, A.. - In: SOIL BIOLOGY & BIOCHEMISTRY. - ISSN 0038-0717. - 89:(2015), pp. 184-195. [10.1016/j.soilbio.2015.07.010]

*Availability:*

This version is available at: 11566/228453 since: 2022-06-06T12:46:54Z

*Publisher:*

*Published*

DOI:10.1016/j.soilbio.2015.07.010

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(Article begins on next page)

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*

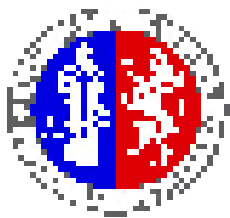
Corresponding Author: Dr. Luisa Massaccesi,

Corresponding Author's Institution: Università degli Studi di Perugia

First Author: Luisa Massaccesi

Order of Authors: Luisa Massaccesi; Gian Maria Niccolò Benucci; Giovanni Gigliotti; Stefania Cocco; Giuseppe Corti; Alberto Agnelli

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 21<sup>st</sup>, 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

*We thank the Reviewer for his/her suggestions addressed to strengthen the message we wanted to address with our findings, and make the manuscript more rigorous and appealing.*

The introduction and discussion section have improved a lot by the revisions. I have some more detailed suggestions to improve the manuscript.

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*Ok, done.*

Line 65-66 'In general, arctic and alpine plants have a higher proportion of their biomass below-ground than trees and bushes from other ecosystems'.

*Ok, done.*

Line 68: 'the proportion of soil that is influenced by the rhizosphere'

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Line 71. Add reference

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*Ok, done.*

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Line 88-90 suggest that the rhizosphere effect is limiting and not different for the three plant species tested, but the hypothesis (lines 91-94) state that they are expected to be different.

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Line 161 'Potential plant-available inorganic P and organic P was determined'

*We changed the phrase on the basis of the suggestion given. However, the term "inorganic" was not insert as we do not exactly know the nature of the available (Olsen) P (line 160 of the new version).*

Line 276 'available water content (AWC)'

*OK, done (line 274 of the new version).*

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Line 377-379 repeats results and can be deleted.

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Line 379-381 'The few differences between rhizosphere and bulk soil of *Silene* indicated that *Silene* 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.'

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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

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

3

4    Massaccesi, L.<sup>1\*</sup>, Benucci G.M.N.<sup>1</sup>, Gigliotti G.<sup>2</sup>, Cocco, S.<sup>3</sup>, Corti, G.<sup>3</sup>, Agnelli, A.<sup>1</sup>

5

6    <sup>1</sup>Department of Agricultural, Food and Environmental Sciences, University of Perugia, Italy

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20    Luisa Massaccesi

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## 27    **Abstract**

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

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

49

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

52

## 53    **1. Introduction**

54    In the rhizosphere, the soil in proximity to the root, processes like rhizodeposition, intense  
55    microbial activity, root nutrient uptake, redox reactions, and CO<sub>2</sub> production induce modifications  
56    of soil components and properties (Hinsinger et al., 2003; Richter et al., 2007). The chemical,  
57    physical and biological differentiation of the rhizospheric soil with respect to the rest of the soil is  
58    called “rhizosphere effect”, which has been investigated in many ecosystems, including those with  
59    environmental constraints and nutrient-poor soils (e.g., Hinsinger et al., 2005; Teixeira et al., 2010).  
60    However, little is known about the rhizosphere effect of pioneer plants in young and poorly  
61    developed soils from periglacial environments (Wookey et al., 2009).

62    Periglacial environments are those affected by severe frost action that dominates geomorphic  
63    processes, and amount to about 25% of the Earth’s land surface. The knowledge of the rhizosphere  
64    effect of pioneer plants in these environments is the basis in understanding how soil-plant  
65    relationships respond to environmental constraints. In general, arctic and alpine plants have a higher  
66    proportion of their biomass below-ground than trees and bushes from other ecosystems (Jackson et  
67    al., 1996; Körner, 2003), and this relatively high below-ground biomass increases the proportion of  
68    rhizosphere soil (Hinsinger et al., 2005; Finzi et al., 2015). Indeed, in poorly developed soils of cold  
69    areas, the presence of vascular plants strongly modifies the soil properties and the structure and  
70    function of the soil microbial community (Yergeau et al., 2007; Teixeira et al., 2010). In these areas,  
71    rhizospheric processes resulting from soil-plant-microbes interactions may improve the ability of  
72    plants to overcome abiotic disturbances such as freezing, high soil daily and seasonal temperature  
73    excursions, freeze–thaw and wet–dry cycles, excessive drainage, and strongly oligotrophic  
74    conditions (e.g., Tscherko et al., 2004, 2005; Edwards et al., 2006; Ciccazzo et al., 2014). The  
75    amount of energy supplied by the plants in form of exudates to rhizosphere heterotrophic microbial  
76    community is key for stimulating rhizospheric processes (Kuzyakov, 2002; Wookey et al., 2009;  
77    Jorquera et al., 2014; Ciccazzo et al., 2014). In fact, most arctic and alpine vascular plants allocate  
78    10-30% of net carbon fixation to establish mycorrhizal 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



105 12,700 and ended about 11,000 years before present (Giraudi, 2004), is mantled by thick morainic  
106 deposits (till) mostly made of limestone, from which the present soils developed. The area is  
107 covered by sparse vegetation mostly made of *Helianthemum nummularium* (L.) Mill. subsp.  
108 *grandiflorum* (Scop.) Sch. and Th., *Dryas octopetala* L., *Silene acaulis* (L.) Jacq. subsp. *cenisia*  
109 (Vierh.) P. Fourn., *Carex kitaibeliana* Degen ex Bech., *Anthyllis vulneraria* L. subsp. *maura* (Beck)  
110 Maire, *Campanula scheuchzeri* Vill., *Minuartia verna* (L.) Hiern subsp. *verna*, *Trifolium pratense*  
111 L. subsp. *semipurpureum* (Strobl) Pign., with spots covered by *Salix retusa* L. and rare dwarf  
112 mountain pines (*Pinus mugo* Turra). Where the vegetation forms a rather continuous mat, the soils  
113 are loamy-skeletal, mixed, frigid Oxyaquic Haplocryolls (SSS, 2010), while in the bare areas the  
114 soils are loamy-skeletal, mixed, frigid Oxyaquic Cryorthents (SSS, 2010). In both cases, the soil is  
115 frozen for meters from December to February/March.

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

125

## 126 2.2. Soil sampling and sample preparation

127 During July 2011, at about 2455 m above sea level, within an area of about 1600 m<sup>2</sup> (40 x 40 m) we  
128 selected three plots with a mean diameter ranging from 5 to 10 m; in each plot all the three plants  
129 were present at once. As a control, for each plot a bare soil was also located at least at 1.5 m from  
130 each plant. The three individual plants for each species were chosen among those showing the

131 maximum development. For *Helianthemum* we considered plants forming patches of 1.5-1.8 m of  
132 diameter, with an estimated age of at least 30-32 years (obtained by the annual ring counting of  
133 basal stems). For *Dryas*, we took into consideration plant mats with a diameter of about 1 m, with  
134 an estimated age of at least 18-22 years (obtained by the annual ring counting of basal stems). In the  
135 case of *Silene*, we selected fully healthy cushions with a diameter comprised between 35 and 40 cm.  
136 According to Benedict (1989) and McCarthy (1992), cushions of *Silene acaulis* have a growth rate  
137 ranging from 0.06 to 3 cm yr<sup>-1</sup>, even though the maximum rate of 2-3 cm yr<sup>-1</sup> is reached in the  
138 intermediate part of their life, which can attain 350 years (Beschel, 1958). Because of this, we  
139 estimated the age of the selected cushions to be more than 50 years.

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

154

### 155 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<sub>2</sub> 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<sub>3</sub> to estimate the “labile Fe”, namely the Fe forming the easily reducible Fe-Mn oxy-  
171 hydroxydes (Berna et al., 2000), 3) NH<sub>4</sub>-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

minutes, and then filtered through a 0.45  $\mu\text{m}$  membrane filter. The obtained supernatant solution received a few drops of concentrated  $\text{H}_3\text{PO}_4$  to eliminate carbonates and was analysed 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 ( $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N) and the most soluble organic N forms, was determined by dry combustion analyser. 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 ( $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N) were measured on soil extracts by a FOSS Fiastar<sup>TM</sup> 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 ( $C_{\text{mic}}$ ) 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  $\text{CO}_2$  developed and titration of the residual  $\text{OH}^-$  with a standardised HCl solution. Basal respiration was expressed as the cumulative amount of  $\text{CO}_2$ -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 analysing 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  $\mu$ m 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, 17:1 $\omega$ 9c and 18:1 $\omega$ 7 (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 18:2 $\omega$ 6 was used as a marker for saprophytic fungi (Federle, 1986), while the fatty acid 16:1 $\omega$ 5 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).

231

## 232 2.6. Statistical analysis

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

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

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

251

### 252 **3. Results**

#### 253 *3.1. RDA and PCA*

254 The RDA plots (Figure 2a,b) showed that the plant species effect (Permutation test,  $F=3.973$ ,  
255  $P=0.001^{***}$ ) explained about 27 % of the total variance, whereas the soil fraction effect  
256 (Permutation test,  $F=3.198$ ,  $P=0.004^{**}$ ) explained about 18 % of the total variance. The PCA  
257 scatter plot (Figure 3) showed the variation of rhizosphere and bulk soil properties under the three  
258 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  $\text{NO}_3^-$ -N (Figure 1a of the  
266 Supplementary Data). PCA-axis 2 was mainly associated with positive relationships to  $\text{CO}_2$ -  
267 C/WEOC ratio and  $\text{qCO}_2$ , and with negative relationships to  $\text{C}_{\text{mic}}/\text{TOC}$  ratio, available P and  $\text{C}_{\text{mic}}$   
268 contents (Figure 1b 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



the basal respiration experiment ( $\Sigma\text{CO}_2\text{-C}$ ) 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  $\Sigma\text{CO}_2\text{-C}$ . The rhizosphere of *Helianthemum* showed the largest percentage of  $\text{C}_{\text{mic}}/\text{TOC}$  (Figure 4a) and the lowest percentage of organic C consumed during the incubation experiment with respect to WEOC ( $\Sigma\text{CO}_2\text{-C}/\text{WEOC}$ ) (Figure 4b). For *Dryas*, the percentage of  $\text{C}_{\text{mic}}/\text{TOC}$  was similar in both rhizosphere and bulk soil, but the rhizosphere showed the largest  $\Sigma\text{CO}_2\text{-C}/\text{WEOC}$  percentage and  $\text{qCO}_2$  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 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* (Figure 5a), and that the synergistic effect of plant species and soil fractions appeared mostly due to bacteria. In fact, as shown in Figure 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 17:1 $\omega$ 9c 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

337 between rhizosphere and bulk soil of *Dryas* and *Silene*. The fungal to bacterial PLFAs ratio was  
338 always much < 1, and showed similar values for all the samples.

339

## 340 **4. Discussion**

### 341 *4.1. Difference in the soil properties of rhizosphere versus bulk soil for the three plant species*

342 Our results indicated that the plant species effect was more significant than the soil fraction effect,  
343 suggesting that both above- and below-ground plant systems play the major role in driving the  
344 changes in soil properties, with significant influences on most measured variables. However, also  
345 the rhizosphere effect, which is closely related to the plant species, plays a decisive role in soil  
346 changes. Among the three species considered in this study, *Helianthemum*, showed the major  
347 differences between rhizosphere and bulk soil, followed by *Dryas* and then *Silene*. As a matter of  
348 fact, *Helianthemum* exerted a relevant “rhizosphere effect” as most of the measured parameters  
349 differed between rhizosphere and bulk: pH, labile Fe, AWC, available P, total and organic N,  $\text{NH}_4^+$ -  
350 N, WEOC,  $C_{\text{mic}}$ ,  $\text{CO}_2$ -C evolved during the basal respiration, total PLFA, total bacteria, Gram-  
351 positive and Gram-negative bacteria, saprophytic fungi, AMF, and non-specific PLFA.

352 *Helianthemum* and *Dryas* had lower pH values in the rhizosphere than in the bulk, so confirming  
353 the acidifying action that the roots exert on the soil in contact with them (e.g., Hinsinger et al.,  
354 2003). The acidification of the rhizosphere can occur by different processes, other than the  $\text{CO}_2$   
355 produced by the root respiration (Richter et al., 2007): 1) excretion of  $\text{H}^+$  following the root  
356 absorption of cations in excess of anions (Haynes, 1990), and 2) release of organic acids to  
357 overcome nutrient deficiency (Rengel and Romheld, 2000; Hinsinger et al., 2003; Sandnes et al.,  
358 2005). For example, in P deficient soils, roots of natural and cultivated plants exude large amounts  
359 of low-molecular weight carboxylates that mobilize P by competing for the same adsorption sites in  
360 the soil matrix (Gerke et al., 2000; Fernández Sanjurjo et al., 2003; Wouterlood et al., 2005). The  
361 higher concentration of available P in the rhizosphere than in the bulk soil of *Helianthemum* may be  
362 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<sub>mic</sub> and CO<sub>2</sub>-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

388 4.2. *Microbial community structure and abundance, and microbial respiration in rhizosphere*  
389 *versus bulk soil within and among the three plant species*

390 Our findings suggested that structure and abundance of the root-associated microbial community, as  
391 measured by PLFAs, were mainly driven by the combined effect of plant species and soil fraction.  
392 The most marked differences in the microbial community structure between rhizosphere and bulk  
393 soil were observed under *Helianthemum*. The large colonization of the *Helianthemum* rhizosphere  
394 by saprophytic fungi and AMF could be due to the ability of this plant species to form mycorrhizal  
395 association with both ectomycorrhizal fungi and AMF (Cornelissen et al., 2001). Although by the  
396 analysis of PLFAs it was not possible to distinguish the ectomycorrhizal by the saprophytic fungi as  
397 they are identified by the same PLFA (Karliński et al., 2006), we recognized a diffuse  
398 ectomycorrhizal infection in the roots of *Helianthemum* by optical microscope observation (Figure  
399 6a). The presence of mycorrhizal fungi could be partly responsible of the more abundant bacterial  
400 community present in the rhizosphere. Indeed, as reported by Marschner et al. (2005), changes in  
401 amount and/or composition of root and fungal exudates due to AMF colonization determine  
402 diversity and abundance of the bacterial community in the rhizosphere. The same authors also  
403 suggested that the influence of AMF on the bacterial population harbouring the rhizosphere can  
404 occur directly through the supply of easily available organic substances due to the growth and  
405 degeneration of the hyphal network or, indirectly, through the rhizodeposition stimulated by root  
406 and shoot growth. Hence, direct or indirect effects induced by the larger presence of the  
407 mycorrhizal fungi, together with rhizodeposition processes and bacterial activity (Buée et al., 2009)  
408 could be responsible of the significantly higher WEOC concentration in the rhizosphere than in the  
409 bulk soil. We also suggest that the larger amount of labile and hydrophilic organic molecules, partly  
410 made of gums and mucilages (Dakora and Phillips, 2002), produced by roots and rhizospheric  
411 microorganisms, together with the fine roots and the mycorrhiza hyphal network, fostered the  
412 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  $\Sigma CO_2-C/WEOC$  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),  $\Sigma CO_2$ -  
 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

### 465 4.3. Conclusions

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

485

### 486 Acknowledgments

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

489

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**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 ( $C_{mic}/TOC$ ), b) percentage of water soluble organic C developed as  $CO_2$ -C ( $\Sigma CO_2$ -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  $P < 0.05$ .

**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. *cenisia*. Cannella valley, Majella massif (Italy).

**Figure 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).

Table 1

**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  $P < 0.05$ .

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

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 *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  $P < 0.05$ .

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

Table 3

**Table 3.** Content of total N, water extractable N (WEN), ammonium (NH<sub>4</sub><sup>+</sup>-N), nitrate (NO<sub>3</sub><sup>-</sup>-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.

	<i>Helianthemum</i>		<i>Dryas</i>		<i>Silene</i>		Bare soil
	Rhizosphere	Bulk	Rhizosphere	Bulk	Rhizosphere	Bulk	
Total N (g kg <sup>-1</sup> )	13.71(0.29) <sup>a</sup>	10.10(0.96) <sup>bc</sup>	14.16(1.73) <sup>a</sup>	9.23(1.02) <sup>bc</sup>	13.00 (1.58) <sup>ab</sup>	7.66(1.11) <sup>c</sup>	5.60(0.51) <sup>c</sup>
WEN (g kg <sup>-1</sup> )	0.20(0.05) <sup>a</sup>	0.20(0.05) <sup>a</sup>	0.13(0.02) <sup>ab</sup>	0.13(0.02) <sup>ab</sup>	0.12(0.03) <sup>ab</sup>	0.12(0.03) <sup>ab</sup>	0.07(0.00) <sup>b</sup>
NH <sub>4</sub> <sup>+</sup> -N (g kg <sup>-1</sup> )	0.04(0.00) <sup>a</sup>	0.03(0.00) <sup>bcd</sup>	0.02(0.00) <sup>d</sup>	0.02(0.00) <sup>cd</sup>	0.03(0.00) <sup>ab</sup>	0.02(0.00) <sup>d</sup>	0.03(0.00) <sup>abc</sup>
NO <sub>3</sub> <sup>-</sup> -N (g kg <sup>-1</sup> )	0.01(0.00) <sup>c</sup>	0.01(0.00) <sup>c</sup>	0.01(0.00) <sup>bc</sup>	0.01(0.00) <sup>b</sup>	0.02(0.00) <sup>ab</sup>	0.01(0.00) <sup>b</sup>	0.02(0.00) <sup>a</sup>
Organic N (g kg <sup>-1</sup> )	13.66(0.28) <sup>a</sup>	10.06(0.96) <sup>bc</sup>	14.13(1.74) <sup>a</sup>	9.20(1.02) <sup>bc</sup>	12.95(1.58) <sup>abc</sup>	7.63(1.11) <sup>bc</sup>	5.55(0.51) <sup>c</sup>

Table 4

**Table 4.** Content of total organic C (TOC), water extractable organic C (WEOC) and microbial biomass C (Cmic), and amount of CO<sub>2</sub> evolved during basal respiration experiments ( $\Sigma$ CO<sub>2</sub>-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  $P < 0.05$ .

	<i>Helianthemum</i>		<i>Dryas</i>		<i>Silene</i>		Bare soil
	Rhizosphere	Bulk	Rhizosphere	Bulk	Rhizosphere	Bulk	
TOC (g kg <sup>-1</sup> )	155.14(4.41) <sup>a</sup>	133.92(12.85) <sup>ab</sup>	118.89(2.07) <sup>abc</sup>	93.78(8.04) <sup>abc</sup>	137.41(17.01) <sup>ab</sup>	66.69(9.25) <sup>bc</sup>	50.09(4.82) <sup>c</sup>
WEOC (g kg <sup>-1</sup> )	1.14(0.25) <sup>a</sup>	0.24(0.03) <sup>bc</sup>	0.31(0.02) <sup>b</sup>	0.14(0.00) <sup>c</sup>	0.34(0.03) <sup>b</sup>	0.17(0.00) <sup>c</sup>	0.17(0.01) <sup>c</sup>
Cmic (mg kg <sup>-1</sup> )	1274.66(7.81) <sup>a</sup>	173.08(20.83) <sup>d</sup>	122.27(17.71) <sup>e</sup>	96.70(1.95) <sup>f</sup>	396.84 (1.97) <sup>b</sup>	210.61(9.34) <sup>c</sup>	126.16(17.95) <sup>e</sup>
$\Sigma$ CO <sub>2</sub> -C ( $\mu$ g kg <sup>-1</sup> )	4106.06(58.67) <sup>b</sup>	2838.46(95.09) <sup>c</sup>	6740.27(156.29) <sup>a</sup>	1693.74(64.68) <sup>d</sup>	1764.38(136.8) <sup>d</sup>	1186.24(149.67) <sup>e</sup>	1278.95(212.12) <sup>e</sup>

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 *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  $P < 0.05$ .

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

**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 <sup>a</sup>	Structure <sup>b</sup>	Roots <sup>c</sup>	Boundary <sup>d</sup>	Other observations
	cm					
Soil under <i>Heliantemum nummularium</i> subsp. <i>grandiflorum</i> 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 <sub>1</sub> m,co	-	Skeleton (by volume): 80%; open work
Soil under <i>Dryas octopetala</i> 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 <i>Silene acaulis</i> subsp. <i>cenisia</i> 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 <sub>1</sub> mi,vf,f; 1m,co	cw	Skeleton (by volume): 85%; open work
C	63-80+	2.5YR 5/6	fragmental	v <sub>1</sub> m,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 <sub>1</sub> 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

<sup>a</sup> moist and crushed, according to the Munsell Soil Color Charts.

<sup>b</sup> 1 = weak, 2 = moderate, 3 = strong; f = fine, m = medium, c = coarse; cr = crumb, abk = angular blocky, sbk = subangular blocky.

<sup>c</sup> 0 = absent, v<sub>1</sub> = very few, 1 = few, 2 = plentiful, 3 = abundant; mi = micro, vf = very fine, f = fine, m = medium, co = coarse.

<sup>d</sup> a = abrupt, c = clear; w = wavy, s = smooth.



Figure 1

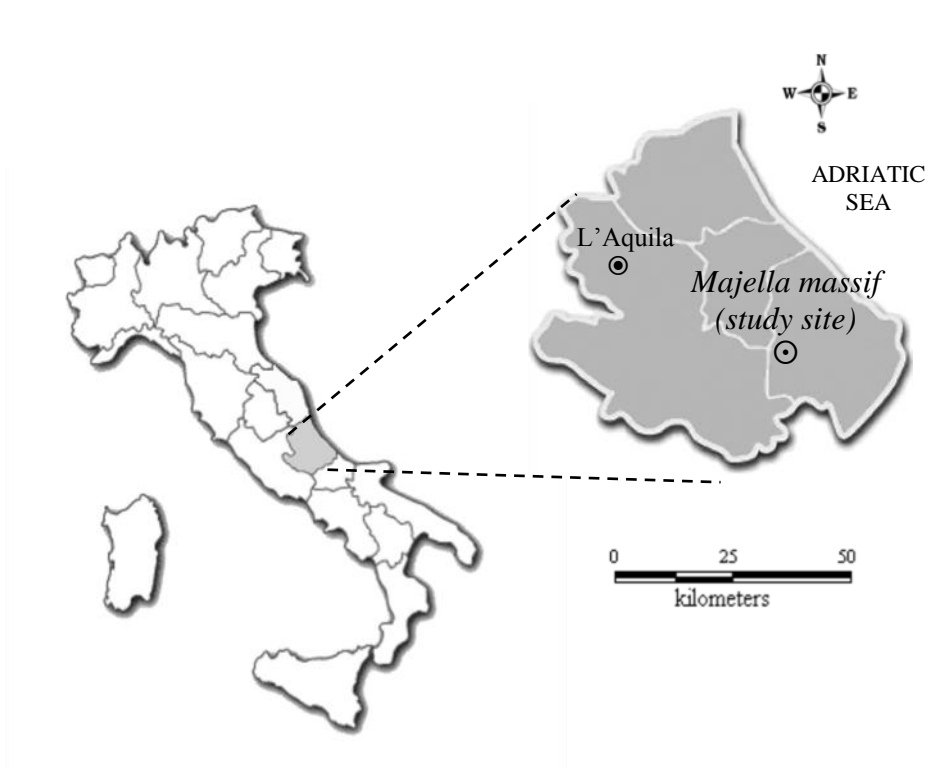


Figure 2

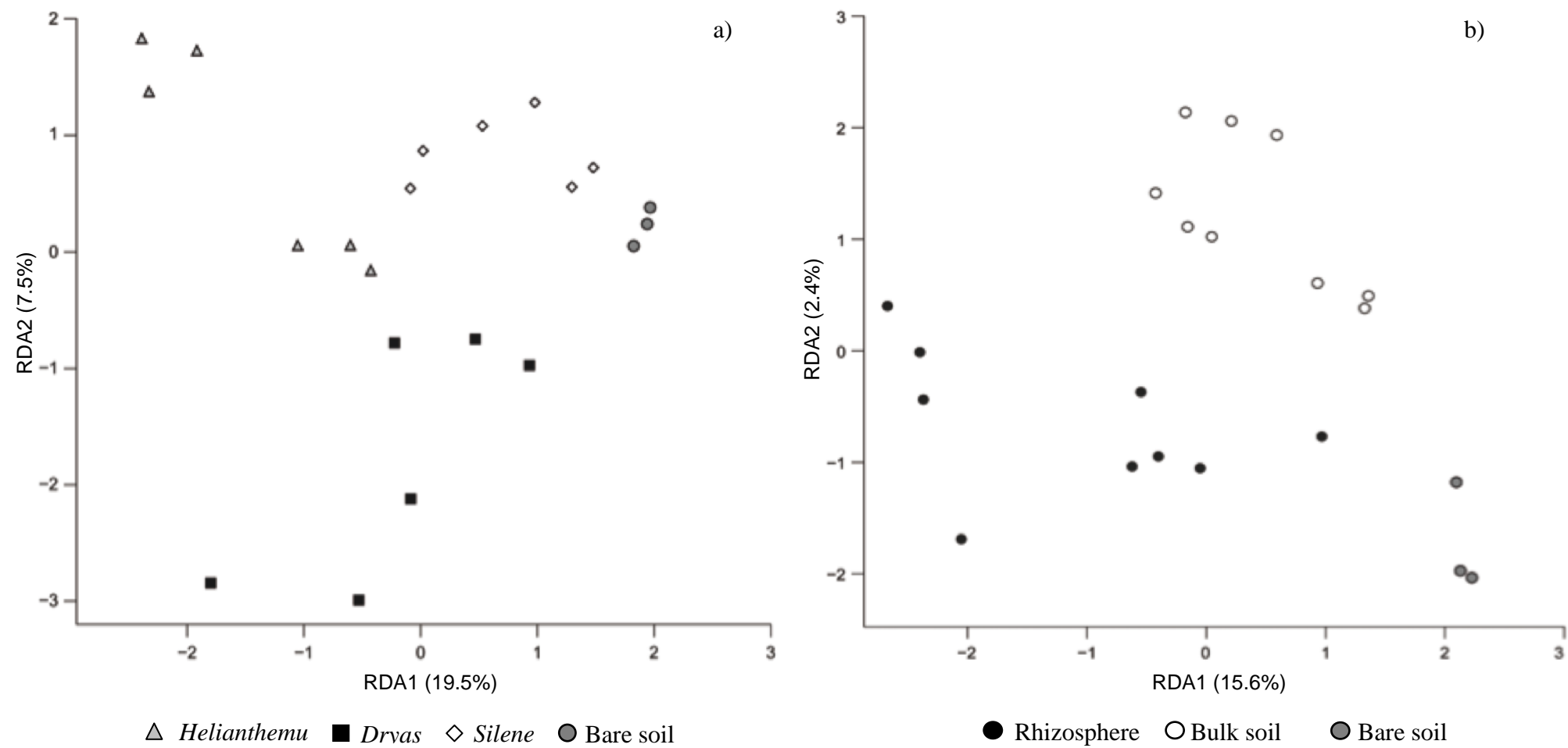
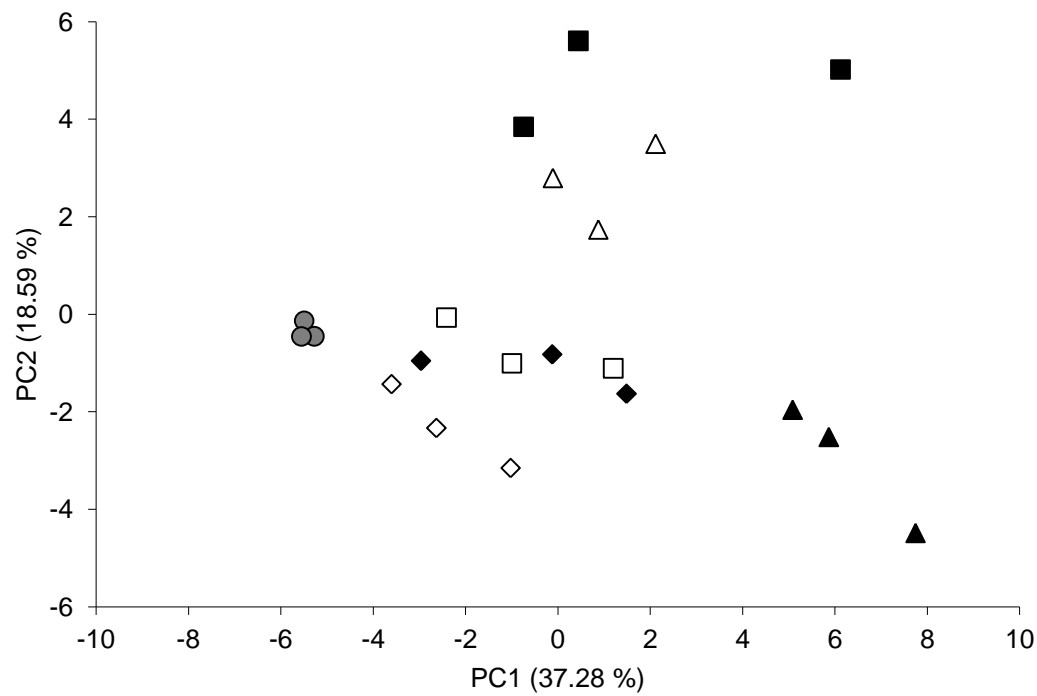


Figure 3



- |                                   |                                 |             |
|-----------------------------------|---------------------------------|-------------|
| ▲ <i>Helianthemum</i> rhizosphere | △ <i>Helianthemum</i> bulk soil | ● Bare soil |
| ■ <i>Dryas</i> rhizosphere        | □ <i>Dryas</i> bulk soil        |             |
| ◆ <i>Silene</i> rhizosphere       | ◇ <i>Silene</i> bulk soil       |             |

Figure 4

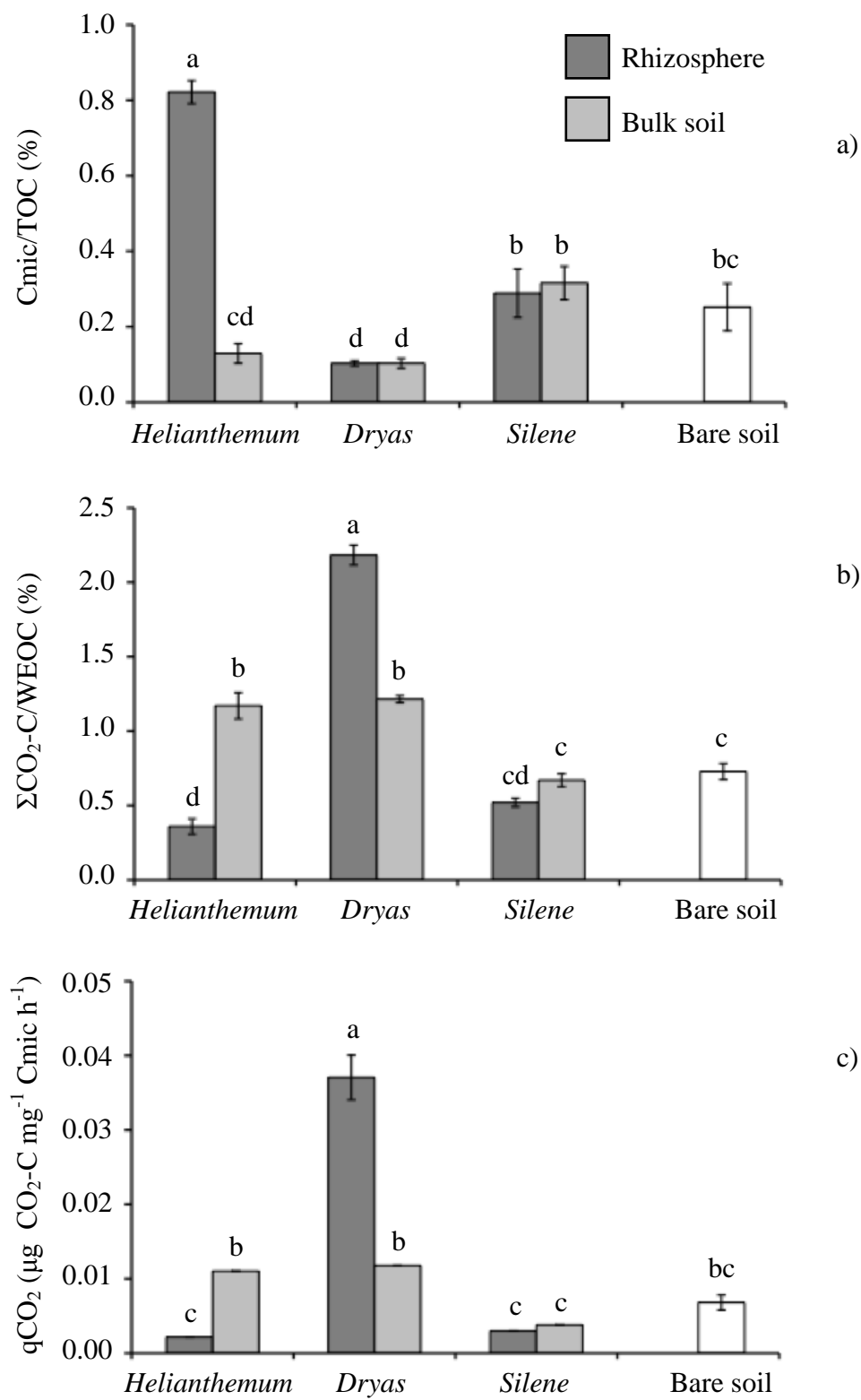


Figure 5

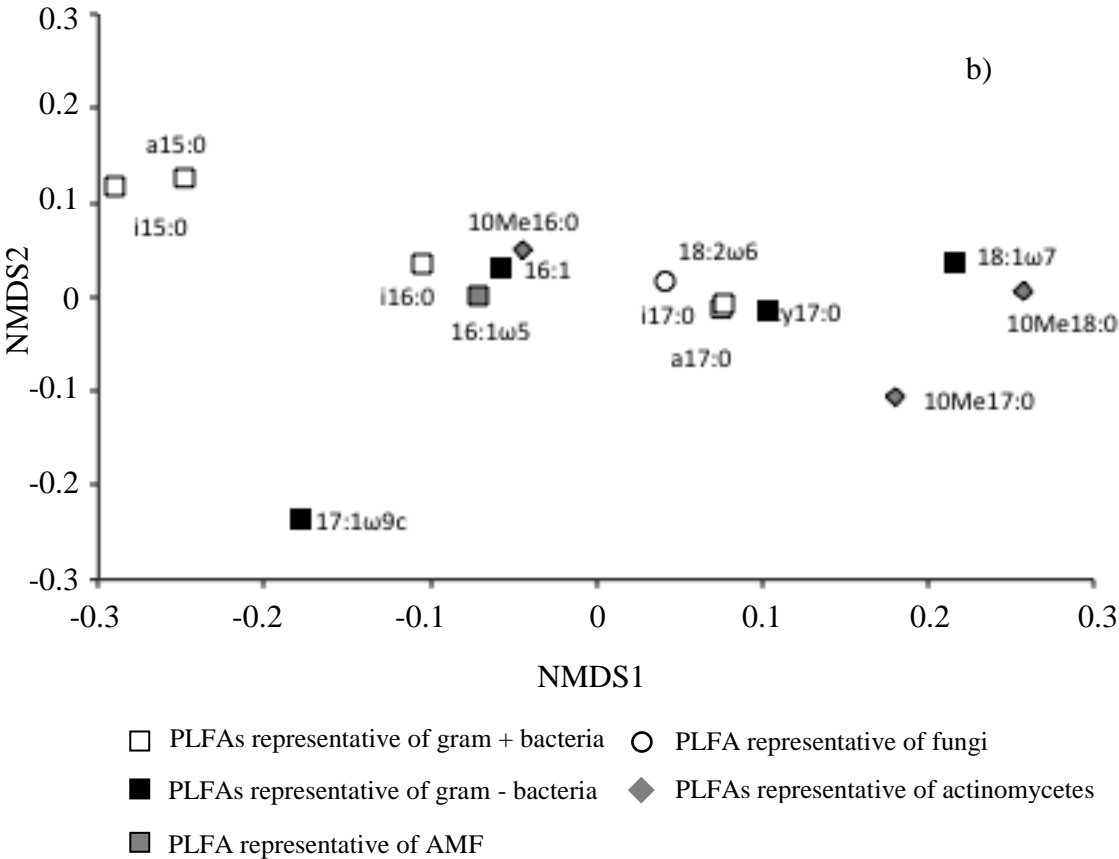
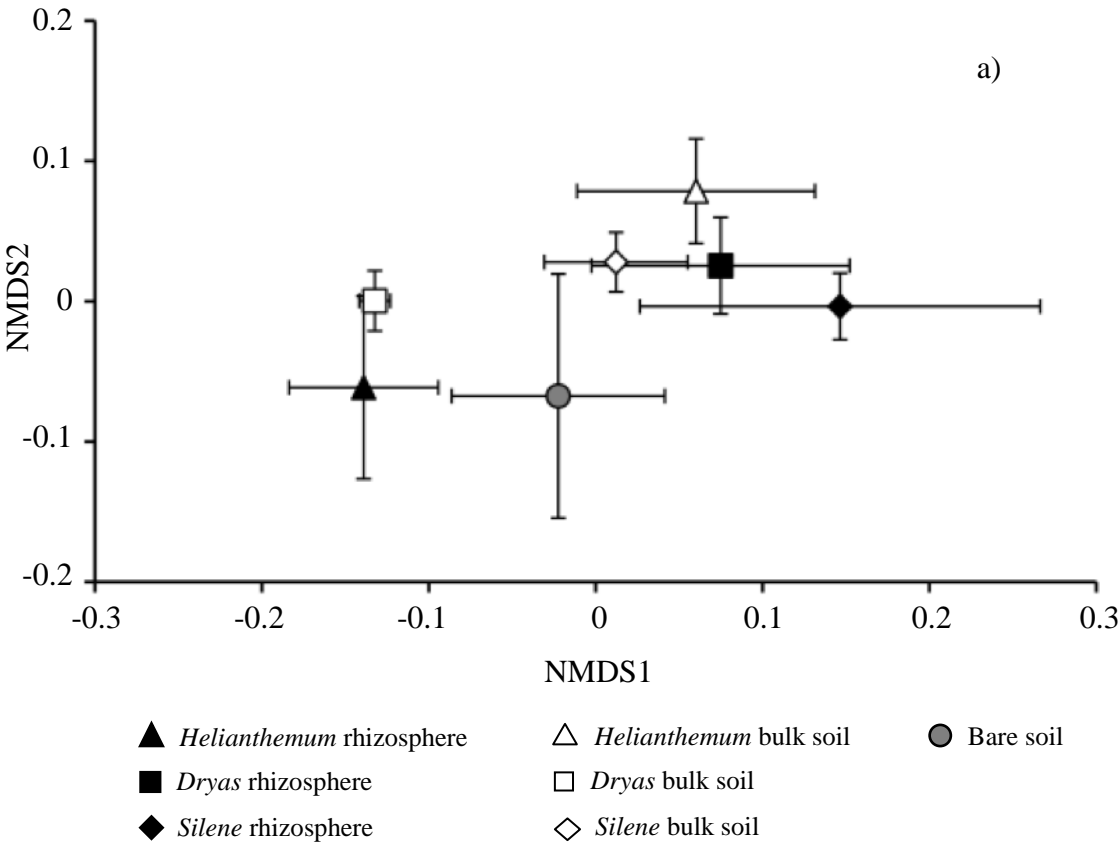


Figure 6

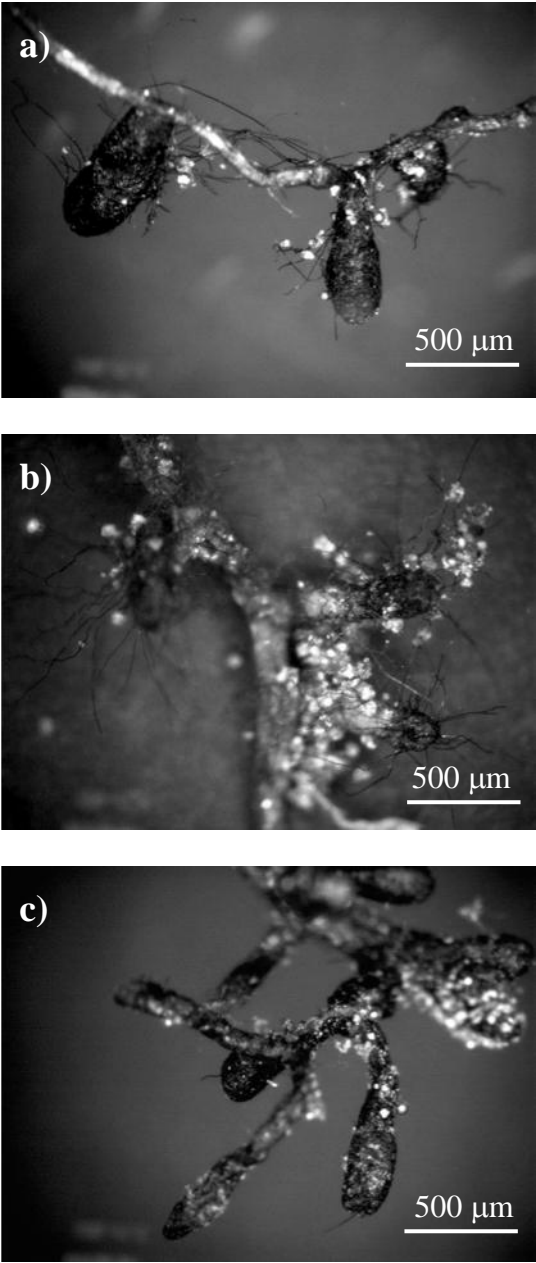
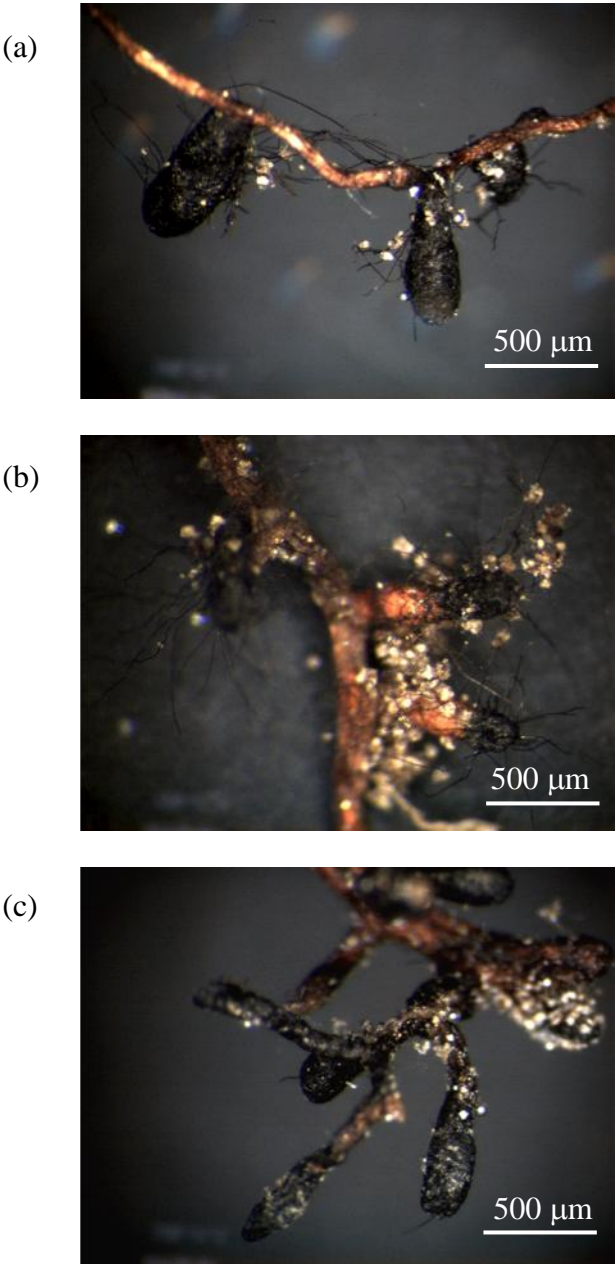
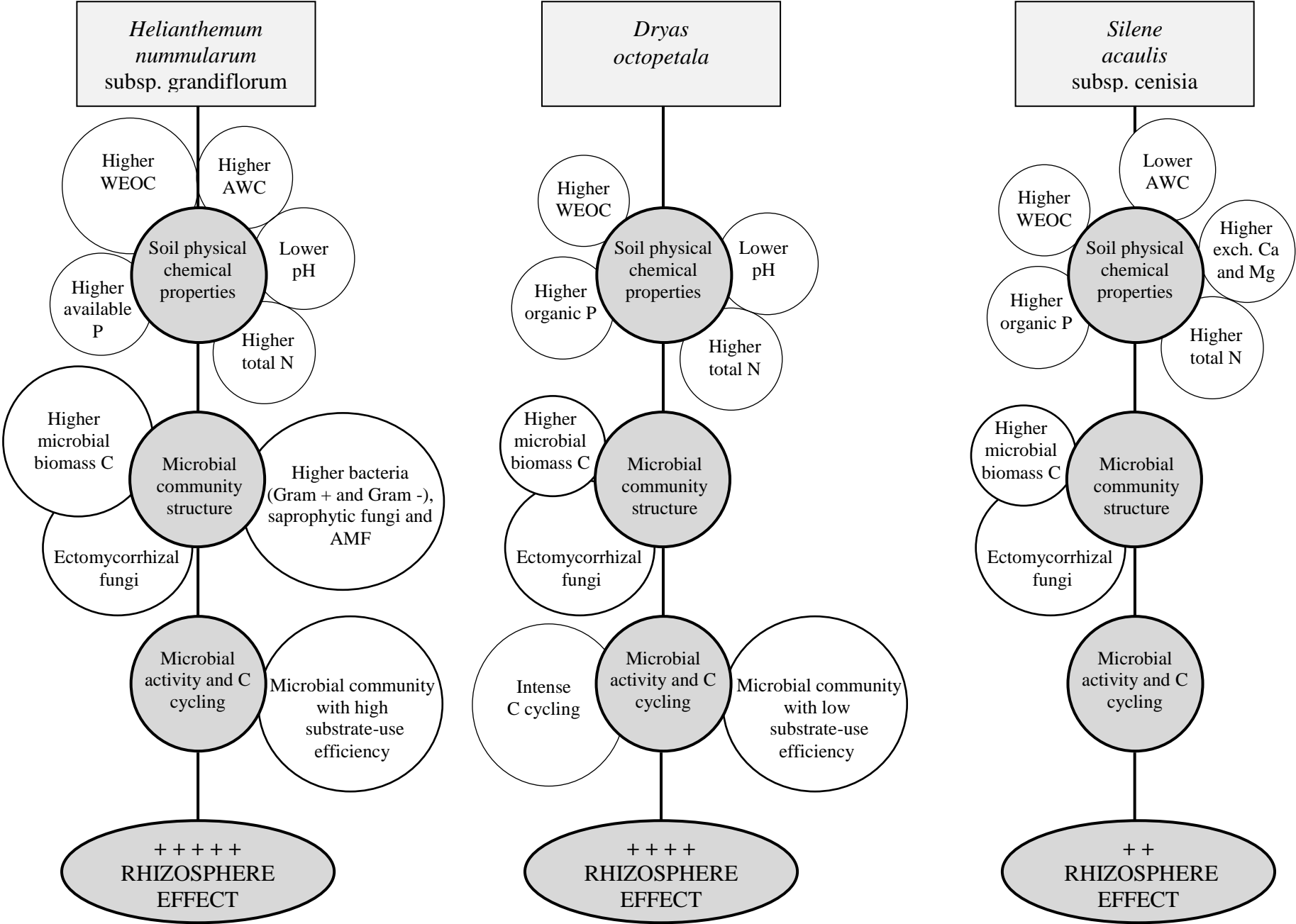


Figure 6 colour

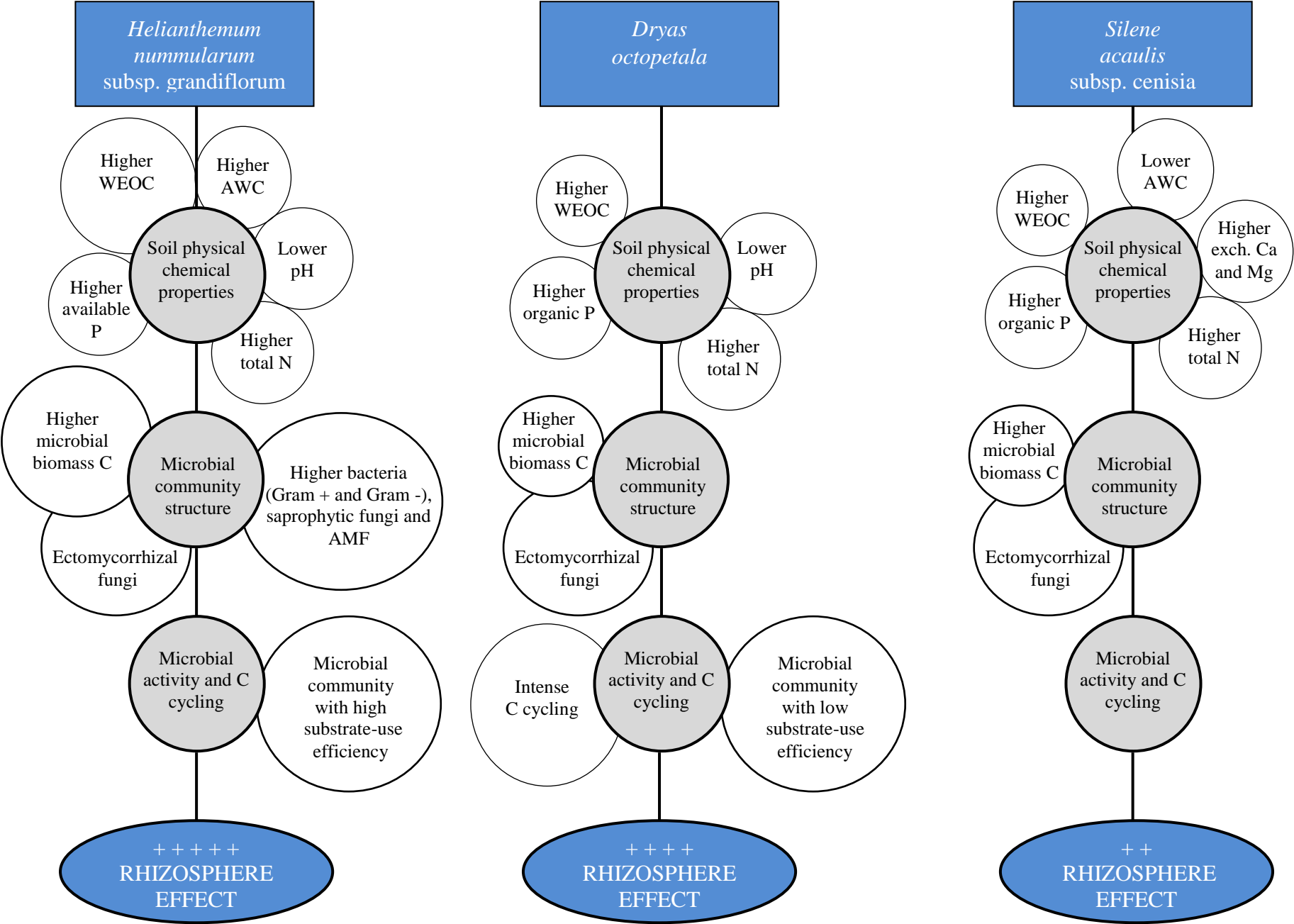


Figure





Figure



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