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Abstract

Water-extractable organic matter (WEOM) is the most dynamic and bioavailable fraction of the soil organic matter pool. Although the litter floor is considered the main source of WEOM, roots also release a great amount of labile organic compounds through rhizodeposition processes. This makes the rhizosphere, the small soil volume in proximity to the roots, a soil compartment relatively enriched in WEOM. Since both the rhizosphere and the labile organic C pool are highly sensitive to the environmental conditions we evaluated the characteristics of WEOM from rhizosphere and bulk soil collected from the A horizons of European beech (*Fagus sylvatica* L.) forest soils of Apennines mountains (central Italy) at two altitudes (800 and 1000 m), using elevation as a proxy for temperature change. Specifically, we tested if *i)* the rhizosphere contains higher amounts of WEOM with a greater diversity of compounds with respect to the bulk soil, and *ii)* this effect is more pronounced at higher altitude. At both 800 m and 1000 m above sea level, the main distinction between WEOM from rhizosphere and bulk soil was the larger amounts of sugars in the soil close to the roots. Further, our results indicated an influence of altitude on rhizospheric processes as suggested by the larger concentrations of organic C and soluble phenols, and richness of tannins in the rhizosphere WEOM than in the bulk soil at 1000 m. We attributed this influence to environmental constraints which enhanced the release of labile organics and secondary metabolites by rhizodeposition and humification processes in the rhizosphere. As a whole, our data draw a picture where the roots are able to affect the characteristics of WEOM and environmental constraints enhance the differentiation between rhizosphere and bulk soil. This view confirms the influence of the rhizosphere on the soil C cycle, and the importance of the rhizospheric processes when environmental conditions become harsher.

Keywords: labile soil C pool; mountain soils; polyphenolic compounds; *ESI FT-ICR MS;* climate change

1. Introduction

Soil water-extractable organic matter (WEOM) is a mixture of macromolecules and low molecular weight compounds produced by decomposition of fresh (leaf litter, root exudates, decaying fine roots) and old soil organic matter (McDowell, 2003). WEOM is an organic fraction that accounts for a small portion on soil organic matter (SOM), and is composed of easily degradable molecules that represent the main C and energy source for the soil microbial community (Smolander and Kitunen 2002; Kaiser and Kalbitz, 2012). Because of this, WEOM is therefore the most dynamic and bioavailable fraction of the SOM (McDowell, 2003) and it is often considered as an indicator of microbial activity (Gutiérrez-Girón et al., 2015).

Although the decomposition of the litter floor is considered the main source of WEOM (Kalbitz and Kaiser, 2007), roots release a wide range of labile and soluble organic compounds into the soil. In fact, plants can release 10 to 40 % of their total net C assimilation per year from their roots through respiration and rhizodeposition in the form of root exudates, mucilage, sloughed-off cells and decaying roots into the soil environment (Lynch and Whipps, 1990; Bertin et al., 2003). The allocation of labile compounds through rhizodeposition processes makes the rhizosphere, the small soil volume in proximity to the root, a soil compartment enriched with WEOM compared to the bulk soil (e.g., Fujii et al., 2012; Massaccesi et al., 2015; Agnelli et al., 2016). Conversely to the rhizosphere, in the soil far from the roots the labile organics mainly derive from the decomposition of SOM (Jones et al., 2009). The easily decomposable organic compounds released by rhizodeposition processes fuel the rhizosphere heterotrophic microbial community (Boddy et al., 2007; Phillips et al., 2011), which in turn may boost SOM decomposition by the priming effect (Kuzyakov, 2002).

WEOM is involved in several biogeochemical processes, and its molecular composition and concentration in soil are highly sensitive to ecosystem disturbances, such as temperature changes. Many authors reported on the influence of temperature on WEOM properties, although their findings were contrasting possibly because the effect of temperature varied depending on the applied methodologies and the nature of the studied ecosystems. For example, Hassouna et al. (2010)

recorded a weak impact of seasonal temperature variations on WEOM quantity in cropland soils, while Roth et al. (2015) found that during the warm seasons forest soils showed a lower abundance of labile organic compounds with respect to colder seasons. Williams et al. (2016) detected no seasonal variation in the amount of soluble phenols in pasture soils. Delarue et al. (2014), by using an in-situ experimental warming with open-top chambers, reported that the soluble organic matter increased in the subsurface of *Sphagnum*-dominated peatland at higher temperatures. Further, in laboratory experiments, Ultra et al. (2013) and Yin et al. (2013a, b) found that high temperatures may increase the amount of WEOM by increasing organic C inputs into the soil via enhanced root exudation. The effect of the temperature was also tested by using altitude as a proxy for temperature change (Vincent et al., 2014; Gutiérrez-Girón et al., 2015). In fact, an increase in the contents of more labile C compounds with altitude was reported in both cultivated (e.g., Chen et al., 2006) and natural (e.g., Fernández Sanjurjo et al., 2003; Xu et al., 2015) soils.

The objective of the present study was to evaluate the effect of the altitude-depending temperature on the quantity and quality of the labile and potentially soluble organic pool in the rhizosphere and bulk soil of Mediterranean mountain areas under European beech (*Fagus sylvatica* L.). We compared WEOM properties of rhizosphere and bulk soil of A horizons under European beech forests at two altitudes (800 and 1000 m) on three mountains in central Italy. It is noteworthy to say that the sites at 800 and 1000 m altitude had a mean annual air temperature that differed by 1°C, which is the expected increase of the air temperature for the year 2050 (IPCC, 2013). Through the combination of chemical and spectroscopic analyses we tested the hypotheses that i) the rhizosphere contains higher amounts of WEOM with a greater diversity of compounds with respect to the bulk soil, and ii) this effect is more pronounced at higher altitude since the rhizosphere is more sensitive than the bulk soil to changes of environmental conditions.

2. Materials and methods

2.1. Study areas and soil features

As study areas, three calcareous massifs were selected on the Apennine chain (central Italy): Mount Terminillo (42°28' N, 12°59' E), Mount San Vicino (43°19' N, 13°03' E), and Mount Acuto (43°28' N, 12°41' E) (Figure S1 of the Supplementary Materials). Two European beech (*Fagus sylvatica* L.) forests were selected at each site at about 800 and 1000 m above sea level, on slopes with an inclination ranging from 25° to 40° and northern aspect. All the forests were coppices in conversion, with the conversion that started from about 20 to about 40 years ago. The mean annual air temperature (MAAT) in the three study areas was 10°C at 800 m and about 9°C at 1000 m with January as the coldest month and July the warmest one. The mean annual precipitation was 1248 mm at Mount Terminillo, 825 mm at Mount San Vicino, and 1430 at Mount Acuto. All the soils had a *mesic* soil temperature regime, and an *udic* soil moisture regime. A detailed description of each site is reported in Table S1 of the Supplementary Materials.

All soils developed from limestone and their morphology, reported in Table S1 of the Supplementary Materials, was obtained per Schoeneberger et al. (2012). Here it suffices to recollect that the mean thickness of the *solum* was 68.8 cm (standard error 15.9) for the soils at 800 m, and 49.3 cm (standard error 9.5) for those at 1000 m. All the soils hosted an *amphimus* type of humus (Baize and Girard, 2008) which is characterized by well-developed O horizons rich in mesofauna that rest on an A horizon with a well-developed crumb structure. According to Soil Survey Staff (2014) the soils at 800 m were classified as Inceptisols (Humudepts) and Mollisols (Hapludolls and Haprendoll), while those at 1000 m were all Mollisols (Haprendoll). Hence, slightly harsher environmental conditions existed at 1000 m because of a lower MAAT and a general reduced soil development than at 800 m.

2.2. Soil sampling

During the late winter 2014 two profiles were opened within a plot of about 100 m^2 at each altitudinal site (3 study areas x 2 altitudes x 2 profiles). The winter sampling was chosen because European beech is a dormant species, and the low rhizosphere and soil microbial community activities occurring during the cold season allow comparing rhizosphere and bulk soil in a more stable condition rather

than in the dynamic seasons like spring or summer (De Feudis et al., 2016). The profiles were opened at about 50-60 cm from the stem of selected beech trees with an age ranging between 40 and 65 years old. The influence of the different age of the selected trees on the rhizosphere properties was assumed to be negligible because we considered as rhizosphere only the soil associated with fine roots, the activity of which is rather independent of plant age because of their periodic turnover (Trumbore and Gaudinki, 2003; Agnelli et al., 2014).

A large amount of sample (at least 3 kg) was collected from the A horizon of each soil and stored in a portable refrigerator. The beech rhizosphere of each sample was isolated in the laboratory by picking up the roots with a diameter lesser than 2 mm together with the adhering soil (Cocco et al., 2013; Massaccesi et al., 2015). The roots with a greater diameter than 2 mm were discarded. After a gentle shaking to detach the weakly adhering soil particles, which were then added to the bulk soil (i.e. the soil not strictly adhering to the roots), the soil material firmly adhering to the fine roots was considered as rhizosphere and recovered by further shaking and soft brushing. The root fragments present in the bulk soil were removed by using tweezers under a magnifying lens. Both rhizosphere and bulk soil samples were air-dried and sieved through a 2-mm mesh.

2.3. Soil chemical analysis

The soil pH was determined potentiometrically in water (pH_{H2O}) and in 1 M KCl solution (pH_{KCl}) (solid:liquid ratio of 1:2.5) after 30 minutes of stirring by a combined glass-calomel electrode.

The content of total organic C (TOC) was estimated by K-dichromate digestion, heating the suspension at 180 °C for 30 minutes (Nelson and Sommers, 1996), and total N content was determined by a Carlo Erba EA1110 dry combustion analyzer (Carlo Erba Instruments, Italy). Available P was estimated according to Olsen et al. (1954).

Total phenol contents were extracted according to Alonso et al. (1998) with a solvent mixture of methanol-distilled water (4:1 v:v ratio) with 2 % trimethylamine. Briefly, 5 mL of solvent mixture were added to 1 g of soil sample, and shaken for 1 h with an orbital shaker (130 rpm). The suspension

was centrifuged at 1400 *g* for 10 min, and then filtered through a 0.45 μm membrane filter. The content of total phenols in the extracts was determined colorimetrically by the Prussian blue method (Graham, 1992; Hagerman, 2002). To a mixture composed of 0.1 mL of extract and 3 mL of distilled water, 1 mL of 0.016 M $K_3Fe(CN)_6$ (Prussian blue) solution was added, followed immediately by the addition of 1 mL of 0.02 M $FeCl_3$ + 0.1 M HCl solution. The mixture was put in a vortex for a while and then left to rest for 15 minutes. Subsequently, 5 mL of stabilizer (800 mL of distilled water + 100 mL of 85 % H_3PO_4 solution + 100 mL of 1 % gum arabic solution) were added and the absorbance was read at 700 nm by means of a Lambda EZ 150 UV/VIS Spectrometer (Perkin Elmer, USA) against a gallic acid standard. The total phenols concentration was normalized against TOC content and expressed as mg gallic acid equivalent g^{-1} organic C.

2.4. Cross-Polarization Magic-Angle-Spinning Carbon-13 NMR Spectroscopy (CPMAS ^{13}C NMR)

Solid-state ^{13}C NMR spectroscopy was employed to assess the main structural components of the SOM in the whole soil samples. Solid-state ^{13}C NMR spectra were acquired with the cross-polarization magic-angle-spinning (CPMAS) technique performed at 15 kHz, using a 7.05 T Varian INOVA™ Unity (Varian Inc., Palo Alto, CA, USA) spectrometer at a frequency of 75.4 MHz. The spectra were collected with a sweep width of 25 kHz and an acquisition time of 20 ms. Contact time for cross-polarization and recycle delay were 1250 μs and 0.5 s, respectively. The ^{1}H radio frequency (RF) field strength was set to 47.0 kHz and the ^{13}C RF field strength to 41.1 kHz. An ascending ramp of 15.3 kHz on the ^{1}H RF field was used (Berns and Conte, 2011).

2.5. Water-extractable organic matter (WEOM)

2.5.1. WEOM extraction

The lack of a standardized method to obtain WEOM makes this organic material an operationally defined fraction as its composition depends on the combination of several factors such as, for example, extracting solution (water or dilute saline solution), sample pre-treatment (fresh or air-dried)

soil:solution ratio, contact time, and energy of the treatment (Jones and Willett, 2006). Therefore, the organic matter obtained by a very mild extraction with a low ionic-strength aqueous solution, a 1:2 solid:liquid ratio, a short contact time (1 min) and a gentle stirring of the mixture is considered representative of the soil solution dissolved organics collected in situ with zero-tension lysimeters (Zsolnay 2003; Chantigny et al., 2008). In contrast, a more vigorous extraction using distilled water with wider solid : liquid ratio (1:5 or 1:10) and overnight shaking solubilizes greater amount of organics because of the partial aggregate disruption and release of organic molecules that are not typically in the soil solution (Kaiser et al., 2015). To this regard, since the goal of this study was to evaluate the effect of altitude on the whole labile and potentially soluble organic pool of rhizosphere and bulk soils, we chose to extract the WEOM by the following procedure (Agnelli et al., 2016): 1 g of rhizosphere or bulk soil sample was placed into a plastic container, submerged with distilled water (solid:liquid ratio 1:10) and shaken (140 rpm) overnight with an orbital shaker. The suspension was centrifuged at 1400 *g* for 10 min, and then filtered through a 0.45 μm membrane filter. The obtained solution was stored in the refrigerator and analyzed within a week.

2.5.2. *Chemical analyses*

The organic C content of the WEOM was analyzed by a TOC-5000A analyzer (Shimadzu Corp., Tokyo, Japan) on aliquots of the extracted solution after the addition of a few drops of concentrated H_3PO_4 to eliminate inorganic C.

The contents of hexose and pentose sugars in the WEOM were colorimetrically estimated (Hofman and Dušek, 2003) by using the anthrone and orcinol methods, respectively. Briefly, for the hexoses, 5 mL of anthrone reagent (0.2 g of anthrone + 5 ml of ethanol + 95 ml of 75% H_2SO_4) were added to 1 mL of extracted solution in a shaking ice-water bath. Once the mixture was cooled it was heated in a boiling water bath for 10 minutes and, then, cooled again in an ice-bath. Once cooled, the absorbance was measured at 625 nm against a glucose standard. For the pentoses, 3 mL of orcinol reagent (0.5 g of orcinol + 50 ml of ethanol + 0.18 g $FeCl_3 \cdot 6H_2O$ in 200 ml of concentrated HCl)

were added to 1 mL of extracted solution, and the mixture was heated in a boiling water bath for 20 minutes. Once cooled, the absorbance was read at 672 nm using ribose as standard. The phenols concentration in the WEOM was determined colorimetrically by the Prussian blue method.

2.5.3. Electrospray Ionization Fourier Transform-Ion Cyclotron Resonance Mass Spectrometry (ESI FT-ICR MS)

WEOM aliquots were analysed by an ESI FT-ICR MS which measures the mass-to-charge ratio of organic molecules after their ionisation. The electrospray ionization induces the breaking of the non-covalent bonds such as Van der Walls forces and electrostatic interaction, and typically allows the detection of molecules with a mass lesser than 1000 Da (Hertkorn et al., 2008; Stubbins and Dittmar, 2015). The ultra-high resolution in combination with exact mass characterisation (here below 1 ppm) of this technology allows identification of thousands of compounds for each sample (Sleighter and Hatcher, 2007). However, the FT-ICR MS cannot be used as quantitative analysis (Sipler and Seitzinger, 2008) because the polarity of the compounds affects the intensity of the signals: the higher the polarity, the higher the intensity. To prevent quenching processes due to soil inherent permanent ions a combined desalting and pre-concentration of the WEOM samples was performed by solid phase extraction (SPE, C18 hydra cartridges, Machery & Nagel, Düren, Germany), using methanol (≥99.98 %, Ultra LC-MS grade; Carl Roth, Germany) as back eluent. The mean C recovery achieved with the SPE cartridges ranged between 66 and 71%, in agreement with the values reported in literature (e.g., Stubbins at al., 2012; Stubbins and Dittmar, 2015).

The ultra-high-resolution mass spectra were acquired using an ESI-LTQ-FT Ultra (ThermoFisher Scientific, San Jose, CA, USA) equipped with a 7 T supra-conducting magnet (Oxford Instruments, Abingdon, UK). The mass spectrometer was used in negative ionization mode with 2.9 kV spray voltage, -50 V capillary voltage, 275 °C transfer capillary temperature, and nitrogen as sheath gas.

The samples were introduced by a syringe pump providing an infusion rate of 8 µl/min, and full FT-ICR MS spectra between 200 and 1000 Da were recorded at a mass resolving power of 400.000 at

m/z 400 Da. The spectra were averaged out of 7 scans, and each scan was accumulated from 50 transients. Moreover, the ICR cell filling was adjusted to 500.000 ions per transient to obtain an accuracy of 0.4 ppm. The formula assignment was performed by an in-house developed calculation program using Scilab routines. The results obtained from the ESI FT-ICR MS were presented by plotting van Krevelen diagrams (Kim et al., 2003) of H/C versus O/C molar ratios of the CHO compounds (Hofmann et al., 2015). To estimate the proportion of WEOM components four regions were delineated within the diagrams according to Sleighter and Hatcher (2007) and Ohno et al. (2010), to cluster the detected molecules within the typical ranges of selected biopolymers: lignins (H/C: 0.7-1.5, O/C: 0.1-0.67); tannins (H/C: 0.5-1.4, O/C: 0.67-0.9); condensed aromatics (H/C: 0.2-0.7, O/C: 0.1-0.67); sugars (H/C: 1.5-2.0, O/C: 0.67-1.0).

2.6. Statistical analysis

Two-way ANOVA was carried out by including rhizosphere and bulk soil (2 levels) and altitudes (2 levels) as fixed factors. The study area (3 levels) was also included as blocking factor. The normality and homoscedasticity of the data were verified by graphical analysis of residuals and transformed if necessary. The transformation was selected by the maximum likelihood procedure suggested by Box and Cox (1964), as implemented in the boxcox function of the package MASS (Venables and Ripley 2002). The results presented and discussed are based on mean values and standard error, and the comparison of means was assessed by Tukey HSD post-hoc test ($P < 0.05$).

Principal component analysis (PCA) was applied only to WEOM properties (contents of water-extractable organic C, pentoses, hexoses and phenols, and richness of lignins, tannins and condensed aromatic compounds) to identify the variables capable of explaining most of the variability between the two altitudes and between rhizosphere and bulk soil. The WEOM dataset was standardized prior to PCA by subtracting the mean and dividing by the standard deviation.

The data were analyzed using R software (R Core Team, 2014).

3. Results

3.1. Rhizosphere and bulk soil properties

The bulk soil showed a higher pH in H_2O and in KCl at 1000 than at 800 m of altitude, while no difference was observed for the pH of the rhizosphere at both altitudes (Table 1). The TOC content was greater in the rhizosphere than in the bulk soil for both altitudes, while the total N content was higher at 1000 than at 800 m, with no difference between rhizosphere and bulk soil (Table 1). The content of available P was similar at the two altitudes, but always with higher contents in the rhizosphere than in the bulk soil (Table 1). Total phenols concentrations, once normalized to the TOC content, showed no difference between rhizosphere and bulk soil, while a greater amount was found at 800 m than at 1000 m (Table 1).

The CPMAS ^{13}C NMR spectroscopy detected six functional groups: alkyl-C like lipids and cutin at 0–45 ppm, N-alkyl-C like amino acids at 45–60 ppm, O-alkyl-C such as carbohydrates, hemicellulose, and cellulose at 60–110 ppm, aryl-C such as lignins, tannins, and aromatic C compounds at 110–160 ppm, carboxyl-C like carboxyl acids at 160 – 190 ppm, and carbonyl-C such as aldehydes and ketons at 190–215 ppm. The relative concentration of these organic functional groups in the rhizosphere and bulk soils showed the following general trend: O-alkyl-C > aryl-C ≥ alkyl-C > N-alkyl-C = carboxyl-C > carbonyl-C (Table 2, and Figure S2 of the Supplementary Materials). Rhizosphere and bulk soil showed no difference in the content of the various C forms, but some difference in the SOM composition occurred between the two altitudes: O-alkyl-C and alkyl-C had a larger relative percentage at 800 m, whereas the aryl-C showed greater proportion at 1000 m.

3.2. WEOM extracted from rhizosphere and bulk soil

3.*2.1. Chemical and spectroscopic (ESI FT-ICR MS) analyses*

The water-extractable organic carbon (WEOC) concentration was similar at 800 and 1000 m above sea level (Table 3). However, whereas for the soils at 800 m no difference occurred between rhizosphere and bulk soil, at 1000 m the rhizosphere had higher WEOC content than the respective

bulk soil. The contents of hexose and pentose sugars were always larger in the rhizosphere than in the bulk soil, although no significant difference between the two altitudes occurred (Table 3). The concentration of water extractable phenols was greater in the rhizosphere at 1000 m, while it did not differ between rhizosphere and bulk soil at 800 m (Table 3).

The analysis of WEOM by ESI FT-ICR MS produced a large data set with thousands of peaks corresponding to molecules with different mass-to-charge ratios (figure not shown). In particular, the data obtained by the mass analyses showed a similar number of compounds (formulas) in all rhizosphere and bulk soil samples from both altitudes (Table 4). Further, the van Krevelen diagrams exhibited similar patterns for the different samples with most of the molecules clustered in the regions corresponding to lignins and tannins (Figure S3 of the Supplementary Materials, Table 4). However, while no difference was found between rhizosphere and bulk soil at 800 m, at 1000 m the rhizosphere showed a greater number of formulas that fell in the tannins and condensed aromatics regions of the van Krevelen diagram (Table 4). The low proportion of sugars detected in the WEOM of all samples was attributed to the fact that the ESI FT-ICR MS weakly ionizes oxygen-rich molecules such as carbohydrates without permanent charge (Hertkorn et al., 2007). Furthermore, such high polar substances could be partly lost in the SPE process.

3.*2.2. Principal Component Analysis (PCA)*

The first two components calculated by PCA explained 69.3% of the total variance (Figure 1). The variability along the PC1 was mainly affected by the content of pentoses, hexoses, WEOC, and the richness of tannins (Table 5). The variability along the second component (PC2) was mainly driven by the richness of lignins and condensed aromatic molecules (Table 5).

In the PCA scoring plot (Figure 1) the observations have been grouped (rhizosphere or bulk soil, 800 or 1000 m above sea level) and the 95% confidence ellipses were drawn for each group. The addition of confidence ellipses improved the interpretation of the scoring plot because they showed the degree of separation of the groups (Hammer and Harper, 2006; Montanari et al., 2016). The lack of

overlapping of the confidence ellipses drawn for the WEOM of rhizosphere and bulk soil at 1000 m indicated a distinction between the two groups. Conversely, this did not happen between the rhizosphere and bulk soil at 800 m. Further, the lack of overlapping of the confidence ellipses between the WEOM from the rhizospheres at the two altitudes showed a clear differentiation of the two groups.

4. Discussion

4.1. Chemical properties of rhizosphere and bulk soil at 800 and 1000 m of altitude

Although literature often reports lower pH in the rhizosphere than in the bulk soil (e.g., Pandey and Palni, 2007; Cocco et al., 2013; Massaccesi et al., 2015), in our case the absence of significant differences between the two fractions was likely due to the calcareous nature of the parent material, as protons released by the roots are neutralized by carbonate dissolution (Richter et al., 2007; Agnelli et al., 2016). However, the lower weathering rate occurring at higher altitudes (Riebe et al., 2004) particularly in soil developed on alkaline substrates in central Italy (e.g., Corti et al., 2012; Agnelli et al., 2016), was responsible for the generally higher pH values at 1000 than at 800 m. Altitude also had an effect on organic matter content (Dieleman et al., 2013; Prietzel and Christophel, 2014; Tashi et al., 2016). Indeed, the lower temperatures occurring at higher altitudes reduce the net primary production and the litterfall input (Zianis and Mencuccini, 2005; Bu et al., 2012), but also soil microbial activity (Yimer et al., 2006; Xu et al., 2014). The positive relationship between altitude and organic matter content was confirmed by the greater TOC and total N contents at 1000 m than at 800 m. At both altitudes, the larger amount of TOC and available P in the rhizosphere compared to the bulk soil was mainly attributed to C input due to root exudates, border cells and decaying roots (Hinsinger et al., 2009, Sokolova, 2015). With regards to P, this element is usually limited in soil because it mostly forms stable organic compounds or calcium- or iron-phosphates (Frossard et al., 1995; Nelson and Janke, 2007). However, plants are able to mobilize this element by the release of phosphatase enzymes (Nannipieri et al., 2011), and through exudation of low molecular weight organic acids (LMWOA), sugars, and phenolic compounds (Carvalhais et al., 2011; Bowsher et al.,

2015). In the same study sites De Feudis et al. (2016) attributed the higher P availability in the rhizosphere to an enhanced organic matter cycling triggered by root exudation.

The chemical structure of the organic matter of rhizosphere and bulk samples, as revealed by the ^{13}C NMR spectroscopy, was similar to that found by previous studies on forest floors (e.g., Rumpel et al., 2002; Cepáková and Frouz, 2015), and this similarity derived from the strong influence of the organic horizons on the underlying A horizon. In particular, the input of slightly decomposed organic residues into the mineral soil during forest floor decay could explain the general high proportion of O-alkyl C (Rumpel et al., 2002) which is the most represented constituent in the ^{13}C NMR spectra of beech leaves and roots (Angst et al., 2016). Hence, the higher amount of O-alkyl-C at the lower altitude suggested relatively greater litter decomposition due to the higher temperatures which could have favored a greater incorporation of cellulose- and hemicellulose-derived substances in the mineral horizons at 800 m than at 1000 m. In addition to the larger proportion of aliphatic compounds, also the higher concentration of extractable total phenols, which we considered as mostly derived from lignins and humic substances decomposition (Santana et al., 2009), would confirm the higher degradation degree of the organic pool at 800 m than at 1000 m. The results obtained from the ^{13}C NMR analysis, together with the TOC contents indicated that temperature affected both the quantity (through mineralization rate) and the quality of organic matter. Indeed, besides reducing the rate of organic matter degradation, the lower temperatures at the higher altitude could have promoted the accumulation of aromatic substances which further fostered a greater organic C storage at 1000 than at 800 m. The NMR analysis did not reveal any difference between the SOM of rhizosphere and bulk soil, although some differences were expected. However, the lack of differences between the two fractions might be masked by the fact that we analyzed the whole organic pool. Indeed, although one of the main roles of the roots is the enrichment of the rhizosphere with labile C compounds (Angst et al., 2016), these latter represent a very small proportion of the whole soil organic carbon pool. In our case, the WEOC was less than 0.5% of TOC.

4.2. WEOM of rhizosphere and bulk soil at 800 and 1000 m of altitude

The rhizosphere had a larger WEOC content than the bulk soil only at 1000 m where slightly harsher environmental conditions might have 1) affected the quality of root exudation (Chen et al., 2006; Guo et al., 2015), 2) increased the amount of labile C compounds (Fernández Sanjurjo et al., 2003; Xu et al., 2015), and 3) accelerated fine roots production and turnover (Graefe et al., 2008; Girardin et al., 2013). In contrast, rhizospheres at 800 and 1000 m showed no significant difference in WEOC content. These findings contrasted with those of Ultra et al. (2013) and Yin et al. (2013a, b) who reported that warming increases the amount of WEOC in the rhizosphere because of enhanced root exudation. However, these studies were run under controlled laboratory conditions where the only variable was the temperature and no other constraints occurred. Instead, the effect of altitude is more complex than that of an experimental warming because factors other than temperature (e.g., soil development and properties, fine roots turnover, nutrient availability) may control processes in the rhizosphere.

As supported by PCA (Figure 1), WEOM of the rhizosphere at 1000 m was qualitatively different from that of the respective bulk soil and the difference was mostly due to a greater richness of phenols, tannins and condensed aromatic compounds in the rhizosphere WEOM (Tables 3 and 4). Among the phenolic compounds, tannins occur in all the different plant tissues, especially in leaves, roots and bark (Kraus et al., 2003). However, as fine roots are a great part of forest biomass and often decay faster than the leaf litter (Vogt et al. 1996; Stevens et al., 2002), they can be considered a significant contributor of tannins to forest soils (Kraus et al., 2003). Hence, as at higher altitude roots undergo to a faster turnover (Graefe et al., 2008; Girardin et al., 2013), we attributed the larger presence of phenolic compounds in the rhizosphere than in the bulk at 1000 m to the harsher environmental conditions occurring at this altitude.

The richness of lignin and tannin compounds indicated a general presence of recalcitrant organic molecules (e.g., Kögel-Knaber, 2002; Ohno et al., 2010) in the WEOM of both rhizosphere and bulk soil. Indeed, these carboxyl-rich alicyclic molecules are considered as products derived from

decomposition of organics and important substrate for the formation of humic substances (e.g., Kraus et al., 2003; Hertkorn et al., 2006; Buondonno et al., 2014). At 1000 m, the rhizosphere displayed a greater diversity of compounds belonging to the condensed aromatics class than the bulk soil (Table 4). This fact was interpreted as the result of an increase of the humification rate in the rhizosphere at the higher altitude, with the consequent indirect increase of the number of recalcitrant compounds (Bayer et al., 2002) in the vicinity of the roots. These larger number of condensed aromatic compounds could be partly due to the mobilization of humic substances promoted by the root exudation of LMWOA which are able to break bridges between metals and humic moieties by complexing metal cations (Takeda et al., 2009).

The higher content of sugars in the rhizosphere than in the bulk soil at both altitudes suggested that the major source of these compounds was root exudation (Gunina and Kuzyakov, 2015). The release of sugars, as well as other low molecular weight organic compounds, is considered as a plant strategy to overcome environmental restrictions through the stimulation of the microbial biomass activity (Bowsher et al., 2015; Kaiser et al., 2015). However, we cannot exclude that the larger content of sugars in the rhizosphere was due to the activity of the microbial biomass that, besides using these compounds as an energetic substrate, is able to produce new carbohydrates both directly and following decomposition of plant residues (Gunina and Kuzyakov, 2015).

In addition to the above considerations the different WEOM characteristics between rhizosphere and bulk soil at 1000 m might be the result of the activity of different microbial communities. For example, the meta-analysis conducted by Blankinship et al. (2011) revealed a higher microbial diversity in soils subjected to lower temperatures, while an increased fungal diversity in the rhizosphere of an annual plant (*Eschscholzia californica* Cham.) was found at higher altitude by de Armas-Ricard et al. (2016).

5. Conclusions

We appraised the effect of altitude (800 and 1000 m) on some characteristics of WEOM extracted from the rhizosphere and bulk soil of beech forests in the Apennine mountains (central Italy). The main distinction occurring at both altitudes between WEOM of rhizosphere and bulk soil was due to the larger amounts of sugars in the former. The greater availability of these compounds in the rhizosphere WEOM was attributed to both rhizodeposition and microbial activity. We hypothesized that the influence of altitude on the rhizospheric processes, as revealed by the larger concentrations of organic C and soluble phenols, and richness of tannins in the rhizosphere WEOM than in the bulk soil at 1000 m, was due to environmental constraints that enhanced the release of labile organics and secondary metabolites by rhizodeposition processes. Furthermore, the presence of these easily degradable compounds in the rhizosphere could promote the degradation of more stable soil organic matter leading to an increase in richness of soluble condensed aromatic moieties. Hence, environmental conditions can affect the belowground C allocation with potential impact on the forms and amounts of the rhizosphere labile organic C pool.

Because of its capability to respond to disturbances, the rhizosphere WEOM could be taken as an index of environmental changes following temperature variations in both time and space. However, more studies on WEOM and its composition are needed to strengthen this aspect.

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Figure caption

Figure 1. Variation of the properties of the water-extractable organic matter (WEOM) of rhizosphere and bulk from soils under European beech *(Fagus sylvatica* L.*)* forest at two altitudes (800 and 1000 m), as analysed by principal component analysis (PCA) using standardized data. Central Apennines, Italy. Also the 95% confidence ellipses are drawn.

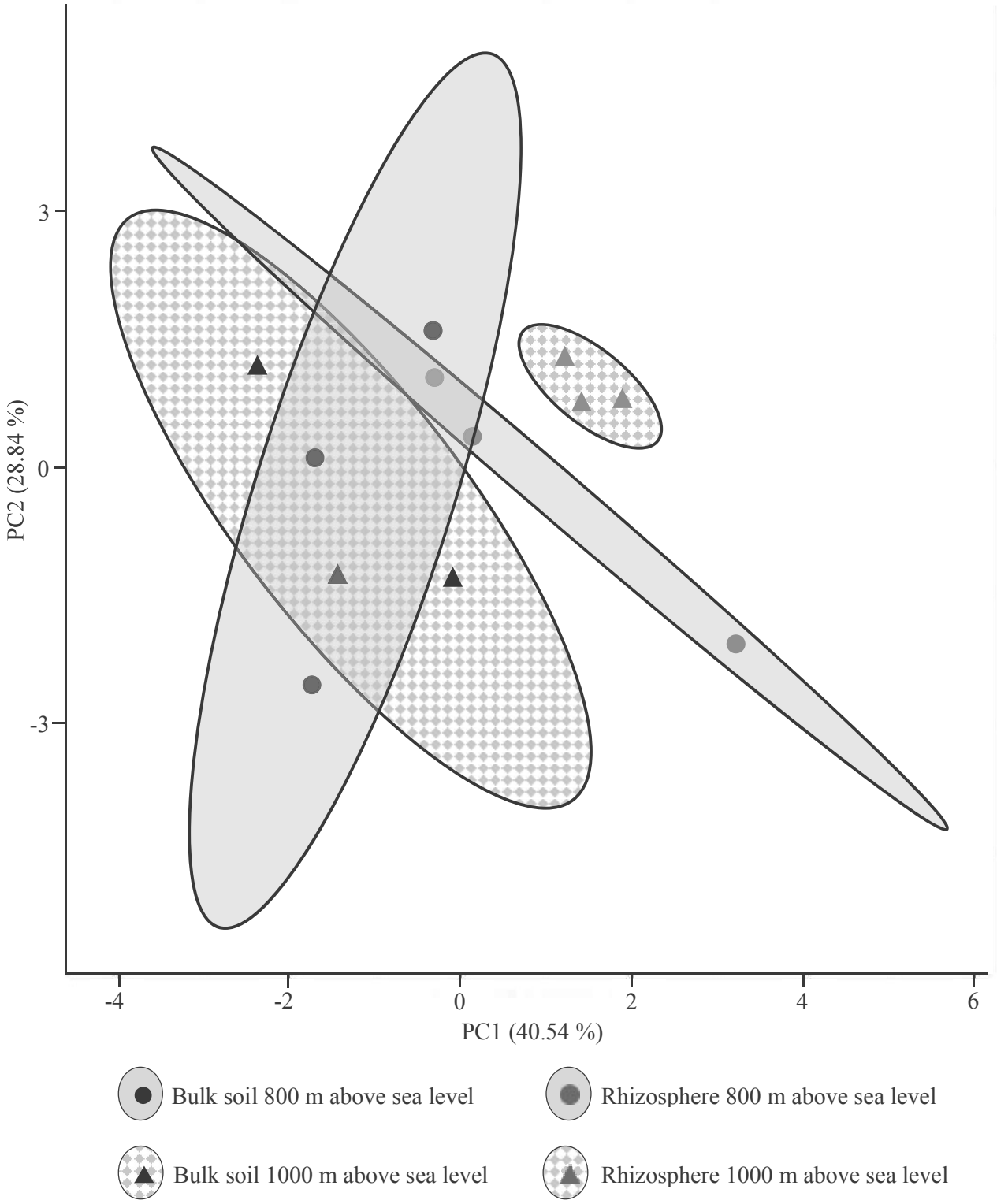
3
0
-3
PC2 (28.84 %)
-4
-2
0
2
4
6
PC1 (40.54 %)
Bulk soil 800 m above sea level
Rhizosphere 800 m above sea level
Bulk soil 1000 m above sea level
Rhizosphere 1000 m above sea level

Table 1. Main properties of rhizosphere and bulk from the soils under European beech (*Fagus sylvatica* L.) forest at two altitudes (800 and 1000 m). Central Apennines, Italy. Numbers in parentheses are the standard errors (n=3). For each line, mean values with different letters significantly differ for $P < 0.05$.

	800 m above sea level		1000 m above sea level	
	Rhizosphere	Bulk soil	Rhizosphere	Bulk soil
pH_{H_2O}	6.69 (0.29) b	6.77 (0.20) b	6.93 (0.14) ab	7.34 (0.15) a
pH_{KCl}	5.98 (0.27) b	5.99 (0.18) b	6.51 (0.13) ab	6.60 (0.12) a
Total organic C (g kg^{-1})	81.11 (5.31) b	68.52 (4.22) c	119.66 (6.25) a	91.69 (8.98) b
Total N (g kg^{-1})	5.44 (0.58) b	4.36 (0.48) b	10.75 (0.85) a	8.76 (0.93) a
Available P (mg kg^{-1})	26.07 (1.92) a	17.29 (1.39) b	29.38 (0.75) a	20.09 (0.69) b
Total phenols-to-total organic C (mg gallic acid equivalent g^{-1} TOC)	4.53 (0.27) a	4.59 (0.26) a	2.87 (0.13) b	3.10 (0.29) b

Table 2. Relative proportions of carbon containing functional groups as detected by CPMAS ^{13}C NMR analysis in rhizosphere and bulk from the soils under European beech (*Fagus sylvatica* L.) forest at two altitudes (800 and 1000 m). Central Apennines, Italy. Numbers in parentheses are the standard errors (n=3). Mean values with different letters significantly differ for $P < 0.05$.

Groups	800 m above sea level		1000 m above sea level	
	Rhizosphere	Bulk soil	Rhizosphere	Bulk soil
	% of spectral area			
Alkyl-C	20.3 (0.5) d	20.7 (0.4) d	17.5 (0.4) e	17.0 (0.4) ef
N-Alkyl-C	13.5 (0.6) gh	13.9 (0.4) g	12.4 (0.3) gh	11.9 (0.4) gh
O-Alkyl-C	34.4 (1.2) a	32.7 (0.5) a	30.9 (0.2) b	30.4 (0.4) b
Aryl-C	19.8 (0.4) de	19.8 (0.4) de	24.6 (0.6) c	25.3 (0.7) c
Carboxyl-C	11.0 (1.0) h	11.9 (0.7) gh	12.6 (0.5) gh	13.1 (0.7) gh
Carbonyl-C	1.1 (0.2) k	1.1 (0.2) k	2.0 (0.2) jk	2.4 (0.5) j

Table 3. Concentrations of water soluble organic C (WEOC), hexose and pentose sugars, and phenols of rhizosphere and bulk from the soils under European beech (*Fagus sylvatica* L.) forest at two altitudes (800 and 1000 m). Central Apennines, Italy. Numbers in parentheses are the standard errors (n=3). For each line, mean values with different letters significantly differ for $P < 0.05$.

	800 m above sea level		1000 m above sea level	
	Rhizosphere	Bulk soil	Rhizosphere	Bulk soil
WEOC (g kg^{-1})	0.36 (0.04) ab	0.30 (0.02) b	0.44 (0.03) a	0.30 (0.04) b
Hexose sugars (g glucose-C kg^{-1}soil)	0.10 (0.02) a	0.04 (0.01) b	0.09 (0.01) a	0.05 (0.01) b
Pentose sugars (g ribose-C kg^{-1}soil)	0.05 (0.01) a	0.03 (0.01) b	0.06 (0.00) a	0.02 (0.00) b
Phenols (μg gallic acid equivalent g^{-1} soil)	10.81 (0.66) b	11.68 (1.98) b	19.95 (1.94) a	10.12 (1.30) b

Table 4. Number of CHO formulas as detected by ESI FT-ICR MS analysis, and relative proportion of lignins, tannins, condensed aromatics, and sugars of water-extractable organic matter (WEOM) of rhizosphere and bulk from the soils under European beech (*Fagus sylvatica* L.) forest at two altitudes (800 and 1000 m). Central Apennines, Italy. Numbers in parentheses are the standard errors (n=3). For each column, mean values with different letters significantly differ for $P < 0.05$.

	N° of formulas	Lignins	Tannins	Condensed aromatics	Sugars	Others
				%		
800 m above sea level						
Rhizosphere	3403 (824) a	54.7 (6.2) a	26.6 (1.8) ab	6.4 (0.5) a	< 1	12.4
Bulk soil	4114 (926) a	67.5 (6.4) a	20.2 (5.0) b	5.8 (2.3) ab	< 1	6.4
1000 m above sea level						
Rhizosphere	2796 (94) a	56.1 (0.3) a	29.6 (1.5) a	5.7 (0.1) a	< 1	8.6
Bulk soil	3928 (249) a	61.8 (6.2) a	24.6 (1.3) b	2.3 (0.4) b	< 1	11.3

Table 5. Contributes of variables (%) and their correlation coefficients with PC1 and PC2 of the water-extractable organic matter (WEOM) from the soils under European beech (*Fagus sylvatica* L.) forest at two altitudes (800 and 1000 m). Central Apennines, Italy.

Chemical properties	PC1		PC2	
	%	Correlation coefficient	%	Correlation coefficient
Water-extractable organic C	17.96	0.71	3.65	-0.27
Pentoses	29.03	0.91	0.06	0.03
Hexoses	23.99	0.83	5.36	-0.33
Phenols	6.52	0.43	14.41	0.54
Lignins	6.40	-0.43	31.57	0.80
Tannins	13.84	0.63	15.14	0.55
Condensed aromatics	2.27	0.25	29.81	0.78

Supplementary materials

Altitude affects the quality of the water-extractable organic matter (WEOM) from rhizosphere and bulk soil in European beech forests

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Figure S1. Map of Italy showing the location of the three study areas (Mt. Terminillo, Mt. San Vicino, and Mt. Acuto). Central Apennines, Italy.

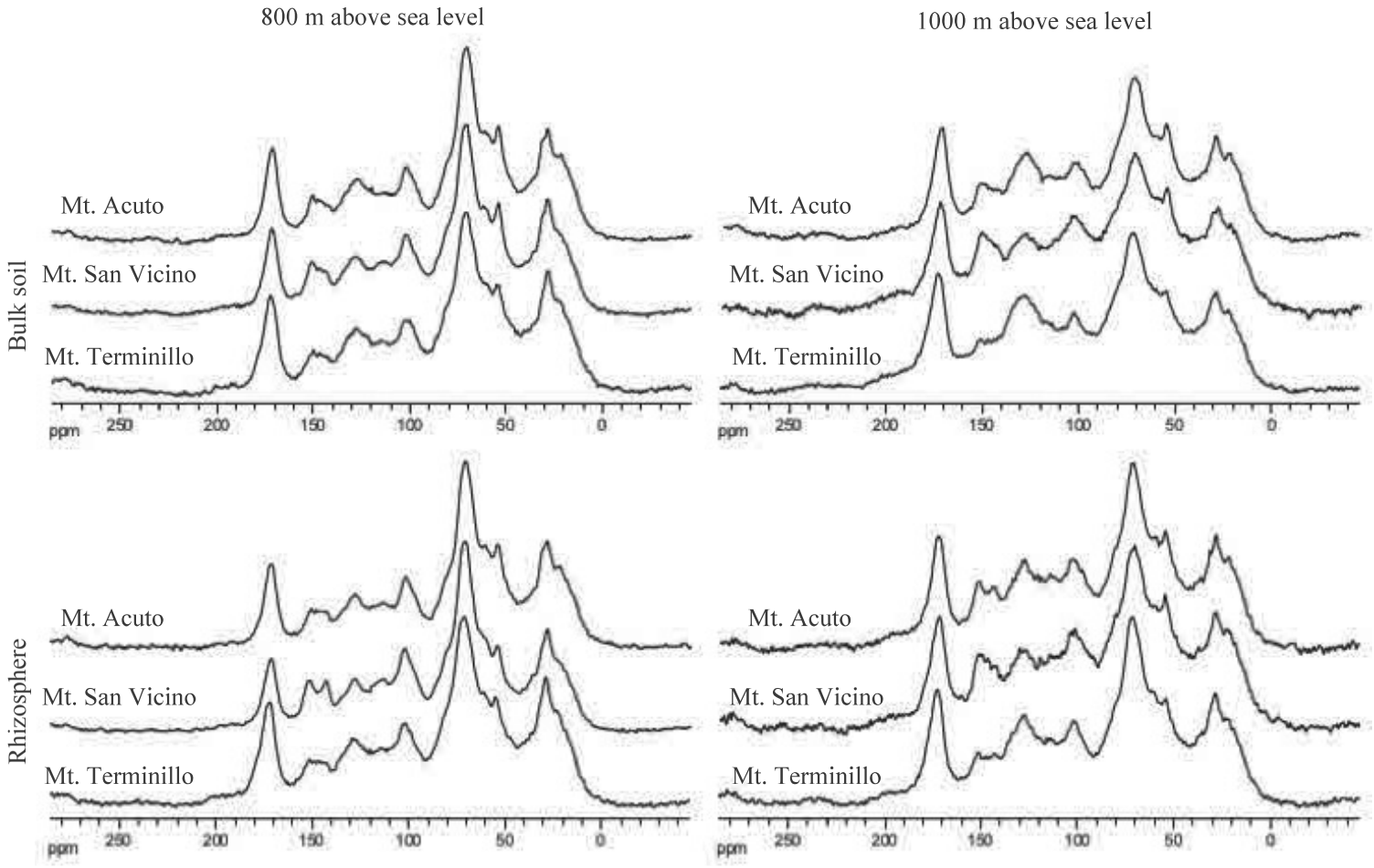

Figure S2. CPMAS ^{13}C NMR spectra of rhizosphere and bulk from the soils under European beech (*Fagus sylvatica* L.) forest at two altitudes (800 and 1000 m). Central Apennines, Italy.

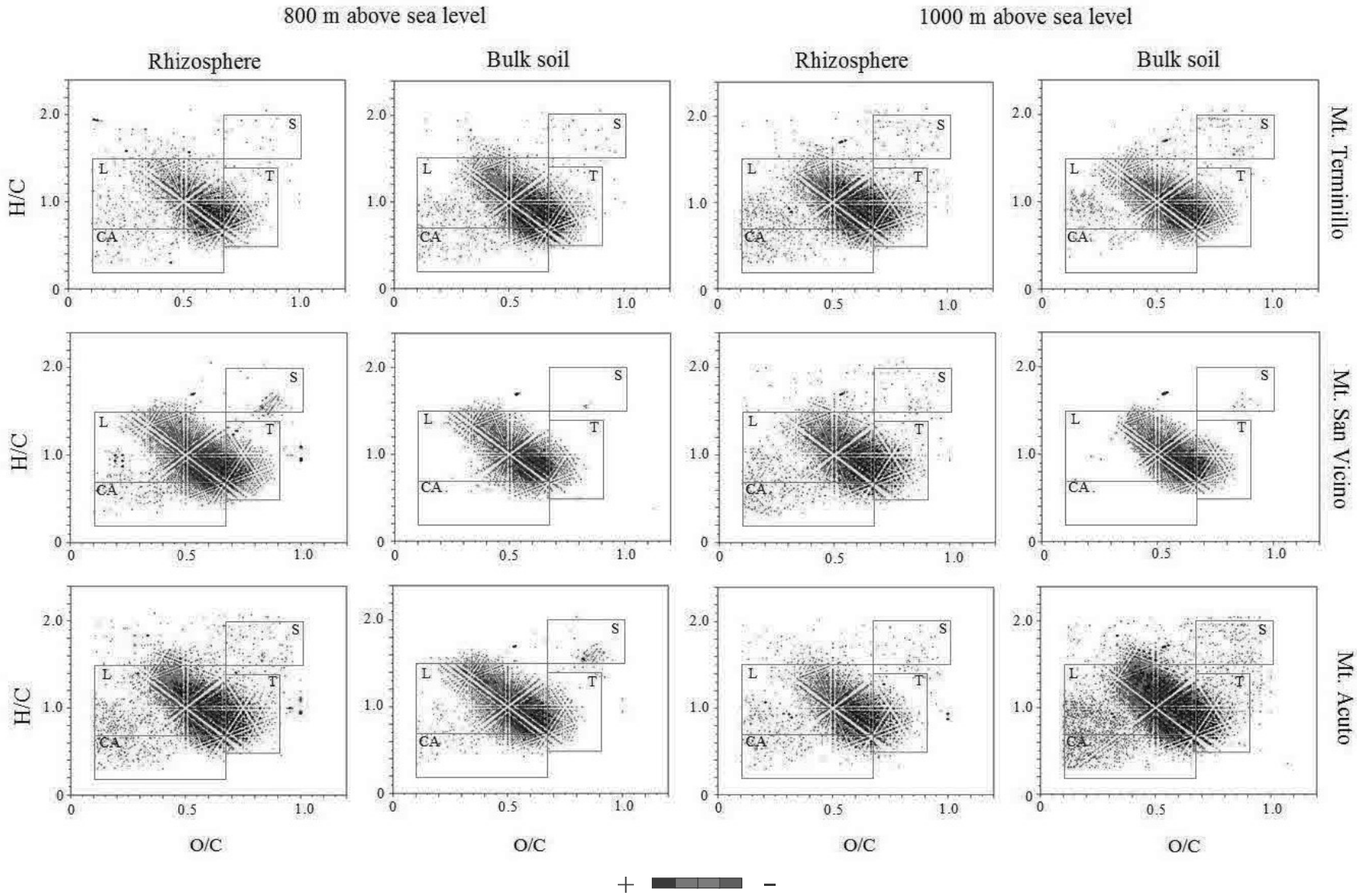

Figure S3. van Krevelen diagrams of H/C versus O/C molar ratios for the CHO compounds detected by the ESI FT-ICR MS in the water-extractable organic matter (WEOM) of rhizosphere and bulk from soils under European beech (Fagus sylvatica L.) forest at two altitudes (800 and 1000 m). Central Apennines, Italy. Boxes overlain on the plots indicate the major biopolymer classes [lignins (L), tannins (T), condensed aromatics (CA), sugars (S)], whereas colors indicate relative mass peak intensity.

Table S1. General information and features for the soils under European beech (*Fagus sylvatica* L.) forest at two altitudes (800 and 1000 m). Central Apennines, Italy. For symbols see legend.

Horizon	Depth	Colour[a]	Structure[b]	Roots[c]	Skeleton
	cm				%

Mount Terminillo. Parent rock: grey limestone with small flintstone layers. Forest management: coppice in conversion to timber forest. Exposure: N. Mean annual precipitation: 1248 mm. Soil temperature regime: *mesic*. Soil moisture regime: *udic*.

Altitude: 800 m
Slope: 40°; Mean annual air temperature: 10.0°C; Mean temperature of the coldest month (January): 1.6°C; Mean temperature of the warmest months (July and August): 19.4°C. Vegetation: *Fagus sylvatica* L. (95%), *Acer* spp., *Laburnum anagyroides* Medik..

Soil: loamy-skeletal, mixed, mesic, Typic Hapludoll (Soil Survey Staff, 2014)

Forest floor	9-0	-	-	0	0
A	0-8	2.5YR 2.5/1	3f,m sbk, fr	3mi,vf,f,m,co	40
AB	8-16	2.5YR	2m pl→2f,m sbk, fr	3mi,vf,f,m,co	10
Bw1	16-23	5YR 3/4	2m pl→2f,m sbk, fr	2mi,vf,f; 3m,co	10
Bw2	23-33	5YR 4/4	2m pl→2f,m sbk, fr	2mi,vf,f,m,co	70
Bw3	33-66	5YR 5/4	1f,m abk, fr	2mi,vf,f,m,co	70
BC	66-77+	10YR 6/6	sg & 1f abk, fr	1mi,vf,f; 3m,co	70

Soil: loamy, mixed, mesic Lithic Haprendoll (Soil Survey Staff, 2014)

Forest floor	4-0	-	-	0	0
A	0-9	2.5YR 2.5/1	3f,m sbk, fr	3mi,vf,f,m,co	10
AB	9-19	5YR 2.5/2	3f,m sbk+abk, fr	2mi,vf,f,m; 3co	40
R	19-22+	-	-	-	-

Altitude: 1000 m
Slope: 40°; Mean annual air temperature: 9.1°C; Mean temperature of the coldest months (January and February): 1.0°C; Mean temperature of the warmest month (July): 18.3°C. Vegetation: *Fagus sylvatica* L. (99%), *Acer* spp..

Soil: loamy-skeletal, mixed, mesic, Lithic Haprendoll (Soil Survey Staff, 2014)

Forest floor	17-0	-	-	0	0
A	0-11	2.5YR 2.5/1	3f cr+sbk, fr	3mi,vf,f,m,co	20
AB	11-20	5YR 2.5/1	3f abk+sbk, fr	2mi,vf,f; 3m,co	40
BA	20-28	5YR 2.5/2	3f abk, fr	1mi,vf,f; 2m; 3co	50
2Bwb	28-38	5YR 4/3	1f,m abk, fr	1mi,vf,f,m,co	20
2Crb	38-49+	10YR 5/4	1f,m abk, fr	1mi,vf,f,m,co	75

Soil: loamy, mixed, mesic, Lithic Haprendoll (Soil Survey Staff, 2014)

Forest floor	16-0	-	-	0	0
A	0-14	2.5YR 2.5/1	3f,m sbk+abk, fr	3mi,vf,f,m; 2co	5
AB	14-24	5YR 2.5/2	3f,m sbk+abk, fr	2mi,vf,f,m,co	30
Cr	24-45+	10YR 6/4	1m abk, fr	2f; 3m,co	75

Mount San Vicino. Parent rock: grey limestone with marl and flintstone layers. Forest management: coppice in conversion to timber forest. Exposure: N. Mean annual precipitation: 825 mm. Soil temperature regime: *mesic*. Soil moisture regime: *udic*.

Altitude: 800 m
Slope: 35°; Mean annual air temperature: 10.0°C; Mean temperature of the coldest month (January): 1.0°C; Mean temperature of the warmest month (July): 21.0°C. Vegetation: *Fagus sylvatica* L. (90%), *Quercus cerris* L.*, Castanea sativa* Mill..

Soil: coarse-loamy, mixed, mesic, Typic Hapludoll (Soil Survey Staff, 2014)

Forest floor	9-0	-	-	0	0
A	0-10	7.5YR 5/2	2ccr	3mi,vf,f,m,co	10
AB	10-16	7.5YR 4/2	2f,m abk+sbk, fr	2mi,mf,f	20
Bw1	16-30	7.5YR 5/4	2f sbk, fr	2mi,vf,f	20
Bw2	30-43	10YR 4/3	2f, cr+sbk, fr	1mi,vf,f,m	20
2Bwb	43-68	10YR 4/2	2f cr, fr	3mi,vf,f; 1m	70
3Bwb	68-81	2.5Y 2/0	1f abk, fr	3mi,vf,f; 2m	70
3Crb	81-90+	7.5YR 4/4	1f abk, fr	1vf,f	90

Soil: coarse-loamy, mixed, mesic, Typic Humudept (Soil Survey Staff, 2014)

Forest floor	7-0	-	-	0	0
A	0-9	7.5YR 5/2	2-3f cr, fr	3mi,vf,f,m	20
AB	9-16	10YR3/2	2f cr, fr	2mi,vf,f,m,co	30
Bw1	16-28	10YR5/4	2f sbk, fr	2mi,vf,f; 1m	40
Bw2	28-37	10YR3/4	3m sbk, fr	2mi,vf; 3f,m	20
2Crb	37-40+	10YR3/2	1f abk, fr	1mi,vf,f; 2m,co	80

Altitude: 1000 m
Slope: 45°; Mean annual air temperature: 9.0°C; Mean temperature of the coldest month (January): 0.0°C; Mean temperature of the warmest month (July): 20.0°C. Vegetation: *Fagus sylvatica* L. (80%), *Sorbus aria* Crantz (10%)*, Acer* spp., *Quercus cerris* L..

Soil: loamy-skeletal, mixed, mesic, Lithic Haprendoll (Soil Survey Staff, 2014)

Forest floor	6-0	-	-	0	0
A	0-7	5YR 2.5/2	3f cr, fr	3mi,vf,f,m,co	25
AB	7-16	5YR 3/3	3m,f cr+sbk, fr	3mi,vf,f,m,co	50
Bw	16-29	7.5YR 3/3	3f cr+sbk, fr	3mi,vf,f,m,co	70
2Bwb	29-40	2.5YR 3/4	3f sbk, fr	2mi,vf,f; 3m,co	25
2BCb	40-47+	10YR 6/5	1m abk, fr	1mi,vf,f; 2m,co	60

Soil: loamy-skeletal, mixed, mesic, Inceptic Haprendoll (Soil Survey Staff, 2014)

Forest floor	4-0	-	-	0	0
A	0-5	5YR 2.5/2	3f sbk+cr, fr	3mi,vf,f,m	25
AB	5-14	5YR 3/2	3f cr+sbk, fr	3mi,vf,f; 2m	50
Bw1	14-36	5YR 3/3	2-3f,m sbk, fr	3mi,vf,f; 1m	70
Bw2	36-43	5YR 3/4	1m sbk, fr	2mi,vf,f; 1m	70
BC	43-52+	10YR 6/5	1m abk, fr	1mi,vf; 2f,m,co	70

Mount Acuto. Parent rock: white limestone with flintstone layers. Forest management: coppice in conversion to timber forest. Exposure: N-NE. Mean annual precipitation: 1430 mm. Soil temperature regime: *mesic*. Soil moisture regime: *udic*.

Altitude: 800 m
Slope: 40°; Mean annual air temperature: 10.0°C; Mean temperature of the coldest month (January): 0.0°C; Mean temperature of the warmest month (July): 21.0°C. Vegetation: *Fagus sylvatica* L. (95%), *Carpinus betulus* L.*, Acer opalus* Mill.*, Quercus* spp..

Soil: coarse-loamy, mixed, mesic, Typic Humudept (Soil Survey Staff, 2010)

Forest floor	7-0	-	-	0	0
A	0-3	10YR 2/1	2-3f cr, vfr	2mi,vf,f	5
AB	3-9	10YR 4/4	3f,m sbk, fr	2vf,f,m,co	20
Bw1	9-25	10YR 5/4	1f,m abk+sbk, fr	3mi,vf,f 2m,co	30
Bw2	25-4	10YR 5/4	1m sbk+abk, fr	3mi,vf,f,m,co	30
Bw3	44-76	10YR 5/4	1m sbk, fr	2mi,vf,f; 3m,co	30
Bw4	76-99	2.5YR 5/6	1f,m sbk, fr	2mi,vf; 3f; 2m,co	50
Bw5	99-108	10YR 7/6	1m sbk, fr	1mi,fv,f,m,co	70
BC	108-115+	10YR 7/6	sg & 1f sbk, fr	1mi,fv,f,m,co	70

Soil: coarse-loamy, mixed, mesic, Typic Humudept (Soil Survey Staff, 2014)

Forest floor	7-0	-	-	0	0
A	0-3	10YR 2/1	3f cr, fr	3mi,vf,f,m,co	10
AB	3-8	10YR 2/2	3f cr, fr	3mi,vf,f,m,co	10
Bw1	8-25	10YR 4/4	1f sbk, fr	3mi,vf,f,m,co	20
Bw2	25-38	10YR 4/4	1m,c sbk, fr	2mi,vf,f,m,co	30
Bw3	38-54	10YR 5/6	3f,m sbk, fr	2mi,vf,f,m,co	30
Bw4	54-73	10YR 5/4	1f,m sbk, fr	2mi,vf,f; 1m,co	40
Bw5	73-102	10YR 6/6	1f,m sbk+abk, fr	1mi,vf; 2f	30
BC	102-113+	10YR 7/6	sg & 1m sbk, fr	1mi,fv,f,m,co	50

Altitude: 1000 m
Slope: 25°; Mean annual air temperature: 9.0°C; Mean temperature of the coldest month (January): -1.0°C; Mean temperature of the warmest month (July): 20.0°C. Vegetation: *Fagus sylvatica* L. (100%).

Soil: coarse-loamy, mixed, mesic, Inceptic Haprendoll (Soil Survey Staff, 2014)

Forest floor	5-0	-	-	0	0
A	0-11	7.5YR 3/2	3f,m sbk, fr	2mi,vf; 3f,m	10
AB	11-23	7.5YR 2.5/1	2f,m sbk, fr	2mi,vf,f; 3m,co	30
2Ab	23-33	7.5YR 3/2	3f,m sbk, fr	3mi,vf,f; 3m,co	50
2Bwb	33-54	10YR 7/6	1f abk, fr	2mi,vf,f	80
3Ab	54-63	10YR 4/3	1f sbk, fr	2mi,vf,f	60
3Bwb	63-70	10YR 5/3	1f abk, fr	2mi,vf; 1f	70
4Ab	70-71	7.5YR 3/2	3f abk, fr	1mi,vf,f	70
4Bwb	71-76	10YR 5/3	2f,m abk, fr	1mi,vf,f	75
4BC	76-86+	10YR 6/6	1f,m abk, fr	v1mi,vf,f	80

Soil: loamy-skeletal, mixed, mesic, Inceptic Haprendoll (Soil Survey Staff, 2014)

Forest floor	13-0	-	-	0	0
A	0-13	7.5YR 2.5/1	3f,m cr+sbk, fr	3mi,vf,f,m; 2co	40
AB	13-20	10YR 3/2	1f,m sbk+abk, fr	2mi,vf,f,m,co	50
2Ab	20-36	10YR 2/2	1f abk, fr	2mi,vf,f; 3m,co	50

2Bwb1	36-69	10YR 4/4	1f abk, fr	2mi,vf,f; 1m	60
2Bwb2	69-75	10YR 6/6	1f abk, fr	1mi,vf	60
2BC2	75-85+	10YR 7/6	1f abk, fr	1mi,vf	75

[a]moist and crushed, according to the Munsell Soil Color Charts.
[b]sg=single grain; 1=weak, 2=moderate, 3=strong; f=fine, m=medium, c=coarse; cr=crumb, abk=angular blocky, sbk=subangular blocky; vfr=very friable, fr=friable.
[c]0=absent, v1=very few, 1=few, 2=plentiful, 3=abundant; mi=micro, vf=very fine, f=fine, m=medium, co=coarse.

HIGHLIGHTS

- Beech rhizosphere effect on WEOM was evaluated at 800 m and 1000 m a.s.l.
- Rhizosphere WEOM had larger amounts of sugars than that of bulk soil
- Organic C and phenols contents were higher in rhizosphere than in bulk soil WEOM at 1000 m
- Higher tannins diversity occurred in rhizosphere than in bulk soil WEOM at 1000 m
- Environmental constraints enhanced the rhizosphere effect on WEOM quality