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(Article begins on next page)

1    **MINERAL WEATHERING AND LESSIVAGE AFFECT MICROBIAL COMMUNITY**  
2    **AND ENZYME ACTIVITY IN MOUNTAIN SOILS**

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## 20    **Abstract**

21    The aim of the study was to assess if pedogenic processes such as mineral weathering and lessivage,  
22    other than organic matter accumulation, can affect soil microbial population and enzyme activities.  
23    This study examines six soil profiles located in a karst region of the North-Eastern Italian Alps and  
24    characterized by a vertical textural differentiation due to lessivage. For each soil, four pedological  
25    layers were recognized according to the dominant soil forming process: *i*) the top soil (Tp layer),  
26    formed by A and AB horizons, characterized by organic matter accumulation; *ii*) the subsurface  
27    eluviated layer (Elu layer), comprising AE and EB horizons; *iii*) the layer dominated by the in-situ  
28    mineral weathering (Wh layer), made by Bw horizons; *iv*) the deepest layer (Ls), subjected to clay  
29    illuviation and comprised by Bt horizons. In the upper layers (Tp and Elu), because of the low pH,  
30    weathering also occurred, as indicated by the presence of disordered smectite and by the high values  
31    of pedogenic Fe oxi-hydroxides to pseudo-total Fe ratio.

32    The microbial biomass content and structure, and the enzyme activities significantly differed in the  
33    four pedological layers. The amount of microbial biomass was, as expected, most abundant in the  
34    Tp layer, where bacteria and actinomycetes abounded. Conversely, in Elu and Wh we observed a  
35    fungal-to-bacterial biomass ratio significantly higher than in Tp and Ls; in Elu, also the gram (+)/  
36    gram (-) ratio was the highest. In the upper layer, the interaction between enzymes and minerals like  
37    disordered smectite and pedogenic Fe-oxides appeared as responsible for the inhibition of the total  
38    enzyme activity per unit of organic C, and of the lipase activity. In Ls layer, where clay illuviation  
39    and high organo-minerals interaction occurred, the potential hydrolysis of organic matter was low,  
40    as revealed by the SEI/TOC ratio, the reduced lipase activity, and the inhibited activity of  $\alpha$ -  
41    fucosidase and  $\alpha$ -mannosidase. Even if the activity of most enzymes depends on the substrate  
42    availability, which decreases with soil depth, those involved in lipid degradation displayed the  
43    maximum activities in Elu and Wh layers, where a relative increase of the fungal population was  
44    observed. In conclusion, our findings showed that the soil functionality, expressed by the microbial

45 community structure and enzymes activity, can vary according to organic matter–mineral  
46 interaction following the weathering and lessivage gradients along the soil profiles.

47

48 *Keywords:* pedogenic processes, microbial biomass, organo-mineral interactions, illuviation, soil  
49 horizons.

## 1. Introduction

Mountain soils are often weakly developed because of several limiting factors such as steep slope and low temperature, which accelerate soil erosion and slow down the mineral weathering and organic matter oxidative kinetics, respectively (Legros, 1992; De Feudis et al., 2019; Cardelli et al., 2019; Massaccesi et al., 2020). Among the soil forming processes occurring in mountain soils, organic matter accumulation at soil surface (e.g., Boča and Miegroet, 2017), decomposition/neoformation of clay minerals, and translocation of clay particles and organics along the soil profile (Bockheim and Gennadiyev, 2000) are frequent. The organic and mineral phases can reciprocally affect themselves, with soil organic matter (SOM) enhancing mineral weathering and controlling the formation of secondary minerals (e.g., Anderson et al., 1982; Dahlgren and Ugolini, 1989), which in turn contribute to SOM stabilization (e.g., Eusterhues et al., 2003). The organo-mineral interactions are one of the main mechanisms driving the stabilization of SOM, and the nature of clay minerals controls this process (e.g., Mikutta et al., 2007; Agnelli et al., 2008; Kögel-Knabner et al., 2008; Barré et al., 2014; Gartzia-Bengoetxea et al., 2020). The ability of clay minerals to stabilize SOM is function of the reactivity of the mineral surfaces (e.g., Mikutta et al., 2006; Wang et al., 2017), which decreases from high-charge phyllosilicates such as vermiculites and smectites to illite and kaolinite (Bruun et al., 2010). As a part of SOM, also enzymes can be stabilized by clay minerals, either by adsorption through enzyme active-sites and occlusion in micro-aggregates (Sollins et al., 1996). These mechanisms limit the substrate accessibility and contribute to form a reservoir of potential enzymatic activity into the soil (Burns et al., 2013) by protecting enzymes against proteolysis and denaturation (Nannipieri et al., 2012). Because of this, the enzyme activity is considered a sensitive indicator of ecotoxicological pollution (Turan, 2019; Bilen et al., 2019). The ecological benefit of the organo-mineral interactions including enzymes has demonstrated to play a key role also in terms of soil resilience (Benitez et al., 2004).

74 In soils affected by lessivage, clay minerals and their colloidal properties can further control *i*) the  
75 possible translocation of the organo-mineral complexes, and *ii*) the formation of eluviated and  
76 illuviated horizons (E and Bt, respectively) due to mobilisation, transport, and deposition of clay  
77 particles or clay-humus complexes within the soil profile (Schaetzl and Anderson, 2005).  
78 Furthermore, decomposition and synthesis of clay minerals are two processes often associated with  
79 lessivage (Presley et al., 2004; Schaetzl and Anderson, 2005). Clay and clay minerals can be  
80 weathered in the upper and more acidic soil compartment by congruent dissolution and the soluble  
81 by-products translocated in the B horizons, where they precipitate to form new minerals (Schaetzl  
82 and Anderson, 2005). When the lessivage is dominant on mineral weathering, the clay  
83 mineralogical assemblage in the eluviated and illuviated horizons is usually similar, whereas under  
84 decomposition/neoformation processes the clay mineralogy can be strongly different. Consequently,  
85 organo-mineral interaction, and thus microbial biomass and enzyme activities, can be affected by  
86 the amount and nature of clay minerals (Torn et al., 1997; Wiseman and Püttmann, 2005; Mikutta et  
87 al., 2006),  
88 Since, as far as we know, few papers focused on the effects of pedogenic processes on soil  
89 biochemical properties (e.g., Vittori Antisari et al., 2018), a combined pedological, chemical, and  
90 biochemical approach has been adopted in this study to increase the knowledge on the soil  
91 microbial community and enzymatic activity along the pedon. Specifically, the aim of this work  
92 was to assess if lessivage and/or mineral weathering, other than SOM accumulation, can influence  
93 microbial community structure and enzyme activity in mountain soils. We hypothesized that,  
94 although SOM accumulation is the main driver of the soil biochemical activity, lessivage and  
95 mineral weathering could act as limiting factors for the enzyme activities involved in the nutrient  
96 cycling through organo-mineral interactions. We tested the hypothesis by a physicochemical,  
97 mineralogical, and biochemical approach on six soil profiles with evident vertical textural  
98 differentiation due to lessivage in a karst region located in the North-Eastern Italian Alps.

## 2. Materials and Methods

### 2.1 Study area and soil sampling

The study area was located close to the Brocon Pass (1600 m above sea level), North-eastern Italian Alps (Figure 1), where the soils developed on the so called “scaglia rossa”, a thinly-layered, red to reddish-pink marly limestone interbedded with clayey micritic limestones and shales (Tosoni, 2011). This rock has variable contents of clay minerals like micas and smectites (ISPRA, 2010), and the colour is due to the dispersion of iron oxides (mainly hematite and goethite) in the limestone mass (Bertola and Cusinato, 2004).

The area is characterized by moderate to steep slopes covered by herbaceous vegetation mainly composed of *Festuca paniculata* (L.) Schinz & Thell. subsp. *paniculata* and *Cirsium eriophorum* L. (Table S1), and it is intensively pastured during the summer-autumn period. The climate is continental with cold winters and hot summers. The mean annual air temperature is 4.4 °C, with July as the warmest month (15.7 °C) and December as the coldest one (-2.7 °C). The mean annual precipitation, including the snow water equivalent, is 976 mm. The soil is covered by snow from mid-October till the end of April.

Six sites were selected within an area of about 0.55 ha (Figure 1). For each site, a soil profile was dug to investigate the solum and the relationship between the soil forming processes and the physicochemical and biochemical soil properties. All the profiles were described by Schoeneberger et al. (2012) (Table S1). The investigated soils had a depth that varied from 37 to 60 cm (Table S1), with the topsoil characterized by well-developed O horizons (1.5-4 cm thick) resting on A, AB, or AE horizons (9-21 cm thick); below the topsoil, rather thick Bw and Bt horizons formed. Even if the soils developed from calcareous parent materials, during the field operations the soil material never showed effervescence with 10% HCl solution, indicating the absence of CaCO<sub>3</sub> in the solum. This feature testified the occurrence of decarbonation along the investigated soil thickness.

124 Soil samples were collected by genetic horizons and maintain in a refrigerated bag for all the field  
125 operations. Once in the laboratory,  $\frac{3}{4}$  of each soil sample were air dried and sieved at 2 mm, while  
126 the rest was kept at 4 °C for the biochemical analyses.

127

## 128 2.2 Soil physical and chemical analyses

129 The particle-size distribution was determined by the pipette method (Gee and Bauder, 1986) after  
130 treatment with NaClO solution at 6% of active chlorine to remove organic cements (Lavkulich and  
131 Wiens, 1970) and with dithionite-citrate-bicarbonate solution to remove Fe-Al oxi-hydroxides  
132 cements (Mehra and Jackson, 1960). The clay fraction was collected for the mineralogical analysis.  
133 The pH was determined potentiometrically in water after one night of solid:liquid (1:2.5 w:v ratio)  
134 contact, using a combined glass-calomel electrode immersed into the suspension. The electrical  
135 conductivity (EC) was determined by a WTW multi 340i conductivity meter (Weilheim, Germany)  
136 in a 1:2.5 soil:water suspensions (w:v). Total organic carbon (TOC) and total nitrogen (TN) were  
137 measured using the dry combustion method with Thermo Soil NC—Flash EA1112 elemental  
138 analyser. The exchangeable cations were displaced by hexamine cobalt (III) chloride (Orsini and  
139 Remy, 1976). The displaced Ca, Mg, K, Na, and Al were determined by Inductive Coupled Plasma  
140 – Optic Emission Spectroscopy (ICP-OES, Ametek Germany). Exchangeable H was calculated as  
141 the pH difference between the 0.2 M BaCl<sub>2</sub> solution before and after contact with the soil samples  
142 (Corti et al., 2019). Effective cation exchange capacity (eCEC) was obtained as the summation of  
143 all exchangeable cations (Ca, Mg, K, Na, Al, and H). The base saturation was obtained by dividing  
144 the sum of exchangeable Ca, Mg, K, and Na by the eCEC value.

145 Pedogenic Fe and Al oxi-hydroxides were measured through extraction with Na-dithionite-citrate-  
146 bicarbonate solution (Fe<sub>DCB</sub> and Al<sub>DCB</sub>) (Mehra and Jackson, 1960). Fe and Al in the extracts were  
147 measured by ICP-OES. The pseudo-total amount of Al, Fe, Ca, Mg, K, Na, Mn, P and S (Al<sub>T</sub>, Fe<sub>T</sub>,  
148 Ca<sub>T</sub>, Mg<sub>T</sub>, K<sub>T</sub>, Na<sub>T</sub>, Mn<sub>T</sub>, P<sub>T</sub>, and S<sub>T</sub>) was obtained digesting finely ground sample aliquots in  
149 polyethylene vials with *aqua regia* (3:1 HCl:HNO<sub>3</sub>) in microwave oven (Milestone, 1200)

150 according to Vittori Antisari et al. (2014); then, the concentration of each element in the extract was  
151 measured by ICP-OES. The  $Fe_{DCB}/Fe_T$  ratio was taken as an index of the amount of pedogenic Fe-  
152 oxides with respect to the total Fe ( $Fe_T$ ) (Qafoku and Amonette, 2017). The ratio between the molar  
153 sum of  $Ca_T$ ,  $Mg_T$ ,  $K_T$ , and  $Na_T$  and the molar sum of  $Al_T$  and  $Fe_T$  [ $(Ca_T+Mg_T+K_T+Na_T)/(Al_T+Fe_T)$ ],  
154 which represent the most and less mobile groups of elements, respectively (Chadwick et al., 1999),  
155 was calculated to assess the redistribution of the elements along soil profile driven by their mobility.

156

### 157 *2.3 Mineralogical analysis*

158 Mineralogical assemblage was determined on powdered and manually compressed aliquots by X-  
159 ray diffraction with a Philips PW 1830 diffractometer, using the Fe-filtered Co  $K\alpha_1$  radiation (35  
160 kV and 25 mA); the step size was  $0.02^\circ 2\theta$ , the scanning speed was 1 sec per step, and sample  
161 aliquots were scanned from  $3$  to  $80^\circ 2\theta$ . The mineralogical composition was obtained by identifying  
162 the minerals based on their characteristic peaks (Brindley and Brown, 1980; Dixon and Schulze,  
163 2002). For each sample, a semi-quantitative estimation was obtained by calculating the area  
164 produced by the primary peak of each mineral by multiplying the peak height by the base at the  
165 half-height. The clay fraction was Mg- or K-saturated; the Mg-saturated clays were  
166 glycerol solvated, while the K-saturated ones were heated at  $550^\circ C$ . The presence of Al-  
167 hydroxopolymers in the interlayers of the 2:1 clay minerals was ascertained, and their thermo-  
168 stability assessed, by K-saturated and heated at  $550^\circ C$  specimens (Brindley and Brown, 1980; Corti  
169 et al., 1997; Dixon and Schulze, 2002).

170

### 171 *2.4 Soil biochemical analysis*

172 Soil microbial biomass C (MBC) and N (MBN) were determined using the fumigation-extraction  
173 method (Brookes et al., 1985; Vance et al., 1987). MBC was obtained by  $eC \cdot k_{eC}$ , where  $eC$  was the  
174 difference between organic C extracted using 0.5 M  $K_2SO_4$  solution (1:4 w/v) from fumigated and

175 not-fumigated samples, and  $k_{eC} = 2.64$  is the extraction efficiency coefficient (Joergensen, 1996).  
 176 The amount of C extracted by  $K_2SO_4$  solution from non-fumigated samples ( $C_{ext}$ ) was considered  
 177 the easily extractable and most labile soil organic C pool. MBN was calculated by  $eN \cdot k_{eN}$ , where  $eN$   
 178 is the difference between N extracted using 0.5 M  $K_2SO_4$  solution (1:4 w/v) from fumigated and  
 179 not-fumigated samples and  $k_{eN} = 2.22$  is the extraction efficiency coefficient (Jenkinson, 1988). The  
 180 extracted C and N were determined with the TOC-V CSN and TNM-1 analysers (Shimadzu, Japan).  
 181 The living microbial biomass was determined as the sum of all microbial groups obtained using  
 182 Ester linked-Fatty Acid Methyl Ester (El-FAME). The microbial community profiles were  
 183 determined, quantified, and converted to  $\mu\text{mol} \cdot \text{g}^{-1}$  using peak areas from internal standard  
 184 (methylnonadecanoate, C19:0) used at known concentrations. A total of 13 El-FAME biomarkers  
 185 were summed into the broad microbial groups Actinobacteria (10Me16:0, 10Me17:0, 10Me18:0),  
 186 Gram-positive (G<sup>+</sup>) bacteria (i15:0, a15:0, i16:0, i17:0, a17:0), Gram-negative (G<sup>-</sup>) bacteria (cy  
 187 17:0, cy 19:0 $\omega$ 8c, 18:1  $\omega$ 7c), and saprophytic fungi (18:1  $\omega$ 9c, 18:2  $\omega$ 6c), according to previous  
 188 studies (Zelles, 1999; Massaccesi et al., 2015; Stazi et al., 2017). The per mil fungal-to-bacteria  
 189 ratio (F/B) was calculated as an index of soil microbial community change, while G<sup>+</sup>/G<sup>-</sup> ratio was  
 190 proposed as an indicator of stressful conditions such as low oxygen availability, suboptimal pH or  
 191 water content, or low nutrient supply because of the greater dependence of G<sup>-</sup> than G<sup>+</sup> on labile C  
 192 (Fanin et al., 2019). Moreover, the sum of El-FAMEs characteristic of general bacteria, G<sup>+</sup> and G<sup>-</sup>  
 193 bacteria, actinomycetes, and fungi was used as broad taxonomic microbial grouping.  
 194 The soil enzyme activities were measured using 4-methylumbelliferine (MUF) and 7-amino-4-  
 195 methylcoumarin (AMC) fluorogenic substrates (Marx et al., 2001; Vepsäläinen et al., 2001). The  
 196 selected 17 enzyme activities (Table S2) are involved in the main biogeochemical cycle of C ( $\beta$ -  
 197 cellobiohydrolase,  $\beta$ -xylosidase,  $\beta$ -glucosidase,  $\alpha$ -glucosidase,  $\alpha$ -galactosidase,  $\beta$ -galactosidase,  $\beta$ -  
 198 glucuronidase,  $\alpha$ -mannosidase,  $\alpha$ -fucosidase, butyrate esterase, esterase lipase, and lipase activities),  
 199 N (leucine-arylamidase, valine arylamidase, and N-acetyl- $\beta$ -glucosaminidase activities), P (acid  
 200 phosphomonoesterase activity), and S (arylsulphatase activity). Even if the pH values of the studied

soil samples ranged from 4.3 to 6.9, enzymes involved in a wide range of substance degradation with optimal pH in acid and alkaline intervals were selected (Table S2). Therefore, specific substrates were prepared using different buffer adjusted to the optimum for each selected enzyme (0.5 M sodium acetate pH 5.5; 0.5 M Tris acetate pH 7.5). Fluorescence (excitation 360 nm, emission 450 nm) was measured with an automatic fluorometric plate reader (Fluoroskan Ascent), and readings were performed after 0, 30, 60, 120, and 180 min at 30 °C. The MUF and the AMC standard curves were prepared and measured for each sample and buffer. The results were expressed as nmoles of product (MUF or AMC) of each enzymatic reaction released per g of soil sample per unit of time in relation to a standard curve prepared with increasing MUF or AMC concentrations and incubated at the same experimental conditions. The Synthetic Enzymatic Index (SEI), which expresses the sum of all enzyme activities, was calculated for all samples as a synthetic measure of microbial functional capacity (Moscatelli et al., 2018). Based on the obtained data, the specific enzyme activities per unit of TOC (SEI/TOC) was calculated to appraise the nutritional status of SOM (Boerner et al., 2005; Trasar-Cepeda et al., 2008).

215

## 2.5 Data treatment

In the studied soils, four pedological layers were recognized according to the dominant soil forming process: *i*) the topsoil layer (Tp), characterized by SOM accumulation and comprising the A and AB horizons; *ii*) the sub-surface layer (Elu), providing indication of past or on going eluvial processes and made of AE and EB horizons; *iii*) the intermediate portion of the soil profile (Wh), characterized by *in-situ* mineral weathering and represented by Bw horizons; *iv*) the deepest layer (Ls), subjected to clay illuviation and formed by Bt horizons. For each layer (Tp, Elu, Wh, and Ls), the physicochemical, mineralogical, and biochemical properties have been calculated as the average of the corresponding horizons for the six soil profiles. Because of the non-parametricity of the data and the impossibility to transform them into parametrically distributed data, the significant

differences among the layers were checked by using the non-parametric Wilcoxon test. To define the soil properties driving the layers differentiation, a principal component analysis (PCA) was run for both physicochemical and biochemical data obtained from 30 soil samples (one per each horizon) collected from the six profiles. This multivariate analysis is based on the linear model of variance analysis and consists of decomposing the total variability among soil properties. The variables were standardized due to the difference in the units of measure. The applicability of the PCA to the data sets was verified through the application of Bartlett's sphericity test. Non-parametric correlation (Spearman coefficient) was performed between physicochemical and biochemical soil properties. Statistical analysis was performed using JMP 11.0 software.

235

### 3. Results

#### 3.1 Soil physicochemical characteristics

The pH values of Tp, Elu, and Wh layers were strongly acid, with average values ranging from 4.55 to 4.82, while the deepest layer (Ls) significantly differed, reaching a moderately acid pH value of 5.97 (Table 1). The TOC and TN contents significantly decreased with depth (Table 1) and the same trend was observed for P<sub>T</sub> and S<sub>T</sub> contents (Table 1). The C<sub>extr</sub> concentration (Table 1), which represented 1.80-3.43% of TOC, showed a decreasing trend with depth. Conversely, the concentrations of Al<sub>T</sub>, Fe<sub>T</sub>, Ca<sub>T</sub>, Mg<sub>T</sub>, and K<sub>T</sub> increased with depth (Table 2), while Mn<sub>T</sub> and Na<sub>T</sub> had a homogenous content all throughout the profiles. The total clay content increased along the profiles (Table 2), with the highest values, as expected, in Ls (692 g kg<sup>-1</sup>). The eCEC values were similar among soil layers (from 29.0 to 31.1 cmol<sub>+</sub> kg<sup>-1</sup>) and the base saturation was always higher than 50%. The EC values were lower in Elu and Wh (on average 0.07 and 0.09 dS m<sup>-1</sup>, respectively) than in Tp and Ls (0.35 and 0.20 dS m<sup>-1</sup>). The content of pedogenic Fe and Al (Fe<sub>DCB</sub> and Al<sub>DCB</sub>) did not displayed a linear trend with depth (Table 2), but the Fe<sub>DCB</sub>/Fe<sub>T</sub> ratio (Figure 2A) had the highest values (p<0.01) in Tp and Elu horizons (0.65 and 0.72, respectively). The

251  $(Ca_T + Mg_T + K_T + Na_T)/(Al_T + Fe_T)$  molar ratio showed lower values ( $p < 0.01$ ) in Tp, Elu, and Wh than  
 252 in Ls (Figure 2B). For these physicochemical data, the PCA has allowed to extract two principal  
 253 components with eigenvalues greater than 2 (Figure 3). The two-component model accounted for  
 254 64.5% of the total variance, with the first and the second axes explaining 41.6% and 22.9% of total  
 255 variation, respectively. The Table inserted in Figure 3 indicates that the first axis showed high  
 256 positive loadings for TOC, TN,  $P_T$ ,  $S_T$ , and  $Fe_{DCB}/Fe_T$  ratio and high negative loading for  $C_{extr}/TOC$   
 257 ratio. The second axis was positively driven by pH, EC,  $(Ca_T + Mg_T + K_T + Na_T)/(Al_T + Fe_T)$  molar  
 258 ratio, and total clay. The PCA highlighted some differences among the four layers: i) Tp layer  
 259 generally showed positive values of both components; ii) Elu layer displayed positive values for the  
 260 first component and negative values for the second one; iii) Wh layer exhibited negative values for  
 261 both components; iv) Ls layer presented negative values for the first component and positive values  
 262 for the second one (Figure 3).

263

### 264 3.2 Soil mineralogy

265 The semi-quantitative mineralogical composition showed that quartz was the predominant primary  
 266 mineral (from 41 to 47%), with plagioclases, orthoclase, and micas present in small amounts (Table  
 267 3). All the samples showed the presence of a peak at 1.4 nm that moved to  $\approx 1.8$  nm after glycerol  
 268 solvation, and partially collapsed at  $\approx 1.0$  nm when the K-saturated specimen was heated at 550 °C,  
 269 indicating the presence of smectite (Figure S1). The 1.0 nm peak in the heated specimens was  
 270 however rather wide and asymmetrical in all samples, with the exception for those of the horizons  
 271 forming the Elu layers. A peak at 0.7 nm was detected in the Mg-saturated specimens and  
 272 disappeared after heating at 550 °C, indicating the presence of kaolinite. Therefore, in all the  
 273 horizons, the clay minerals were mainly represented by smectite (from 31 to 35%) and kaolinite  
 274 (from 2 to 5%). Smectite was also present as disordered layer minerals, as deduced from the broad  
 275 diffraction band between 1.4 and 1.5 nm of the Mg-saturated specimens and between 1.8 and 1.95

276 nm in the Mg-saturated and glycerol-solvated specimens. The pronounced asymmetry of the 1.0 nm  
277 peak after specimen heating was indicative of Al polymers in the smectite interlayers, which  
278 prevented the complete collapse of smectite at 550 °C (Table 3). In particular, disordered smectite  
279 was present in the Tp and Elu layers, whereas HIS were absent in Elu layer.

280

### 281 3.3 Microbial biomass and enzyme activities

282 The amounts of MBC and MBN were significantly higher ( $p < 0.05$ ; Table 4) in Tp (874 and 246 mg  
283  $\text{kg}^{-1}$ , respectively) than in the deeper soil layers (Wh and Ls; 118 and 227 mg MBC  $\text{kg}^{-1}$ ; 45 and 81  
284 mg MBN  $\text{kg}^{-1}$ ), while Elu layer displayed intermediate amounts (445 and 151 mg  $\text{kg}^{-1}$ ,  
285 respectively). Conversely, the amount of the living microbial biomass expressed as the sum of the  
286 microbial groups assessed by El-FAME and of bacteria and actinomycetes were the highest in Tp  
287 (464, 449, and 53.5 nmol  $\text{g}^{-1}$ , respectively) and the lowest in Elu (63, 59, and 6.8 nmol  $\text{g}^{-1}$ ,  
288 respectively). Furthermore, the F/B ratio was greater in Elu and Wh (47.5 and 39.1%, respectively)  
289 than in Tp and Ls (28.2 and 24.4%), while the G+/G- ratio displayed the highest value in Elu (1.02)  
290 (Table 4).

291 The enzyme activities involved in the C cycle ( $\beta$ -cellobiohydrolase,  $\alpha$ - and  $\beta$ -glucosidase,  $\beta$ -  
292 xylosidase,  $\beta$ -galactosidase, and  $\beta$ -glucuronidase) were the highest in Tp (76.3, 79, 389, 189, 89,  
293 and 203 nmol MUF  $\text{g}^{-1} \text{h}^{-1}$ , respectively), and showed a significant reduction of their activity  
294 starting from Elu (Table 5). Conversely, enzymes involved in N, P, and S cycles (N-acetyl- $\beta$ -  
295 glucosaminidase, leucine arylamidase, butyrate esterase, acid phosphomonoesterase, and  
296 arylsulphatase) showed the highest activities in Tp and Elu layers and significantly decreased in Wh  
297 or Ls (Table 5). Two over 17 enzyme activities, esterase lipase and valine arylamidase, did not  
298 show any significantly change along the profiles, whereas  $\alpha$ -mannosidase and  $\alpha$ -fucosidase showed  
299 a very low activity in Ls (9.7 and 8.2 nmol MUF  $\text{g}^{-1} \text{h}^{-1}$ , respectively; Figure 4A and B).  
300 Conversely, the lipase had the lowest activity in Tp and Ls (154 and 197 nmol MUF  $\text{g}^{-1} \text{h}^{-1}$ ,

301 respectively) and the highest in Wh (359 nmol MUF g<sup>-1</sup> h<sup>-1</sup>; Figure 5A). The total enzyme activity  
302 expressed per unit of organic carbon (SEI/TOC) displayed a similar trend of lipase (Figure 5B),  
303 reaching in Ls an average value similar to that of Tp (110 vs. 77 nmol MUF mg<sub>TOC</sub><sup>-1</sup> h<sup>-1</sup>;  
304 respectively).

305 Compared to the others, lipase and esterase lipase activities were not correlated with pH, TOC, TN,  
306 C<sub>extr</sub>/TOC, clay content, and Fe<sub>DCB</sub>/Fe<sub>T</sub> (Table 6); lipase activity only showed negative correlations  
307 with electrical conductivity (EC) and (Ca<sub>T</sub>+Mg<sub>T</sub>+K<sub>T</sub>+Na<sub>T</sub>)/(Al<sub>T</sub>+Fe<sub>T</sub>) ratio. The PCA run with the  
308 soil biochemical data showed that 10 over the 17 enzyme activities had positive loading values  
309 along the first component, which explained 62.8% of the total variance (Figure 6). Conversely, the  
310 other seven enzyme activities ( $\alpha$ -mannosidase,  $\alpha$ -fucosidase, butyrate esterase, esterase lipase,  
311  $\alpha$ -galactosidase,  $\beta$ -galactosidase, and lipase) were mainly correlated with the second component,  
312 explaining 14.1% of the total variance.

313

#### 314 4. Discussion

315 The clay coatings on soil pedis observed in the profiles (Table S1) and the increasing amount of clay  
316 particles with depth (Table 2) proved that clay illuviation occurred in these soils and that this  
317 process was responsible for the formation of Bt (illuviated) horizons. It is well known that clay  
318 eluviation (with the formation of eluviated horizons) occurs mainly at pH values ranging between  
319 4.5/5 and 6; below this range clay flocculates because of a high Al<sup>3+</sup> and H<sup>+</sup> activity in the soil  
320 solution, while at higher pHs clay flocculates because of a high concentration of Ca<sup>2+</sup> or other  
321 divalent cations in the soil solution (Quénard et al., 2011). Accordingly, in the acid horizons (pH  
322 4.55-4.82) of our soils clay eluviation could occurred, whereas in the deep Ls layer the slight  
323 increase of soil reaction (pH 5.97) induced clay to flocculate so to form illuviated horizons. In Ls,  
324 the clay flocculation was possibly enhanced by the leached soluble elements (Levy et al., 1993;

325 Kaplan et al., 1997), which were able to increase the EC values with respect to the overlying Elu  
 326 and Wh layers.

327 The similar mineralogical assemblage of the four layers supported the occurrence of lessivage and  
 328 suggested that clay decomposition/neoformation processes along the soil profiles were limited. The  
 329 presence of small amounts of hydroxy-Al interlayered smectite (HIS) indicated that weathering has  
 330 occurred through the intercalation of hydroxy-Al polymers into the smectite interlayers. This  
 331 transformation is rather common in soil affected by lessivage (e.g., Bonifacio et al., 2009). In  
 332 particular, the large presence of disordered smectite and very small amounts of HIS in the Elu layer  
 333 indicated the occurrence of weathering processes promoted by low pH values and of accumulation  
 334 of organic matter in the upper part of the soil profiles, as reported in several works carried out on  
 335 Italian mountains soils (e.g., Vittori Antisari et al., 2016; De Feudis et al., 2016; 2017a, b; Cardelli  
 336 et al., 2019). The occurrence of mineral weathering in Elu, as well as in Tp, is also confirmed by the  
 337 relatively high  $\text{Fe}_{\text{DCB}}/\text{Fe}_{\text{T}}$  ratio and by the PCA on soil physicochemical properties, which grouped  
 338 Tp and Elu layers into two well distinguished ellipses with respect to Wh and Ls (Figure 3).

339 The soil microbial biomass and the total enzyme activity (SEI) decreased with soil depth. These  
 340 results are usually found in soil since both these parameters largely depend on the amount and  
 341 quality of soil organic matter (Fierer et al., 2003; Sidari et al., 2008; Agnelli et al., 2016). However,  
 342 according to the PCA loading values on the first two PCs (Figure 6), soil biochemical properties  
 343 mainly depended on SOM accumulation process in Tp and Elu layers. Therefore, the highest values  
 344 of TOC content, coupled with the accumulation of N, P, and S in Tp and Elu, favoured the soil  
 345 microbial community (Likens et al., 2002; De Feudis et al., 2016; Adams et al., 2018), as indicated  
 346 by the higher MBC and SEI. Nonetheless, other processes than SOM accumulation affected the  
 347 biochemical properties of the investigated soils. Indeed, while TOC, TN,  $\text{P}_{\text{T}}$ , and  $\text{S}_{\text{T}}$  contents did not  
 348 significantly differ between Wh and Ls, SEI differed between them. As the main pedogenic process  
 349 at depth was the formation of Bt horizons due to clay illuviation, the lower SEI in Ls than in Wh  
 350 was ascribed to a higher inhibition of the enzyme activity due to the sorption of organics (including

351 enzymes) onto clay minerals (Singh et al., 2018), as demonstrated by the significant negative  
352 correlation between SEI and clay content. By expressing the enzyme activity per unit of organic  
353 carbon (SEI/TOC), it was possible to stress the effect of both lessivage and weathering processes  
354 (Marinari et al., 2020). In Ls, the hydrolytic activity per unit of TOC was lower than in Wh,  
355 probably due to the organo-mineral interactions among substrates, enzymes, and the illuviated clay.  
356 However, the SEI/TOC ratio was low also in Tp, where SOM accumulation was coupled to  
357 relatively intense weathering conditions, as testified by the presence of disordered smectite and a  
358 relatively high  $Fe_{DCB}/Fe_T$  ratio. According to Singh et al. (2018), in the Tp layer a strong inhibition  
359 of the enzyme activities (per unit of TOC) probably occurred because of the interactions between  
360 enzymes and smectite and/or pedogenic oxides.

361 A specific behaviour was observed for enzyme activities involved in the lipid degradation, lipase  
362 and esterase lipase, which appeared not related to TOC content and, thus, to SOM accumulation.  
363 Lipase showed the highest activity in Elu and Wh, and was low in Ls. As reported in a previous  
364 study (Eichlerova et al., 2015), fungal groups have different patterns of enzymes such as esterase,  
365 lipase,  $\alpha$ -mannosidase, and  $\alpha$ -fucosidase, so that the decrease of both F/B and G+/G- ratios in Ls  
366 may justify the variations of soil biochemical activity. Furthermore, it has been shown that a  
367 reduction in enzyme activity can occur when arbuscular mycorrhizal fungi (AMF) attach to lignin-  
368 derived material such as lignin-derived biochar (Khan et al., 2020). The different enzyme activities  
369 among soil layers may also indicate the presence of different microbial metabolic pathway as  
370 consequence of selective sorption of aromatic and hydrophobic compounds such as lipids onto clay  
371 mineral surfaces, so becoming less available to microbial attack (Kaiser and Guggenberger, 2000).  
372 This was most possible in the Ls layer, where clay accumulated. In addition, the lowest activities of  
373  $\alpha$ -fucosidase and  $\alpha$ -mannosidase in Ls suggested the occurrence of a specific inhibition of these  
374 enzymes by clay, as suggested by the high negative correlation coefficient between these enzyme  
375 activities and clay content (Table 6).

376

## 5. Conclusions

Our findings showed that, in mountain soils developed from calcareous parent materials, pedogenic processes such as mineral weathering and lessivage affect the soil biochemical properties along the solum. The mineral fraction stabilized SOM in Tp and Ls, where the organo-mineral interactions were more effective through the involvement of minerals like smectite and iron oxides in Tp and illuviated clays in Ls. In Wh, where both organic substrates and enzymes were adsorbed onto clay minerals, the microbial functions related to SOM degradation were more conservative, contributing to the incipient phase of carbon sequestration in the horizons forming this layer. In this layer, the microbial community was dominated by fungi, which were probably responsible for a higher activity of the enzymes involved in the lipid degradation, particularly lipase and esterase lipase. Therefore, soil functionality, expressed by microbial community and enzyme activities, varies following weathering and lessivage processes, which differently affect the occurrence of organo-mineral interactions along the soil profile. The lessivage, responsible for the formation of Bt horizons, appeared to be a relevant process able to affect the activities of microbial biomass and enzymes involved in SOM degradation.

Our results advocate that soil forming processes are key to understand the functioning of microbial biomass and enzyme activities involved in SOM decomposition. Further, the used approach, which considers the biochemical properties in relation to the pedogenic processes, can allow the transferability of the results to other environments with similar factors of soil formation.

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Table 1. Values of pH, total organic C (TOC), total N (TN), extractable C ( $C_{\text{extr}}$ ),  $C_{\text{extr}}$ /TOC ratio, total P ( $P_T$ ), and total S ( $S_T$ ) for the investigated soil layers. Values within brackets represent the standard errors. Different letter indicates significant difference among soil layers ( $p < 0.05$ ). Brocon Pass, north-eastern Italian Alps.

| Soil layers | <i>n</i> | pH               | TOC                | TN                 | $C_{\text{extr}}$   | $C_{\text{extr}}$ /TOC | $P_T$               | $S_T$               |
|-------------|----------|------------------|--------------------|--------------------|---------------------|------------------------|---------------------|---------------------|
|             |          |                  | g kg <sup>-1</sup> | g kg <sup>-1</sup> | mg kg <sup>-1</sup> | %                      | mg kg <sup>-1</sup> | mg kg <sup>-1</sup> |
| Tp          | 8        | 4.82 b<br>(0.21) | 83.5 a<br>(15.4)   | 9.67 a<br>(1.71)   | 1328 a<br>(177)     | 1.80 b<br>(0.26)       | 1282 a<br>(192)     | 700 a<br>(157)      |
| Elu         | 4        | 4.55 b<br>(0.05) | 49.9 ab<br>(12.5)  | 5.28 a<br>(1.30)   | 1188 a<br>(220)     | 2.62 ab<br>(0.56)      | 1197 a<br>(69)      | 644 a<br>(79)       |
| Wh          | 9        | 4.80 b<br>(0.16) | 24.9 b<br>(5.4)    | 2.81 b<br>(0.61)   | 738 b<br>(96)       | 3.43 a<br>(0.46)       | 749 b<br>(101)      | 299 b<br>(56)       |
| Ls          | 9        | 5.97 a<br>(0.38) | 27.6 b<br>(13.0)   | 2.42 b<br>(0.63)   | 524 c<br>(83)       | 3.27 a<br>(0.61)       | 767 b<br>(85)       | 262 b<br>(45)       |

*n*: number of replicates for each layer.

$C_{\text{extr}}$ : C extracted by 0.5 M K<sub>2</sub>SO<sub>4</sub> solution .

Table 2. Concentrations of pseudo-total elements (Al<sub>T</sub>, Fe<sub>T</sub>, Ca<sub>T</sub>, K<sub>T</sub>, Mg<sub>T</sub>, Mn<sub>T</sub>, and Na<sub>T</sub>), clay, Fe and Al extracted by DCB (Fe<sub>DCB</sub>, Al<sub>DCB</sub>), cation exchange capacity (eCEC), base saturation (BS), and electrical conductivity (EC) in the investigated soil layers. Values within brackets represent the standard errors. Different letter indicates significant difference among soil layers (p<0.05). Brocon Pass, north-eastern Italian Alps.

| Soil layers | <i>n</i> | Al <sub>T</sub>    | Fe <sub>T</sub>  | Ca <sub>T</sub> | K <sub>T</sub>  | Mg <sub>T</sub> | Mn <sub>T</sub> | Na <sub>T</sub>     | Clay               | Fe <sub>DCB</sub> | Al <sub>DCB</sub>                  | eCEC            | BS              | EC                 |
|-------------|----------|--------------------|------------------|-----------------|-----------------|-----------------|-----------------|---------------------|--------------------|-------------------|------------------------------------|-----------------|-----------------|--------------------|
|             |          | g kg <sup>-1</sup> |                  |                 |                 |                 |                 | mg kg <sup>-1</sup> | g kg <sup>-1</sup> |                   | cmol <sub>+</sub> kg <sup>-1</sup> |                 | %               | dS m <sup>-1</sup> |
| Tp          | 8        | 52.6 b<br>(2.6)    | 29.8 b<br>(1.6)  | 2.5 b<br>(0.4)  | 10.6 c<br>(0.4) | 7.6 c<br>(0.6)  | 2.5 a<br>(0.4)  | 502 a<br>(52)       | 628 b<br>(39)      | 19.3 ab<br>(0.7)  | 4.1 b<br>(0.3)                     | 30.9 a<br>(3.3) | 54.4 b<br>(8.6) | 0.35 a<br>(0.08)   |
| Elu         | 4        | 50.1 b<br>(1.9)    | 32.0 ab<br>(2.5) | 1.5 b<br>(0.5)  | 9.8 c<br>(0.3)  | 6.6 c<br>(0.2)  | 1.6 a<br>(0.2)  | 494 a<br>(24)       | 634 b<br>(37)      | 23.3 a<br>(2.1)   | 5.3 a<br>(0.5)                     | 33.9 a<br>(3.4) | 79.3a<br>(8.0)  | 0.09 b<br>(0.01)   |
| Wh          | 9        | 60.6 a<br>(2.0)    | 35.2 a<br>(0.9)  | 2.3 b<br>(0.4)  | 11.5 b<br>(0.5) | 9.3 b<br>(0.5)  | 2.6 a<br>(0.4)  | 487 a<br>(38)       | 667 ab<br>(26)     | 20.4 a<br>(0.5)   | 5.1 a<br>(0.2)                     | 29.0 a<br>(3.2) | 56.2 b<br>(9.1) | 0.07 b<br>(0.02)   |
| Ls          | 9        | 61.7 a<br>(1.3)    | 34.8 a<br>(0.6)  | 6.1 a<br>(1.3)  | 12.6 a<br>(0.4) | 10.7 a<br>(0.2) | 2.5 a<br>(0.4)  | 532 a<br>(29)       | 692 a<br>(24)      | 18.4 b<br>(0.7)   | 3.4 c<br>(0.3)                     | 31.1 a<br>(3.8) | 65.8 a<br>(7.1) | 0.20 a<br>(0.05)   |

*n*: number of replicates for each layer.

Table 3. Semi-quantitative mineralogical composition of the investigated soil layers. Values within brackets represent the standard errors. Brocon Pass, north-eastern Italian Alps.

| Soil layers | <i>n.</i> | Q       | P      | O      | M      | S        | HIS     | Kao    |
|-------------|-----------|---------|--------|--------|--------|----------|---------|--------|
|             |           | %       |        |        |        |          |         |        |
| Tp          | 8         | 46(5) a | 5(1) a | 4(1) a | 4(1) a | 31(1)* b | 7(2) a  | 3(1) a |
| Elu         | 4         | 47(2) a | 6(2) a | 5(1) a | 7(1) a | 32(1)* b | 1(0) b  | 2(1) a |
| Wh          | 9         | 41(2) a | 6(2) a | 5(0) a | 8(1) a | 31(1) b  | 4(1) ab | 5(1) a |
| Ls          | 9         | 42(2) a | 5(2) a | 4(1) a | 5(1) a | 35(1) a  | 5(0) ab | 4(1) a |

*n*: number of replicates for each layer.

Q = quartz, P = plagioclases, O = orthoclase, M = micas, S = smectite, HIS = hydroxy-aluminum interlayered smectite, Kao = kaolinite.

\* mainly disordered smectite.

Table 4. Soil microbial biomass C (MBC) and N (MBN), and results of El-FAME analysis for the investigated soil layers. Values within brackets represent the standard errors. Different letter indicates significant difference among soil layers ( $p < 0.05$ ). Brocon Pass, north-eastern Italian Alps.

| Soil layers | <i>n</i> | MBC                 | MBN            | LMB- El-<br>FAME | B             | F                    | P              | Act             | G+               | G-               | F/B             | G+/G-           |
|-------------|----------|---------------------|----------------|------------------|---------------|----------------------|----------------|-----------------|------------------|------------------|-----------------|-----------------|
|             |          | mg kg <sup>-1</sup> |                |                  |               | nmol g <sup>-1</sup> |                |                 |                  |                  | %               | ratio           |
| Tp          | 8        | 874 a<br>(187)      | 246 a<br>(129) | 464 a<br>(20)    | 449 a<br>(21) | 11.8 a<br>(1.5)      | 2.8 a<br>(0.3) | 53.5 a<br>(5.5) | 130.6 a<br>(7.9) | 178.2 a<br>(8.1) | 28.2 b<br>(4.1) | 0.73 b<br>(0.0) |
| Elu         | 4        | 445 ab<br>(267)     | 151 a<br>(55)  | 63 c<br>(14)     | 59 c<br>(13)  | 3.1 b<br>(0.4)       | 0.6 b<br>(0.2) | 6.8 c<br>(1.4)  | 18.8 b<br>(4.9)  | 18.1 c<br>(4.2)  | 47.5 a<br>(3.9) | 1.02 a<br>(0.1) |
| Wh          | 9        | 118 b<br>(30)       | 45 c<br>(10)   | 120 b<br>(37)    | 116 b<br>(36) | 3.6 b<br>(0.9)       | 1.0 b<br>(0.2) | 17.2 b<br>(6.2) | 31.1 b<br>(9.7)  | 41.9 b<br>(13.2) | 39.1 a<br>(5.7) | 0.80 b<br>(0.0) |
| Ls          | 9        | 227 b<br>(102)      | 81 b<br>(14)   | 112 b<br>(24)    | 109 b<br>(23) | 2.3 b<br>(0.4)       | 0.4 c<br>(0.0) | 12.8 b<br>(2.8) | 26.8 b<br>(5.6)  | 42.4 b<br>(9.3)  | 24.4 b<br>(3.5) | 0.69 b<br>(0.0) |

*n*: number of replicates for each layer.

LMB-El-FAME: living microbial biomass determined by El-FAME, B: bacteria, F: saprophytic fungi, P: protozoa, Act: actinomycetes, G+: Gram positive bacteria, G-: Gram negative bacteria, F/B: fungi/bacteria ratio, G+/G-: Gram positive bacteria/Gram negative bacteria ratio.

Table 5. Enzyme activities in the investigated soil layers. Values within brackets represent the standard errors. Different letter indicates significant difference among soil layers ( $p < 0.05$ ). Brocon Pass, north-eastern Italian Alps.

| Soil layers                              | <i>n</i> | Cell                                     | Chit          | BG             | AG             | AP                                          | Sulph                                       | Xylo                                               | But                                           | a-Gal         |
|------------------------------------------|----------|------------------------------------------|---------------|----------------|----------------|---------------------------------------------|---------------------------------------------|----------------------------------------------------|-----------------------------------------------|---------------|
| nmol MUF g <sup>-1</sup> h <sup>-1</sup> |          |                                          |               |                |                |                                             |                                             |                                                    |                                               |               |
| Tp                                       | 8        | 76.3 a<br>(20.3)                         | 304 a<br>(70) | 389 a<br>(98)  | 79 a<br>(16)   | 1506 a<br>(293)                             | 900 a<br>(166)                              | 189 a<br>(47)                                      | 1553 a<br>(301)                               | 128 a<br>(29) |
| Elu                                      | 4        | 22.8 b<br>(7.9)                          | 113 b<br>(33) | 142 b<br>(48)  | 40 b<br>(14)   | 1034 a<br>(282)                             | 704 a<br>(233)                              | 91 ab<br>(30)                                      | 1204 a<br>(299)                               | 100 a<br>(35) |
| Wh                                       | 9        | 19.8 b<br>(5.1)                          | 105 b<br>(25) | 126 b<br>(28)  | 37 b<br>(9)    | 571 b<br>(114)                              | 369 b<br>(107)                              | 58 b<br>(21)                                       | 880 b<br>(201)                                | 46 b<br>(11)  |
| Ls                                       | 9        | 12.2 b<br>(3.1)                          | 78 b<br>(28)  | 69 c<br>(16)   | 17 b<br>(4)    | 292 c<br>(45)                               | 193 b<br>(59)                               | 23 c<br>(8)                                        | 474 c<br>(107)                                | 19 c<br>(4)   |
| Soil layers                              | <i>n</i> | b-Gal                                    | b-Gluc        | E-Lip          | Lip            | LeuAryl                                     | ValAryl                                     | SEI                                                | SEI/MBC                                       |               |
|                                          |          | nmol MUF g <sup>-1</sup> h <sup>-1</sup> |               |                |                | nmol AMC<br>g <sup>-1</sup> h <sup>-1</sup> | nmol AMC<br>g <sup>-1</sup> h <sup>-1</sup> | nmol<br>MUF/AMC<br>g <sup>-1</sup> h <sup>-1</sup> | nmol MUF mg <sup>-1</sup> MBC h <sup>-1</sup> |               |
| Tp                                       | 8        | 89 a<br>(25)                             | 203 a<br>(29) | 254 a<br>(104) | 154 b<br>(30)  | 132 a<br>(18)                               | 26 a<br>(5)                                 | 6067 a<br>(1054)                                   | 7.9 b<br>(1.8)                                |               |
| Elu                                      | 4        | 64 ab<br>(22)                            | 114 b<br>(22) | 301 a<br>(37)  | 300 a<br>(108) | 160 a<br>(19)                               | 22 a<br>(2)                                 | 4515 ab<br>(1178)                                  | 16.5 b<br>(5.0)                               |               |
| Wh                                       | 9        | 37 b<br>(7)                              | 126 b<br>(15) | 271 a<br>(54)  | 359 a<br>(79)  | 61 b<br>(12)                                | 17 a<br>(2)                                 | 3151 b<br>(507)                                    | 32.5 a<br>(5.9)                               |               |
| Ls                                       | 9        | 18 c<br>(4)                              | 82 c<br>(14)  | 192 a<br>(42)  | 198 b<br>(52)  | 51 b<br>(11)                                | 18 a<br>(2)                                 | 1755 c<br>(279)                                    | 27.1 ab<br>(10.7)                             |               |

*n*: number of replicates for each layer.

Cell:  $\beta$ -cellobiohydrolase; Chit: N-acetyl- $\beta$ -glucosaminidase; BG:  $\beta$ -glucosidase; AG:  $\alpha$ -glucosidase; AP: Acid phosphomonoesterase; Sulph: arylsulphatase; Xylo: xylosidase; But: butyrate esterase; a-Gal:  $\alpha$ -galactosidase; b-Gal:  $\beta$ -galactosidase; b-Gluc:  $\beta$ -glucuronidase; E-Lip: esterase lipase; Lip: Lipase; LeuAm: leucine arylamidase; ValAryl: valine arylamidase; SEI: Synthetic Enzymatic Index; SEI/MBC: Synthetic Enzymatic Index per unit of microbial biomass carbon.

Table 6. Spearman correlation coefficient between physicochemical and biochemical properties for the investigated soil horizons (n=29). Brocon Pass, north-eastern Italian Alps.

|         | pH        | TOC       | Total N  | C <sub>ext</sub> /TOC | Clay      | EC        | Fe <sub>DCB</sub> /Fe <sub>T</sub> | (Ca <sub>T</sub> +Mg <sub>T</sub> +K <sub>T</sub> +Na <sub>T</sub> )/(Al <sub>T</sub> +Fe <sub>T</sub> ) |
|---------|-----------|-----------|----------|-----------------------|-----------|-----------|------------------------------------|----------------------------------------------------------------------------------------------------------|
| MBC     | -0.399 *  | 0.643***  | 0.704*** | -0.521 **             |           |           | 0.467*                             |                                                                                                          |
| MBN     |           | 0.589**   | 0.541**  | -0.462 *              |           |           | 0.318 ns                           |                                                                                                          |
| Cell    |           | 0.851***  | 0.903*** | -0.736***             |           |           | 0.572 **                           |                                                                                                          |
| Chit    |           | 0.727***  | 0.815*** | -0.696***             |           |           | 0.510 **                           |                                                                                                          |
| BG      |           | 0.825***  | 0.884*** | -0.656***             |           |           | 0.586 **                           |                                                                                                          |
| AG      |           | 0.781***  | 0.833*** | -0.630***             |           |           | 0.552 **                           |                                                                                                          |
| AP      | -0.501 ** | 0.836***  | 0.897*** | -0.649***             | -0.392 *  |           | 0.764***                           | -0.397 *                                                                                                 |
| Sulph   |           | 0.830***  | 0.889*** | -0.692***             |           |           | 0.767***                           |                                                                                                          |
| Xylo    | -0.392 *  | 0.842***  | 0.902*** | -0.674***             |           |           | 0.704***                           |                                                                                                          |
| But     | -0.489 ** | 0.814***  | 0.853*** | -0.634 ***            | -0.398 *  |           | 0.738***                           | -0.441 *                                                                                                 |
| a-Gal   | -0.581 ** | 0.737***  | 0.782*** | -0.572 **             | -0.456 *  |           | 0.672***                           | -0.541 **                                                                                                |
| b-Gal   | -0.563**  | 0.761***  | 0.811*** | -0.607 **             | -0.461 *  |           | 0.694                              | -0.474 **                                                                                                |
| b-Gluc  | -0.501**  | 0.682***  | 0.736*** | -0.615 **             |           |           | 0.559 **                           |                                                                                                          |
| a-Man   | -0.658*** | 0.589**   | 0.608*** | -0.423 *              | -0.512 ** |           | 0.654***                           | -0.656***                                                                                                |
| a-Fuc   | -0.636*** | 0.510**   | 0.515**  | -0.394 *              | -0.563 ** |           | 0.659***                           | -0.707***                                                                                                |
| E-Lip   |           |           |          |                       |           |           |                                    |                                                                                                          |
| Lip     |           |           |          |                       |           | -0.464*   |                                    | -0.480 **                                                                                                |
| LeuAryl | -0.440 *  | 0.629***  | 0.702*** | -0.530 **             |           |           | 0.772***                           | -0.376 *                                                                                                 |
| ValAryl |           | 0.579**   | 0.616**  | -0.593 **             |           |           | 0.606***                           |                                                                                                          |
| SEI     | -0.535 ** | 0.794***  | 0.858*** | -0.622***             | -0.428 *  |           | 0.753***                           | -0.419 *                                                                                                 |
| SEI/TOC | -0.433 *  | -0.428*   | -0.366 * | 0.395 *               |           | -0.711*** |                                    |                                                                                                          |
| SEI/MBC |           | -0.515 ** | -0.561** | 0.435 *               |           |           |                                    |                                                                                                          |

\*\*\* p<0.001; \*\*p<0.01; \*p<0.05; TOC: total organic C; C<sub>ext</sub>/TOC: extractable C/TOC ratio; Fe<sub>DCB</sub>/Fe<sub>T</sub>: Fe extracted by Na-dithionite-citrate-bicarbonate solution/pseudo-total Fe ratio; (Ca<sub>T</sub>+Mg<sub>T</sub>+K<sub>T</sub>+Na<sub>T</sub>)/(Al<sub>T</sub>+Fe<sub>T</sub>): (Ca<sub>T</sub>+Mg<sub>T</sub>+K<sub>T</sub>+Na<sub>T</sub>)/(Al<sub>T</sub>+Fe<sub>T</sub>) molar ratio; MBC and MBN: microbialbiomass C and N, respectively; Cell: β-cellobiohydrolase; Chit: N-acetyl-β-glucosaminidase; BG: β-glucosidase; AG: α-glucosidase; AP: Acidphosphomonoesterase; Sulph: arylsulphatase; Xylo: xylosidase; But: butyrate esteras; a-Gal: α-galactosidase; b-Gal: β-galactosidase; b-Gluc: β-glucuronidase; a-Man: α-mannosidase; a-Fuc: α-fucosidase; E-Lip: Esterase lipase; Lip: Lipase; LeuAryl: Leucine arylamidase; ValAryl: valine arylamidase; SEI: Synthetic Enzymatic Index; SEI/TOC: Synthetic Enzymatic Index per unit of organic carbon; Synthetic Enzymatic Index per unit of microbial biomass carbon.

## FIGURE CAPTIONS

Figure 1. The study area.

Figure 2. Boxplot of  $\text{Fe}_{\text{DCB}}/\text{Fe}_{\text{T}}$  ratio, where  $\text{Fe}_{\text{DCB}}$  and  $\text{Fe}_{\text{T}}$  are amount of Fe extractable with Na-dithionate-citrate-bicarbonate and pseudo-total, respectively (A) and molar ratio between the sum of the  $\text{Ca}_{\text{T}}$ ,  $\text{Mg}_{\text{T}}$ ,  $\text{K}_{\text{T}}$ ,  $\text{Na}_{\text{T}}$  and  $\text{Al}_{\text{T}}$  plus  $\text{Fe}_{\text{T}}$  (B). Different letters mean significant difference at p-level <0.01.

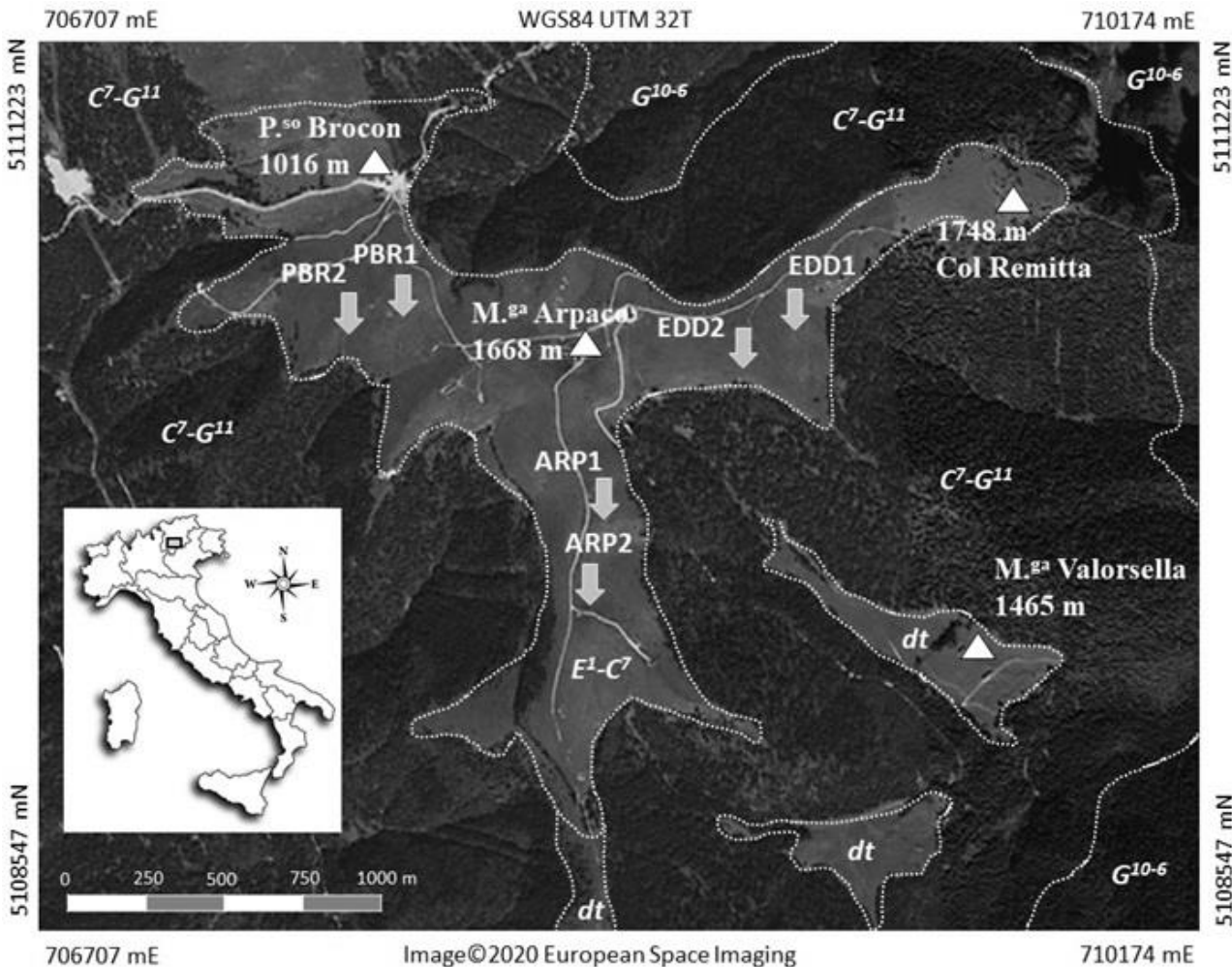
Figure 3. Principal component analysis of the physicochemical properties of soil layers (Tp, Elu, Wh and Ls). On left, plots of first and second components grouping variables, on right table of rotated loading values for the first two PCs from soil samples (in bold significant values  $p < 0.01$ ).

Figure 4. Boxplot of  $\alpha$ -mannosidase (A) and  $\alpha$ -fucosidase (B) activities. Different letters mean significant difference at p-level <0.05.

Figure 5. Boxplot of lipase activity (A) and Synthetic Enzymatic Index per unit of organic carbon - SEI/OC (B). Different letters mean significant difference at p-level <0.01.

Figure 6. Principal component analysis of the biochemical properties of soil layers (Tp, Elu, Wh, and Ls). On left, plots of first and second components grouping variables, on right table of rotated loading values for the first two PCs from soil samples (in bold significant values  $p < 0.01$ ).

Figure 1



Soil profile  
location

EDD2



Litological formations in succession from the Upper to Lower Cretaceous

$G^{10-6}$

«Rosso Ammonitico»  
(Malm-Dogger)  
White or brick red  
limestones

$C^7-G^{11}$

«Biancone»  
(Cenomanian-Malm)  
Greyish – white or  
white limestones

$E^1-C^7$

«Scaglia rossa»  
(Eocene-Cretaceous)  
Red or pink marls and  
clayey limestones

$dt$

Heterogeneous  
glacial debris

Figure 2

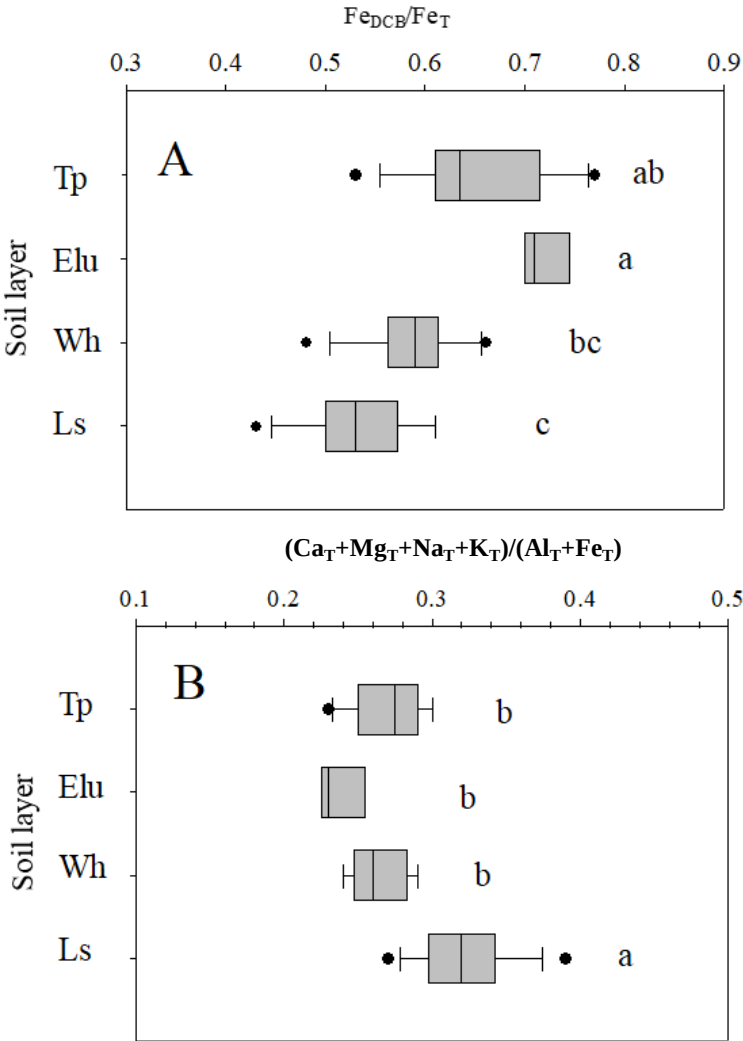
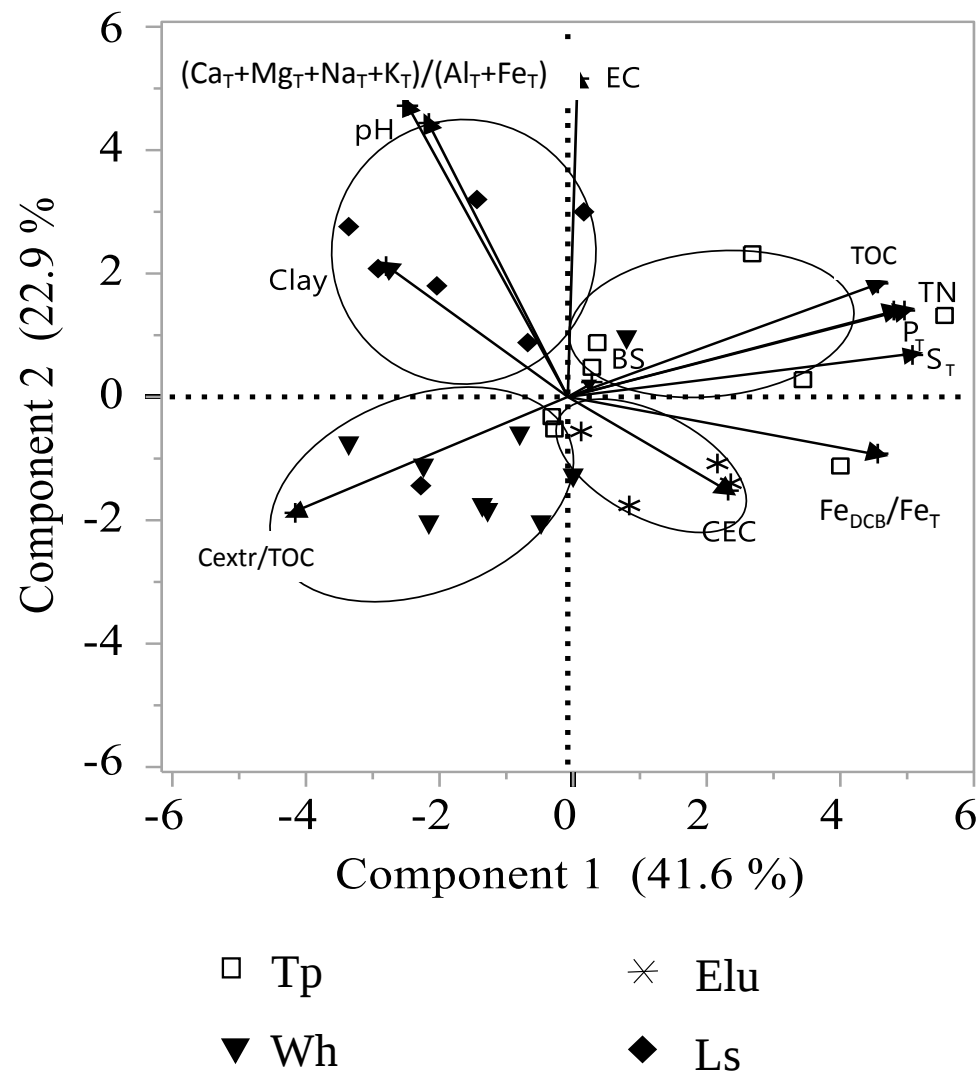


Figure 3



| Soil properties                              | Component 1   | Component 2  |
|----------------------------------------------|---------------|--------------|
| pH                                           | -0.137        | <b>0.939</b> |
| TOC                                          | <b>0.878</b>  | -0.002       |
| TN                                           | <b>0.928</b>  | -0.095       |
| $C_{extr}/TOC$                               | <b>-0.735</b> | -0.060       |
| $P_T$                                        | <b>0.870</b>  | -0.048       |
| $S_T$                                        | <b>0.880</b>  | -0.179       |
| Clay                                         | -0.325        | <b>0.431</b> |
| CEC                                          | 0.258         | -0.335       |
| BS                                           | 0.028         | 0.026        |
| EC                                           | 0.321         | <b>0.847</b> |
| $Fe_{DCB}/Fe_T$                              | <b>0.680</b>  | -0.379       |
| $(Ca_T + Mg_T + Na_T + K_T) / (Al_T + Fe_T)$ | -0.076        | <b>0.802</b> |
| Eigenvalue                                   | 4.99          | 2.74         |
| Accumulated variance                         | 41.6%         | <b>64.5%</b> |

Figure 4

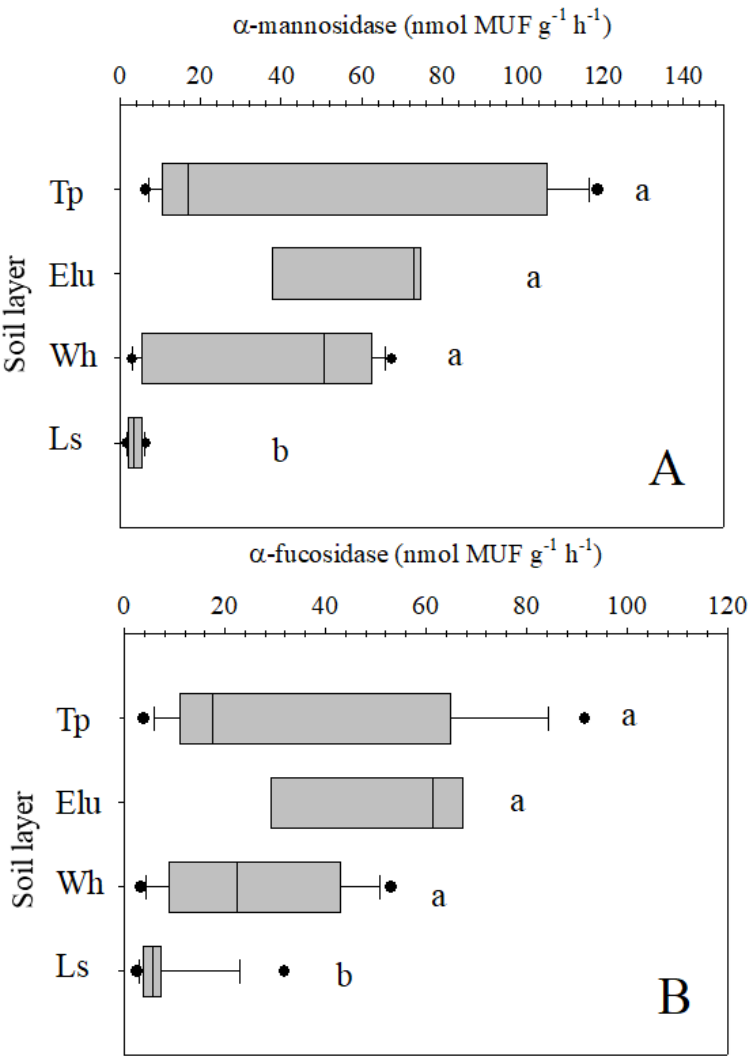


Figure 5

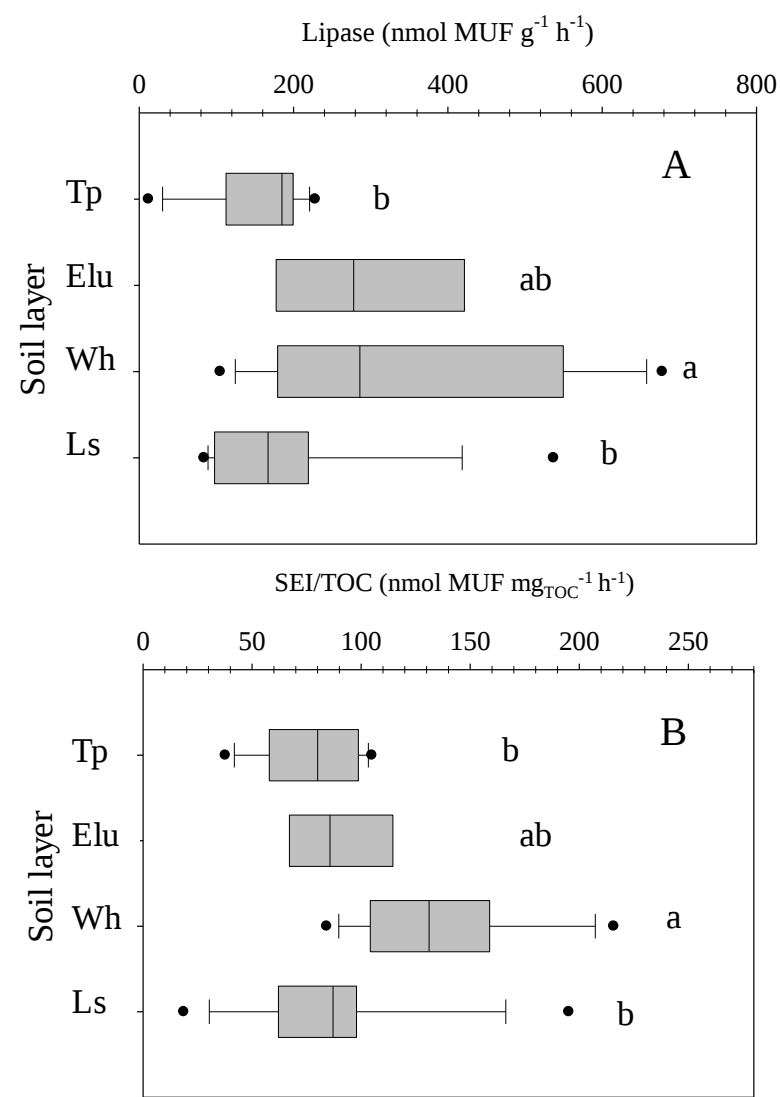
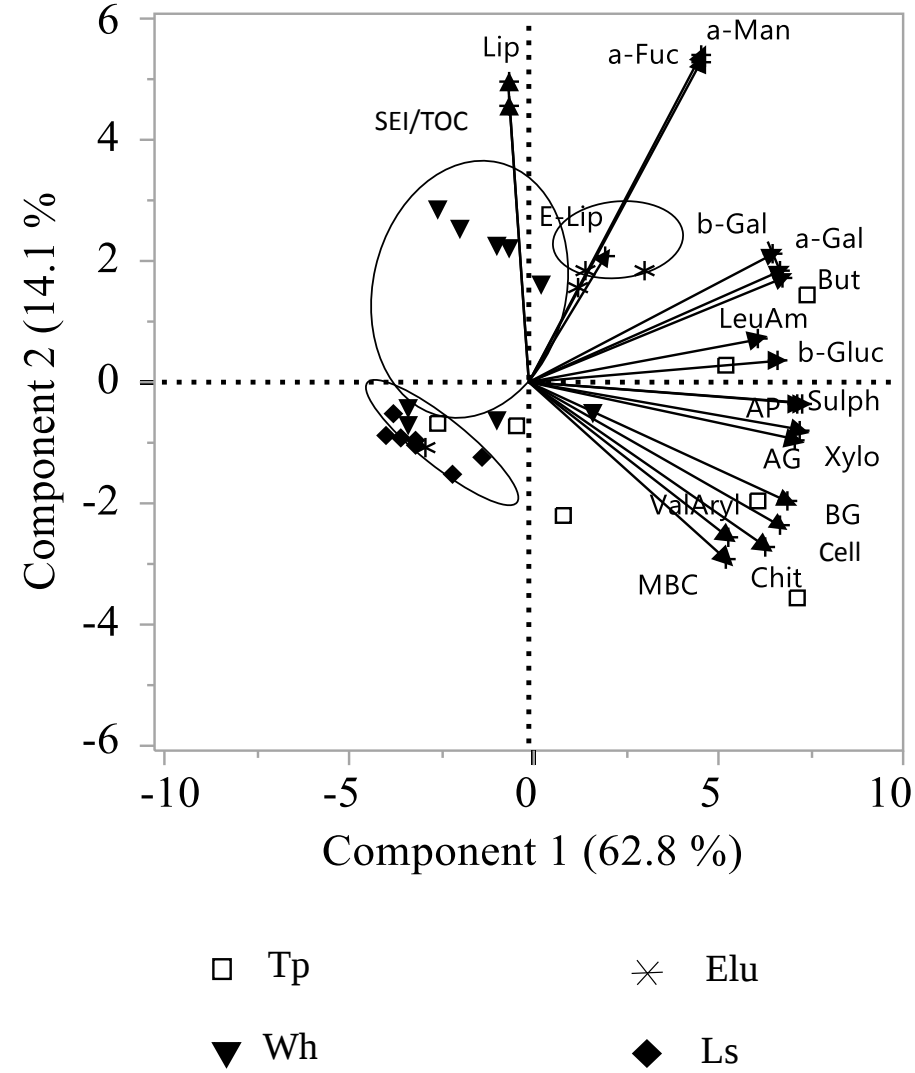


Figure 6



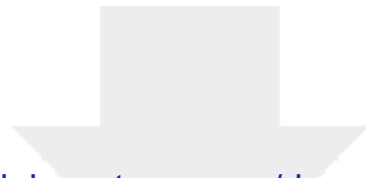
| Soil properties      | Component 1  | Component 2  |
|----------------------|--------------|--------------|
| SEI/TOC              | -0.251       | <b>0.555</b> |
| MBC                  | <b>0.79</b>  | -0.152       |
| Cell                 | <b>0.955</b> | -0.023       |
| Chit                 | <b>0.92</b>  | -0.083       |
| BG                   | <b>0.966</b> | 0.036        |
| AG                   | <b>0.949</b> | 0.174        |
| AP                   | <b>0.951</b> | 0.258        |
| Sulph                | <b>0.93</b>  | 0.251        |
| Xylo                 | <b>0.958</b> | 0.199        |
| But                  | <b>0.801</b> | <b>0.497</b> |
| a-Gal                | <b>0.752</b> | <b>0.537</b> |
| b-Gal                | <b>0.786</b> | <b>0.508</b> |
| b-Gluc               | <b>0.833</b> | 0.317        |
| a-Man                | 0.371        | <b>0.875</b> |
| a-Fuc                | 0.375        | <b>0.859</b> |
| E-Lip                | 0.176        | <b>0.344</b> |
| Lip                  | -0.274       | <b>0.606</b> |
| LeuAm                | <b>0.752</b> | 0.339        |
| ValAryl              | <b>0.789</b> | -0.104       |
| Eigenvalue           | 11.92        | 2.68         |
| Accumulated variance | 62.8%        | <b>76.9%</b> |



**Declaration of interests**

xThe authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper entitled **MINERAL WEATHERING AND LESSIVAGE AFFECT MICROBIAL COMMUNITY AND ENZYME ACTIVITY IN MOUNTAIN SOILS**

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:



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**Supplementary Material**

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