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**Relative Importance of Mineralogy and Organic Matter Characteristics on Macroaggregate and Colloid Dynamics in Mg-Silicate Dominated Soils**

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1 **Title**

2 **RELATIVE IMPORTANCE OF MINERALOGY AND ORGANIC MATTER CHARACTERISTICS**  
3 **ON MACROAGGREGATE AND COLLOID DYNAMICS IN Mg-SILICATE DOMINATED SOILS**

4  
5 **Short title**

6 **AGGREGATION IN Mg-SILICATE DOMINATED SOILS**

7  
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17  
18 **ABSTRACT**

19 Soil aggregation and organic matter conservation are important in the prevention of land degradation.  
20 Aggregation processes and organic matter turnover influence each other and depend on the characteristics of  
21 both minerals and organic pools. We assessed the relative importance of the organic and mineral phases at the  
22 macroaggregate and colloidal scale in two soils (CHL and SRP, chlorite and serpentine-rich, respectively)  
23 where Mg-silicates dominated, by incubating them with a relatively degraded and oxidized organic fraction,  
24 i.e. the humic acids (HA) extracted from the organic horizons of both CHL and SRP. The HA from SRP were  
25 more aromatic and richer in phenolic groups, whereas HA from CHL were N-richer, more aliphatic and richer  
26 in carboxyl groups. The SRP soil formed larger amounts of macroaggregates, more stable than in CHL. At the  
27 colloidal scale, SRP was more flocculated and clay had a lower electrophoretic mobility than CHL. HA  
28 enhanced aggregate formation in both samples but improved aggregate stability only in CHL. In CHL slight  
29 differences in electrophoretic mobility were visible, while in SRP differences were more pronounced, with a  
30 PZC at lower pH and larger hydrodynamic diameter. The abundance of Mg in SRP may have favoured the  
31 formation of weaker outer-sphere interactions and the release of clay-HA associations upon water dispersion,  
32 while in CHL Ca formed more stable bonds with HA. In SRP ligand exchange reactions can be ruled out,  
33 conversely to the dominant bonding mechanism occurring in Al-silicate dominated soils, with important  
34 consequences on the release of organic matter-loaded clay particles.

35  
36 **KEYWORDS:** oxidized organic matter, aggregate stability, zeta potential, soil mineralogy, serpentine

37

## INTRODUCTION

39 Soils are a finite resource sustaining terrestrial life and providing a wide set of ecosystem functions that are  
40 vital for the human kind, such as food and biomass production, carbon regulation, filtering pollutants,  
41 biodiversity and natural/cultural heritage (Adhikari & Hartemink, 2016; Berendse et al., 2015; Keesstra et  
42 al., 2012). However, soil degradation is an ongoing process often leading to permanent loss, putting at risk  
43 soil quality and availability for future generations. Land degradation is the result of a large number of natural  
44 and man-induced processes (Seeger & Ries, 2008; Zhang et al., 2015) that affect the topsoil chemical and  
45 physical quality and globally decrease soil functionality (Brevik et al., 2015). Among the main effects of  
46 land degradation, the breakdown of soil structure and disruption of aggregates are widely studied (De Clercq  
47 et al., 2015) as they are good indicators of poor soil quality (Zornoza et al., 2015). Soil structure and  
48 aggregate stability are directly impacted by land use (Cerdà, 2000; de Oliveira et al., 2015) and enhanced in  
49 natural and forest ecosystems or upon afforestation (Muñoz-Rojas et al., 2015) and tree plantation (Gümüs &  
50 Şeker, 2015). By affecting soil infiltration capacity, soil aggregates play a key role in controlling erosion  
51 processes and soil losses in semiarid areas and in areas that have lost their original vegetation cover  
52 (Arjmand Sajjadi & Mahmoodabadi, 2015; Cerdà, 1996; Mataix-Solera et al., 2011).

53 In addition to the well-known effect that soil structure and aggregates have on soil fertility, gas exchanges, and  
54 on the hydrological cycle, they significantly influence organic matter stocks and are thus fundamental in the  
55 carbon cycle (Six & Paustian, 2014; Chaplot & Cooper, 2015; O'Rourke et al., 2015). The inclusion of organic  
56 residues increases the stability of macroaggregates, although the highest residence time is recorded for clay-  
57 associated OM (Gelaw et al., 2013). A complex relationship between OM and aggregates therefore exists in  
58 soils: aggregation is favoured by OM, but in turn aggregates largely control the dynamics of organic pools (Six  
59 et al., 2004; Kögel-Knabner et al., 2008; Stanchi et al., 2015). Typically, OM in microaggregates is more stable  
60 and less sensitive to changes in land use (Debasish-Saha et al., 2014). Macroaggregates instead include also  
61 fresh residues that may undergo further transformation if aggregate turnover is slow enough to allow the  
62 physical protection of OM (Six et al., 2004). The addition of fresh, bio-labile organic compounds has a short  
63 term effect on soil aggregation processes, while treating the soil with more degraded and oxidized organic  
64 materials may lead to a stable improvement of soil physical characteristics (Piccolo & Mbagwu, 1999; Abiven  
65 et al., 2009; Mamedov et al., 2014; Mikutta et al., 2015). The effect of these molecules on soil aggregation  
66 depends, however, on the availability of reactive mineral surfaces and dispersion/flocculation behaviour of the  
67 colloidal fraction (Amezketta, 1999; Vogel et al., 2014). Deneff et al. (2002) observed that soils with dominating  
68 variable charge clay minerals have a greater potential to form stable macroaggregates when OM concentrations  
69 are low, while the greatest response in stable macroaggregate formation with additional organic inputs was  
70 found in soils with mixed mineralogy, likely because of the occurrence of different binding mechanisms. Also  
71 the characteristics of organic material affect the mechanisms involved in the reactions with mineral surfaces  
72 by determining both bonding extent and strength (Mikutta et al., 2007). The presence of this large set of  
73 controlling variables makes the study of the effect of organic matter on aggregation challenging, and field  
74 studies are mostly limited to the description of statistical correlations (Regelink et al., 2015). Laboratory

75 experiments of aggregate building up may be more suitable for understanding the relative role of several  
76 controlling variables without having to deal with excessive complexity, and therefore widely used to evaluate  
77 the effect of different pools of organic matter on aggregation (Ding et al., 2013; Andruschkewitsch et al., 2014;  
78 Vogel et al., 2014). However, most studies were carried out on kaolinites, smectites and vermiculites, or on  
79 soils where these Al-silicates dominated. Magnesium-rich phyllosilicates, such as serpentines (1:1) and  
80 lithogenic chlorites (2:1:1), are less frequent, but are often found in alpine regions and on peri-Alpine  
81 floodplains (Legros, 1992). Due to the different chemical properties of Al and Mg, the extent of direct bonding  
82 of OM to phyllosilicate surfaces should sharply differ and the results obtained with Al-silicates might not be  
83 directly applied to chlorite- or serpentine-dominated soils.

84 From previous studies, we found that the aggregation process induced by water-only wet and dry cycles  
85 differed in serpentinitic and chloritic soils; clay-sand interactions prevailed in aggregate building up when the  
86 clay fraction was dominated by serpentine, while interactions between clay particles prevailed in the chloritic  
87 soil (Falsone et al., 2007). In this study, we hypothesized that the input of degraded, oxidized organic  
88 substances during wet and dry cycles might promote particle aggregation depending on their characteristics  
89 and soil mineralogy. The aim of this work was therefore to evaluate the relative importance of soil mineralogy  
90 and of the characteristics of added OM on aggregation and aggregate stability, both at the macroaggregate and  
91 colloidal size-scale. We also evaluated the differences in aggregate properties caused by the addition of organic  
92 matter by comparing the results with those obtained in the previous wet and dry experiment.

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## MATERIALS AND METHODS

### *The study area*

The study area was located in north-western Italy (45°10'N, 7°23'E; Col del Lys). Two soils were selected in the area belonging to the greenstone belt of the Alps, a complex mix of ophiolites with micaceous, chloritic and calcareous schists. In the study area the soil parent material differs therefore in mineral abundance. One selected soil developed from chlorite-schists (CHL), and the other one was on serpentinite (SRP). They were described and classified as Typic Udorthent and Typic Hapludalf, respectively (Soil Survey Staff, 2014). The dominant vegetation consisted of grassland, although some larch trees were present at SRP. Soil samples were collected from the second horizon (AC and A2, respectively) after discarding the first A horizon to reduce the effect of native organic matter. The amount of organic carbon was less than 30 g kg<sup>-1</sup> and quite similar between the two soil samples; the soils differed for the dominant cation on the exchange complex (Ca in CHL and Mg in SRP), in pH (5.4 vs 6.1 in CHL and SRP, respectively) and CHL was sandy-loam, while SRP was loamy. Additional details can be found in Falsone et al. (2007).

### *Extraction and characterization of mineral and organic phases*

The mineralogical composition of the samples was evaluated through X-ray diffraction using a PW1810 diffractometer (40kV and 20 mA, Co K $\alpha$ , graphite monochromator) on both the <2 mm sieved samples and

112 the clay fraction. The <2 mm samples were finely ground in an agate mortar and analyzed as air-dried randomly  
113 oriented powders from 3 to 80 °2θ at a speed of 0.6 °2θ min<sup>-1</sup>. The clay fraction was separated by centrifugation  
114 after addition of sodium hexametaphosphate [(NaPO<sub>3</sub>)<sub>6</sub>] and analyzed as air-dried, ethylene glycol solvated,  
115 K-saturated, and heated (550°C) oriented mounts. Scans were made from 3 to 35 °2θ at a speed of 1 °2θ min<sup>-1</sup>.  
116 A semiquantitative evaluation of mineral abundance was performed after background subtraction and  
117 calculation of peak intensities and positions using the second derivative option of the PowderX software  
118 (Dong, 1999).

119 The organic matter fraction was extracted from the organic horizons of both soils by treating the samples with  
120 0.5M NaOH, followed by acidification to pH 1 with HCl and purification with a 0.1N HCl/0.3N HF solution  
121 (Swift, 1996). The purified fraction, i.e. the humic acid fraction, (HA) was then washed with deionized water  
122 until the solution pH was 3, freeze-dried and stored for further analysis. The two obtained HA fractions were  
123 labelled as HA-CHL and HA-SRP.

124 The elemental composition (C, N, H, S) of HAs was determined with an elemental analyzer (CE Instruments  
125 NA2100, Rodano, Italy); the O content was calculated by difference. The amount of total acidity and carboxyl  
126 groups was determined according to Schnitzer and Gupta (1965); the amount of phenolic -OH groups was  
127 calculated by difference. The absorbance ratio of HA solutions at 465 and 665 nm was used to determine the  
128 E<sub>4</sub>/E<sub>6</sub> ratio (Chen et al., 1977). The absorbance was measured with an UV-Vis spectrophotometer (U-2000,  
129 Hitachi, Tokio, Japan).

130 The Fourier-Transform Infrared (FT-IR) spectra of HAs were recorded using pellets prepared by pressing 0.5  
131 mg of humic substances with 200 mg of anhydrous KBr. Spectra were acquired from 4000 to 400 cm<sup>-1</sup>, at 4  
132 cm<sup>-1</sup> resolution and 16 scans were averaged.

133

#### 134 *Incubation conditions*

135 One hundred grams of the <2 mm air-dried samples of each soil were evenly distributed in a container and  
136 sprayed with a solution of HA containing 0.5 g of C<sub>HA</sub>. To evaluate the effect of HA composition, each soil  
137 (CHL and SRP) was incubated with both HA fractions. Four aggregation tests were thus performed: CHL plus  
138 HA-CHL, CHL plus HA-SRP, SRP plus HA-CHL and SRP plus HA-SRP. The samples were allowed to dry  
139 for 2 weeks at room temperature. The experiment was replicated three times. At the end of the incubation  
140 period, the samples were sequentially dry sieved on 5- and 2-mm sieves, the aggregates remaining on each  
141 sieve (i.e. newly formed aggregates, NF) were weighed, and the result was expressed as percentage of the total  
142 mass (i.e., sum of <2, 2-5 and >5 mm fractions). The aggregation state was described by the Mean Weight  
143 Diameter (MWD) computed across *n* sieve size fractions using the relation:

$$144 \quad MWD = \sum_{i=1}^n \bar{x}_i w_i \quad (1)$$

145 where  $\bar{x}_i$  is the assumed diameter of the *i*<sup>th</sup> fraction and *w<sub>i</sub>* is the weight fraction retained on the *i*<sup>th</sup> sieve-size.

146 The aggregate diameter classes used in the MWD calculation were 0-2, 2-5 and 5-10 mm. The upper limit of  
147 the last aggregate diameter class was fixed on the basis of the observed greatest size of the NF-aggregates.

148

149 *Characterization of newly formed aggregates*

150 The analyses were done on the 2-5 mm NF-aggregates. The particle size distribution was determined by the  
151 pipette method (Gee & Bauder, 1986) after: (i) dispersion with  $(\text{NaPO}_3)_6$ ; (ii) dispersion with deionized water  
152 only. The  $<2 \mu\text{m}$  fractions obtained were labelled as sodium-dispersible clay (NaDC) and water-dispersible  
153 clay (WDC). A first estimate of the colloidal stability was obtained by the clay dispersion ratio (CDR)  
154 calculated as the ratio between the mass ( $\text{g kg}^{-1}$ ) of water-dispersible and sodium-dispersible clay  
155 (WDC/NaDC). On the WDC fraction, the electrophoretic mobility (EM) as a function of pH (from 2 to 6) was  
156 measured by laser Doppler velocimetry coupled with photon correlation spectroscopy using a DELSA 440  
157 spectrometer (Beckman Coulter Electronics, Fullerton, CA) equipped with a 5-mW HeNe laser (632.8 nm).  
158 The point of zero charge was estimated by interpolating the two pH values for which the EM values were the  
159 closest to zero. The pH of the clay suspension was measured immediately before the electrochemical  
160 measurements. The Doppler shift arising from Brownian motion was used to determine the particle diffusion  
161 coefficient, which was converted to an equivalent hydrodynamic diameter using Stokes-Einstein equation as  
162 described by Hunter (1986). All measurements were run in duplicate.

163 The wet 2-5 mm NF-aggregate stability (WAS) was evaluated after 10 min in rotating 0.2-mm sieves. The  
164 material remaining on the sieves was then dried and weighed. The amount of stable aggregates ( $>0.2 \text{ mm}$ ) was  
165 corrected for the content of coarse sand determined after  $\text{H}_2\text{O}_2$  oxidation. The WAS index was calculated as  
166 follows:

$$167 \text{ WAS} = \frac{\text{weight retained} - \text{weight of coarse sand}}{\text{total sample weight} - \text{weight of coarse sand}} \cdot 100 \quad (2)$$

168 To test the wet cohesion independently from slaking, thus regardless of the ruptures due to water saturation,  
169 10 g of NF aggregates were gently immersed into 95% solution of ethanol for 10 min (Le Bissonnais, 1996)  
170 before being wet sieved for 10 min. Then, the amount of aggregates that had resisted the sieving ( $\text{WAS}_{\text{et}}$ ) was  
171 determined according to the equation (2).

172 The cumulative pore volume curve of NF-aggregates was determined using a Hg porosimeter (Porosimeter  
173 2000 WS equipped with a Macropore unit 120, CE Instruments, Rodano, Italy). The total volume of intruded  
174 Hg (i.e. total pore volume) was expressed on a mass basis ( $V_{\text{HgTot}}, \text{mm}^3 \text{g}^{-1}$ ). The cumulative curve of intruded  
175 Hg volume as a function of the pore radius was used to calculate the variation of the pore volume as a function  
176 of the log-transformed radius, finding the slope between the pore radii  $R_i$  and  $R_j$  (Bruand & Prost, 1987):

$$177 \text{ slope}_{ij} = \frac{V_i - V_j}{\log_{10} R_j - \log_{10} R_i} \quad (3)$$

178 where the volumes  $V_i$  and  $V_j$  correspond to the radii  $R_i$  and  $R_j$  at two successive positions  $i$  and  $j$ . The values  
179 of the slopes were plotted against the mean log radius. The relative maxima of the slope curve indicated the  
180 most represented classes of pores. Each peak thus corresponded to a radius that was termed modal radius  
181 (expressed in  $\mu\text{m}$ ). The volume of each modal class of pores was thus included between two minimum values  
182 of the slope, which defined the limits of the class. This data treatment typically returns a bimodal distribution.

183 We thus labelled the modal pore radii as  $R_f$  and  $R_c$  and attributed them to the packing of finer (clay) and  
184 coarser (sand and silt), according to Fiès & Bruand (1998). The corresponding volumes of pores were labelled  
185 as  $V_{Hg_f}$  and  $V_{Hg_c}$ . The packing density, i.e. the volume occupied by solid particles with respect to the total  
186 volume, was calculated according to Falsone & Bonifacio (2009) as:

$$187 \text{ packing density} = \frac{\text{particle mass}}{\text{particle density}} \cdot \frac{1}{\frac{\text{particle mass}}{\text{particle density}} + \text{volume of intruded Hg}} \quad (4)$$

188 where particle density was taken equal to  $2.65 \times 10^{-3} \text{ g mm}^{-3}$ , the mass was expressed in  $\text{g (100 g)}^{-1}$ , the volume  
189 of intruded Hg in  $\text{mm}^3 \text{ g}^{-1}$ , and the resulting packing density was dimensionless. We calculated the packing  
190 density of clay using the NaDC fraction from textural analyses for particle mass and the modal volume  
191 corresponding to clay–clay interactions. The packing density of the sand and silt phases was also calculated,  
192 following the same assumptions.

193

#### 194 *Data analysis*

195 To evaluate the relative importance of mineralogy and OM characteristics, the results of this study were  
196 matched to those obtained previously (Falsone et al., 2007) after one water-only wet-dry cycle. The parameters  
197 we considered were: % of 2-5 mm NF-aggregates, wet aggregate stability indexes, CDR, porosity data, and  
198 PZC and hydrodynamic diameter of WDC. The changes of each parameter were expressed as % variation  
199 calculated as:

$$200 \% \text{ variation} = \frac{\text{parameter value}_{NF HA} - \text{parameter value}_{NF WD}}{\text{parameter value}_{NF WD}} \cdot 100$$

201 where *NF HA* and *NF WD* refer to the present and previous data, respectively. Differences in the % variation  
202 among samples were evaluated by means of the analysis of variance (ANOVA and Duncan's test). Before  
203 analysis, the homogeneity of variances was checked using the Levene test and the normality of data through  
204 the Shapiro-Wilks test. The threshold used for significance in all statistical tests was set at 0.05. All data  
205 treatments were carried out using SPSS 20 (SPSS Inc., Chicago, IL).

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208

## 208 **RESULTS**

209

### 210 *Mineralogical and soil organic matter characteristics*

211 The two samples showed similar mineralogical species but sharply differ in their proportions (Figure 1). In the  
212 <2 mm fraction, serpentines were the most abundant minerals in SRP (>30%), followed by chlorite, quartz,  
213 Na-feldspars (albite), amphiboles, lithogenic micas (phlogopite), K-feldspars (orthoclase) and illite. In the  
214 CHL sample instead the most abundant mineral was chlorite (~20%), but large amounts of feldspars (around  
215 15%, both orthoclase and albite) were present, as well as lithogenic micas (margarite), talc and amphiboles  
216 (~10%). Small amounts of serpentines and quartz were found as well. More differences between the two  
217 samples were visible in the clay fraction: in SRP serpentine dominated (~60%), associated with minor amounts  
218 of chlorite and swelling mixed-layer minerals, with traces of amphiboles, illite and illitic mixed-layers. In CHL



219 chlorite was the most abundant mineral (~50%), followed by talc, serpentine, illite, chlorite-illite randomly  
220 interstratified minerals and traces of amphiboles.

221 The carbon content of oxidized organic matter fractions from the CHL organic horizon was significantly lower  
222 ( $p < 0.05$ ,  $n=6$ ) than that of SRP (Table 1), while the opposite was observed for the N content. The C/N molar  
223 ratio was consequently minor in CHL, indicating a higher degree of decomposition and/or a larger  
224 incorporation of N containing organic compounds. The H/C and O/C molar ratios, significantly higher in CHL  
225 than in SRP ( $p < 0.05$ ,  $n=6$ ), suggested a larger contribution of the cellulosic component in the former. Higher  
226 H/C ratios indicate indeed that the organic matter fraction from CHL is more aliphatic than SRP, while the  
227 O/C ratio specifies the presence of O-containing functional groups, such as the -OH groups in the cellulose  
228 polymers and carboxyl (-COOH) groups deriving from oxidation processes. This was confirmed by chemical  
229 titration and FT-IR spectra (Figure 2). The HA from CHL had more carboxyl groups than SRP, but less  
230 phenolic -OH groups ( $p < 0.05$ ,  $n=6$ , Table 1). The higher acidity found in SRP was coupled to a higher  $E_4/E_6$   
231 ratio, indicating a lower molecular size and a higher charge/mass ratio with respect to CHL. This ratio gives  
232 indeed an estimate of the molecular size and presence of charged functional groups: the higher the  $E_4/E_6$  ratio,  
233 the smaller the size and the higher the charge (Chen et al., 1977). Although the FT-IR spectra of HAs appeared  
234 similar (Figure 2), they showed some specific differences, such as the peaks at about 1631 and 1514  $\text{cm}^{-1}$   
235 (aromatic C=C stretching), more pronounced in SRP, a band at 1534  $\text{cm}^{-1}$  (-CONH- stretching of II amides)  
236 visible only in CHL, and a band in the region 1060-1034  $\text{cm}^{-1}$  (polysaccharide C-O stretching) more  
237 pronounced in CHL. The HA from CHL appeared therefore to be richer in cellulosic and proteinaceous  
238 residues, more oxidized, with a higher content of carboxyl groups, whereas HA-SRP was more aromatic and  
239 richer in phenolic groups.

240

#### 241 *Properties of the NF- macroaggregates*

242 From 100 g of the CHL fine earth we obtained on the average 18.8 and 9.8 g of 2-5 and >5 mm NF aggregates,  
243 whereas the yield was 29.7 and 15.3 g for SRP, respectively (Table 2). The MWD value was consequently  
244 significantly higher ( $p < 0.05$ ,  $n=6$ ) in SRP samples than in CHL. No relevant differences between the two HAs  
245 were found.

246 After 10 min of wet sieving, the 2-5 mm NF aggregates of CHL were more fragile than those of SRP, with  
247 WAS values of 63.0 and 71.8% on average, respectively (Table 3). However, when the initial slaking was  
248 avoided, the samples had similar  $\text{WAS}_{\text{et}}$  values (72.8 and 74.6% on average) showing a comparable resistance  
249 against water abrasion. The total volume of Hg intruded in the pores of 2-5 mm NF aggregates ( $V_{\text{HgTot}}$ ) ranged  
250 from 177 to 188  $\text{mm}^3 \text{g}^{-1}$ . The analysis of the cumulative curve of the intruded Hg volume allowed to identify  
251 a bimodal distribution of pores in all samples (data not shown) attributed to the coarser particles, i.e. silt and  
252 sand, and to the finer colloidal phase according to Fiès & Braund (1998). The modal pore of coarser particles  
253 ( $R_c$ ) was more than three times higher in CHL than in SRP ( $p < 0.05$ ,  $n=12$ ). The packing density of coarser  
254 particles, i.e. silt and sand, was similar, with no effect of the origin of HAs, and corresponded to orthorhombic-  
255 rhombohedral particle arrangement (Lal & Shukla, 2004).

256

257 *Properties of the colloidal phases*

258 The colloidal behaviour greatly differed between samples. The CDR values were  $<0.4$  in CHL and  $>0.8$  in  
259 SRP ( $p<0.05$ ,  $n=12$ , Table 3), thus less than 40% clay was water dispersible (WDC) in CHL and more than  
260 80% in SRP. The electrophoretic mobility of WDC varied as a function of pH from  $+0.25$  to  $-0.65 \mu\text{m cm s}^{-1}$   
261  $\text{V}^{-1}$  in CHL; in SRP from  $+0.27$  to  $-0.52 \mu\text{m cm s}^{-1} \text{V}^{-1}$  (Figure 3). No effect of the origin of HAs was visible  
262 in CHL, while in SRP the addition of HA-SRP induced a lower electrophoretic mobility at the lowest pH than  
263 HA-CHL ( $p<0.05$ ,  $n=6$ ). The point of zero charge (PZC) of both samples was however around pH 2-2.5, and  
264 only slightly lower ( $<2$ ) in the case of SRP with HA-SRP. At the lowest pH, the hydrodynamic diameter of  
265 CHL particles was on average slightly smaller than that of SRP (Table 3), and with increasing pH, changes in  
266 the electrophoretic mobility were accompanied by a decrease in particle size.

267 The size of  $R_f$ , i.e. the modal radius of voids originated by the arrangement of clay particles, was always lower  
268 than  $0.02 \mu\text{m}$  and its variability was small among samples (data not shown). The volume of the modal fine  
269 pores ( $V_{\text{Hgf}}$ ) was small, and CHL had lower values than SRP (Table 3). The packing density of the clay phase  
270 was instead significantly higher in CHL ( $p<0.05$ ,  $n=12$ ), and solids may occupy as much as 87-94% of the  
271 total volume, corresponding to a very close rhombohedral arrangement of clays.

272

273 *HA addition effect*

274 In order to compare the results obtained in the four treatments (i.e. two soils and two HAs), hence to understand  
275 both the effect of HA addition and differing mineralogical composition, the data were matched to those  
276 obtained in a previous experiment in which the wet-dry cycle was performed with deionized water only  
277 (Falsone et al., 2007).

278 The mass of the 2-5 mm NF aggregates generally increased after the addition of HA with respect to the water-  
279 only wet-dry cycle (Figure 4A). SRP showed the smallest positive variation, but no significant differences  
280 among samples were visible. Aggregate stability (both WAS and  $\text{WAS}_{\text{et}}$  indexes) was negatively or poorly  
281 affected by HA addition in SRP (Figure 4B, C). This was coupled with a decrease in the volume of coarser  
282 pores and an increase in packing density of coarser fraction (Figure 4D, E). Instead CHL HA-added samples  
283 were more stable to water abrasion (i.e.  $\text{WAS}_{\text{et}}$  increased), and no variations in  $V_{\text{HgC}}$  and  $\text{PDc}$  were found. The  
284 results obtained in CHL were thus generally significantly different ( $p<0.05$ ,  $n=12$ ) from those in SRP (Figure  
285 4). The differences at macroaggregate scale hence appeared more related to the soil mineralogical composition.

286 A significantly different response between samples ( $p<0.05$ ,  $n=12$ ) was also found for the colloidal phase. In  
287 fact, with respect to the water-only wet-dry cycle, the dispersibility and size at the lowest pH of the clay fraction  
288 of HA-added SRP samples increased (Figure 5A, E) and the PZC decreased (Figure 5D). The  $V_{\text{Hgf}}$  increased  
289 in SRP (Figure 5B) and the clay particles had a more open arrangement than in the case of the wet-dry cycle  
290 incubation (Figure 5C). In CHL an opposite trend was generally observed, with the exception of PZC values.  
291 In fact, upon HA addition, the PZC was reached at lower pH also in CHL although the differences were smaller  
292 than in SRP. No significant differences related to the HA origin were observed, with the exception of the

293 hydrodynamic diameter (Figure 5).

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## DISCUSSION

297 Differences in sample response upon addition of organic matter, at both macroaggregate and colloidal size  
298 scale, were found to be largely dependent on mineralogical composition. The serpentinitic soil (SRP) had a  
299 higher macroaggregate formation capacity, and aggregates were more stable than in the chloritic one (CHL),  
300 especially against slaking losses. Moreover, the colloidal phase was less dispersible and WDC had a higher  
301 electrophoretic mobility in CHL than SRP, although both had a relatively low negative charge with respect to  
302 the most common Al-phyllsilicates (Celi et al., 1999). All these findings reflect the aggregation pathways  
303 observed in the previous experiment on the effect of wet-dry cycles that can be summarized as dominated by  
304 clay-clay interactions in CHL and clay-sand interactions in SRP (Falsone et al., 2007).

305 With respect to the water-only wet-dry cycle, the addition of oxidized organic matter enhanced aggregate  
306 formation in both samples but improved aggregate stability towards abrasion only in CHL. In addition, the  
307 clay fraction of HA-added CHL samples was less dispersible, although only slight differences in charge  
308 properties of the dispersed particles were visible. The  $V_{Hgf}$  decreased in CHL and the clay particles had a  
309 denser arrangement than in the case of the previous experiment. In HA added SRP an opposite trend was  
310 observed, and generally the differences were more pronounced, with a PZC at lower pH and larger  
311 hydrodynamic diameter than the water-only wet-dry treated SRP samples. The addition of humic substances  
312 on soil (Plaza et al., 2014), oxides (Li et al., 2015) and clay minerals (Majzik & Tombácz, 2007) has a strong  
313 effect on surface charge and particle dispersibility, mostly because of the polyelectrolyte nature of HA that  
314 increases the net negative charge. The higher flocculation in CHL may be related to a larger abundance of  
315 reactive clay surfaces (Vogel et al., 2014), also favoured by the greater presence of Ca on the exchange  
316 complex, which can thus form more stable bonds with the organic material (Bronick & Lal, 2005). In CHL,  
317 clay particles and HA could thus form a more compact arrangement through clay-Ca-HA, as shown by the PDF  
318 increase. The possibility of direct bonding between negatively charged clay surfaces and positive amino groups  
319 of proteinaceous remains, abundant in HA-CHL, as well as the interaction of  $-\text{COO}^-$  groups with mineral edge  
320 Me-OH by ligand exchange, cannot be ruled out from electrophoretic mobility data, but cation bridges seem  
321 to dominate as no differences between the two HAs were found. Calcium bridges may therefore add to clay-  
322 clay interactions in aggregate formation and contribute to the greater stability against abrasion in CHL. The  
323 added organic material contribute therefore in this soil to the aggregation process while, being retained into  
324 aggregates, had little or no effect on the water dispersible fraction.

325 The larger abundance of Mg, more hydrated than Ca, in the SRP soil might have favoured instead weaker  
326 outer-sphere interactions with HA. The presence of HA on clay surfaces has probably hindered the formation  
327 of clay coatings on sand grains (Falsone et al. 2007), decreasing aggregate stability and allowing the dispersion  
328 of more negatively charged mineral-HA associated particles with respect to the water-only wet-dry conditions.  
329 The chemical composition of HA influenced therefore the charge characteristics of WDC: the higher contents

330 of amino groups in HA-CHL than in HA-SRP induced a higher positive charge at low pH, while the higher  
331 carboxyl content made the surfaces more negative at pH higher than the point of zero charge. Phenolic groups  
332 may contribute to the negative charge only at pH > 6.0 (Alvarez-Puebla & Garrido, 2005) justifying the less  
333 negative electrophoretic mobility obtained with HA-SRP at pH 4.0. As upon HA addition the surface of WDC  
334 still carried a positive charge at low pH, stable bonds such as ligand exchange may be ruled out. In addition,  
335 since low amounts of HA are sufficient to reverse the mineral charge even at low pH, the amount of HA on  
336 clay surfaces was probably very low (Magnacca et al., 2014).

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### CONCLUSIONS

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340 Organic matter inputs on the fine earth fraction of a serpentinitic and a chloritic soil promoted the formation  
341 of new aggregates in the size range of 2-5 and >5 mm, even if their wet stability was not always positively  
342 affected. The soil response however mostly depended on mineralogical composition, with no effect of HA  
343 composition on aggregates but only on the surface charge of the dispersible clay particles.

344 In soils dominated by Mg-silicates, weak chemical interactions between organic matter and mineral particles  
345 are the most important mechanism in controlling both aggregation processes and clay dispersibility. Ligand  
346 exchange reactions, if any, seem to be of only minor importance, stressing the differences with Al-silicates.

347 Because of the complex interlink between mineralogy, organic matter characteristics and dominant cations,  
348 the addition of organic matter may even favour the dispersion of OM-loaded clay particles, confounding the  
349 expected positive effects against land degradation.

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351

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494



495 Table 1. Mean ( $\pm$  standard deviation) of elemental content, total acidity, carboxyl groups, and  $E_4/E_6$  ratio of  
 496 purified, ash and moisture-free HAs extracted from the CHL and SRP organic horizons (HA-CHL and HA-  
 497 SRP, respectively). The letters show the significant differences at  $p$  level  $<0.05$  (Duncan's test) between HA  
 498 (n=6)

	HA-CHL	HA-SRP
ash (%)	1.6 $\pm$ 0.3	1.2 $\pm$ 0.1
C (%)	50.51 $\pm$ 0.31 <sup>b</sup>	54.59 $\pm$ 0.61 <sup>a</sup>
N (%)	5.51 $\pm$ 0.27 <sup>a</sup>	3.59 $\pm$ 0.26 <sup>b</sup>
H (%)	6.13 $\pm$ 0.04	6.16 $\pm$ 0.09
S (%)	0.64 $\pm$ 0.01 <sup>a</sup>	0.30 $\pm$ 0.03 <sup>b</sup>
O (%)	37.21 $\pm$ 0.33	35.35 $\pm$ 0.80
C/N	10.69 $\pm$ 0.54 <sup>b</sup>	17.72 $\pm$ 1.07 <sup>a</sup>
H/C	1.45 $\pm$ 0.01 <sup>a</sup>	1.35 $\pm$ 0.04 <sup>b</sup>
O/C	0.55 $\pm$ 0.01 <sup>a</sup>	0.49 $\pm$ 0.02 <sup>b</sup>
total acidity (cmol kg <sup>-1</sup> )	566 $\pm$ 8 <sup>b</sup>	608 $\pm$ 11 <sup>a</sup>
-COOH (cmol kg <sup>-1</sup> )	254 $\pm$ 6 <sup>a</sup>	232 $\pm$ 3 <sup>b</sup>
Phenolic OH (cmol kg <sup>-1</sup> )	312 $\pm$ 1 <sup>b</sup>	376 $\pm$ 8 <sup>a</sup>
$E_4/E_6$	6.20 $\pm$ 0.08 <sup>b</sup>	6.57 $\pm$ 0.08 <sup>a</sup>

499 -COOH: carboxyl groups; Phenolic OH: phenolic OH-groups;  $E_4/E_6$ : ratio of absorbance at 465 and 665 nm

500

501

502 Table 2. Mean ( $\pm$  standard deviation) of percentage of newly-formed aggregate classes and aggregate Mean  
 503 Weight Diameter (MWD). The letters show the significant differences at  $p$  level  $<0.05$  (Duncan's test)  
 504 between treatments within each site ( $n=6$ ; lowercase letters) and between sites ( $n=12$ ; uppercase letters)

505

soil sample	treatment	2-5 mm (%)	>5 mm (%)	MWD (mm)
CHL	HA-CHL	19.6 $\pm$ 1.6	9.1 $\pm$ 0.7	2.08 $\pm$ 0.01
	HA-SRP	17.5 $\pm$ 3.6	10.3 $\pm$ 2.5	2.10 $\pm$ 0.23
	<i>mean<math>\pm</math>st. dev</i>	18.8 $\pm$ 2.9 <sup>B</sup>	9.8 $\pm$ 1.9 <sup>B</sup>	2.10 $\pm$ 0.16 <sup>B</sup>
SRP	HA-CHL	33.2 $\pm$ 3.4 <sup>a</sup>	14.4 $\pm$ 1.6	2.77 $\pm$ 0.19
	HA-SRP	27.4 $\pm$ 2.5 <sup>b</sup>	15.9 $\pm$ 4.6	2.72 $\pm$ 0.28
	<i>mean<math>\pm</math>st. dev</i>	29.7 $\pm$ 4.0 <sup>A</sup>	15.3 $\pm$ 3.4 <sup>A</sup>	2.74 $\pm$ 0.22 <sup>A</sup>

506

507 Table 3. Mean ( $\pm$  standard deviation) of stability indexes of 2-5 mm newly-formed aggregates (WAS and  
508 WAS<sub>et</sub>) and of the colloidal phase (CDR), porosity data of coarser particles (i.e. sand and silt), and  
509 characteristics of the colloidal phases obtained from newly-formed aggregates. The letters show the  
510 significant differences at  $p$  level  $<0.05$  (Duncan's test) between treatments within each site (n=6; lowercase  
511 letters) and between sites (n=12; uppercase letters)

soil sample	CHL			SRP		
	HA-CHL	HA-SRP	<i>mean<math>\pm</math>dev.st</i>	HA-CHL	HA-SRP	<i>mean<math>\pm</math>dev.st</i>
WAS (%)	61.0 $\pm$ 1.4 <sup>b</sup>	64.9 $\pm$ 1.1 <sup>a</sup>	63.0 $\pm$ 2.5 <sup>B</sup>	71.6 $\pm$ 0.7	72.0 $\pm$ 1.3	71.8 $\pm$ 0.9 <sup>A</sup>
WAS <sub>et</sub> (%)	73.8 $\pm$ 1.8 <sup>a</sup>	71.8 $\pm$ 1.8 <sup>b</sup>	72.8 $\pm$ 1.9	77.0 $\pm$ 1.7 <sup>a</sup>	72.2 $\pm$ 2.5 <sup>b</sup>	74.6 $\pm$ 3.3
V <sub>Hg</sub> Tot (mm <sup>3</sup> g <sup>-1</sup> )	180 $\pm$ 11	177 $\pm$ 9	178 $\pm$ 10	177 $\pm$ 25	188 $\pm$ 23	183 $\pm$ 43
V <sub>Hg</sub> c (mm <sup>3</sup> g <sup>-1</sup> )	178 $\pm$ 9	176 $\pm$ 9	177 $\pm$ 10	173 $\pm$ 22	175 $\pm$ 18	174 $\pm$ 43
Rc ( $\mu$ m)	1.494 $\pm$ 0.272	1.809 $\pm$ 0.279	1.651 $\pm$ 0.349 <sup>A</sup>	0.450 $\pm$ 0.129	0.470 $\pm$ 0.012	0.460 $\pm$ 0.076 <sup>B</sup>
PDc	0.67 $\pm$ 0.01	0.67 $\pm$ 0.01	0.67 $\pm$ 0.01	0.68 $\pm$ 0.03	0.68 $\pm$ 0.02	0.68 $\pm$ 0.05
CDR	0.38 $\pm$ 0.04	0.30 $\pm$ 0.03	0.34 $\pm$ 0.06 <sup>B</sup>	0.86 $\pm$ 0.05	0.94 $\pm$ 0.04	0.90 $\pm$ 0.07 <sup>A</sup>
hydrodynamic diameter at the lowest pH ( $\mu$ m)	1.05 $\pm$ 0.14 <sup>a</sup>	0.83 $\pm$ 0.04 <sup>b</sup>	0.94 $\pm$ 0.15	0.92 $\pm$ 0.10	1.08 $\pm$ 0.17	1.00 $\pm$ 0.17
hydrodynamic diameter at the highest pH ( $\mu$ m)	0.72 $\pm$ 0.10	0.78 $\pm$ 0.07	0.75 $\pm$ 0.08	0.71 $\pm$ 0.03	0.71 $\pm$ 0.02	0.71 $\pm$ 0.02
V <sub>Hg</sub> f (mm <sup>3</sup> g <sup>-1</sup> )	2 $\pm$ 1	1 $\pm$ <1	1 $\pm$ 1	5 $\pm$ 4	14 $\pm$ 5	9 $\pm$ 6
PDF	0.87 $\pm$ 0.08	0.94 $\pm$ 0.04	0.91 $\pm$ 0.06 <sup>A</sup>	0.73 $\pm$ 0.17	0.52 $\pm$ 0.09	0.62 $\pm$ 0.16 <sup>B</sup>

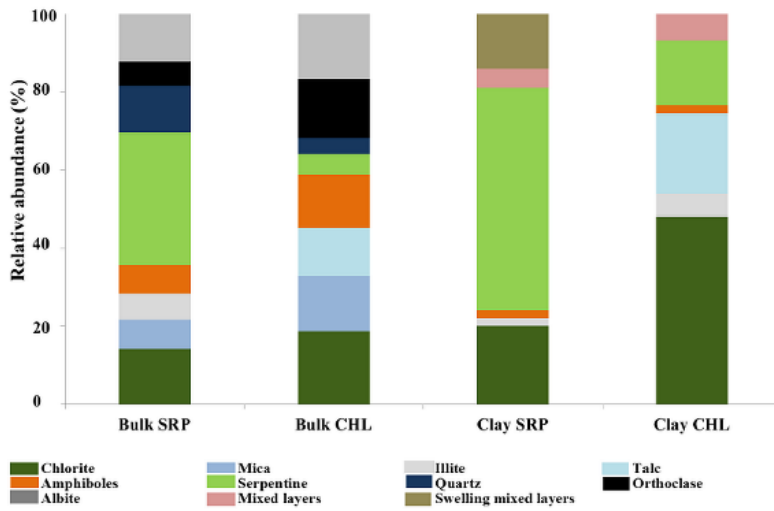
512 WAS: wet aggregate stability; WAS<sub>et</sub>: wet aggregate stability after ethanol saturation; V<sub>Hg</sub>Tot: total volume of intruded  
513 Hg (i.e. total pore volume); V<sub>Hg</sub>c: volume of pores within the coarser particles; Rc: modal radius of packing of coarser  
514 particles; PDc: packing density of the coarser particles; CDR: clay dispersion ratio; V<sub>Hg</sub>f: volume of pores within the  
515 finer particles; PDF: packing density of the finer particles

516

517 Captions to Figures

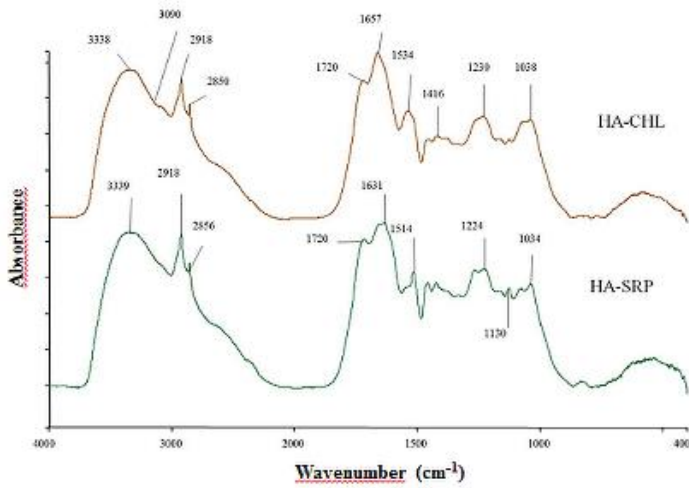
518

519 Figure 1 – Mineral composition of bulk (<2 mm) and clay fractions of the CHL and SRP soil samples



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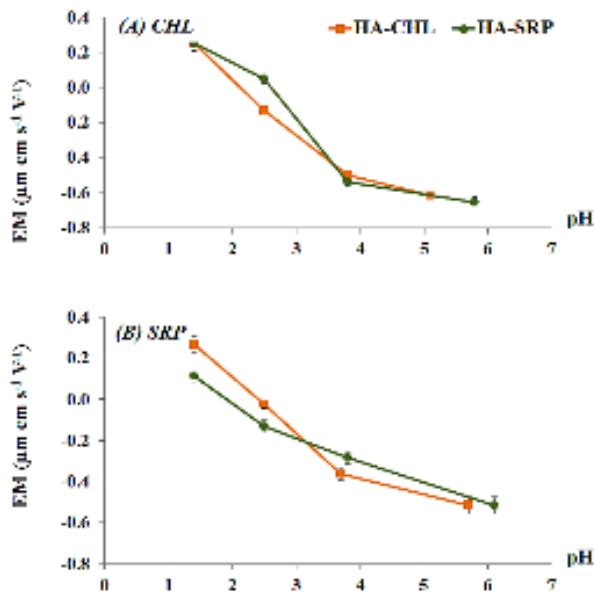
521 Figure 2 - FT-IR spectra of humic acids from the topsoil of CHL and SRP



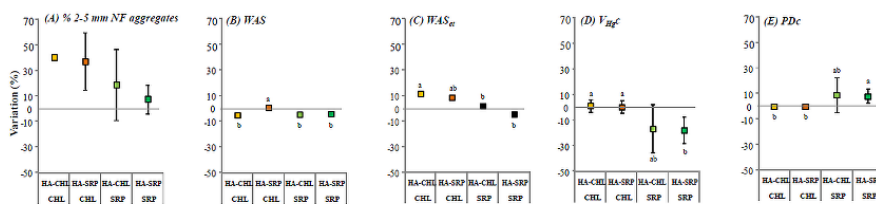
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524 Figure 3 - Electrophoretic mobility (EM) values of WDC fraction of 2-5 NF aggregates obtained after HA  
 525 addition in CHL (A) and SRP (B). Bars represent standard deviation  
 526

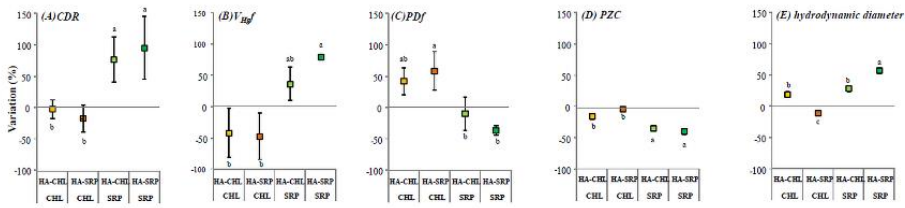


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 529 Figure 4 – Variations in the characteristics of 2-5 NF aggregates after HA addition with respect to water-only  
 530 wet-dry experiment. Percentage changes in mass of aggregates (A), WAS (B), WAS<sub>et</sub> (C) and porosity data of  
 531 coarser particles of NF-macroaggregates (D, E). Bars represent standard deviation (SD). For graphical reasons  
 532 SDs <1% were not reported. The letters show the significant differences at p level <0.05 (Duncan's test) among  
 533 the four treatments



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536 Figure 5 – Variations in the characteristics of water dispersible clay from NF aggregates after HA addition  
 537 with respect to water-only wet-dry experiment. Percentage changes in CDR (A), porosity data of finer particles  
 538 (B, C), PZC (D) and equivalent hydrodynamic diameter (E). Bars represent standard deviation (SD). For  
 539 graphical reasons SDs <5% were not reported. The letters show the significant differences at p level <0.05  
 540 (Duncan's test) among the four treatments



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