Micromorphological analysis on the influence of the soil mineral composition on short-term aggregation in semi-arid Mediterranean soils

Analisis micromorfológico de la influencia de la composición mineral del suelo sobre la agregación a corto plazo en suelos semiáridos de clima mediterráneo

Análise micromorfológica da influencia da composição mineral do solo na agregação a curto prazo em solos semiáridos de clima mediterrânico

AUTHORS

Virto I.[@ 1]
inigo.virto@unavarra.es

Fernández-Ugalde O.[1, 2]

Barré P.[2]

Imaz M.J.[1]

Enrique A.[1]

Bescansa P.[1]

Poch R. M.[3]

@ Corresponding Author

[1] Departmento Ciencias del Medio Natural. ETSIA, Universidad Pública de Navarra. 31006 Pamplona, Spain.

[2] Laboratoire de Géologie, École Normale Supérieure. 24 rue Lhomond. 75005 Paris, France.

[3] Departament de Medi Ambient i Ciències del Sòl. ETSEA, Universitat de Lleida. Rovira Roure 191. 25198 Lleida, Spain.

ABSTRACT

Aggregation in soils is the result of the interaction of the soil organic components and soil minerals. The reactivity of the mineral phase is acknowledged to interfere with aggregates formation and stabilization, but its influence on aggregation in semi-arid Mediterranean soils remains mostly unknown. In this study, we used micromorphological analysis of aggregates formed in a 28-d incubation in two agricultural soils differing only in the composition of the mineral phase in the upper Ap horizon (a carbonate-depleted *Palexeralf* with 21.5% clay, and a contiguous carbonate-rich *Typic Calcixerept* with 20.9% clay before decarbonation which was reduced to 10.4% upon decarbonation). The two soils belong to the same agricultural field and have had similar management for decades. Soil samples were completely disaggregated into their fractions < 250 μm, and incubated with fresh organic matter to stimulate re-aggregation. Macroaggregates (> 2 mm) formed during the incubation were separated at days 3, 7, 14, 21 and 28 and used to prepare thin sections. Macroaggregates were more abundant at day 3, and then decreased in number in the two soils, which indicates a dependency between organic matter decomposition and stable macroaggregates formation. They contained a greater proportion of smaller aggregates in the decarbonated soil. Micromorphological analysis revealed significant differences in the fabric and physical characteristics of these macroaggregates, in which bonds among primary particles were observed to be led by clays in the *Palexeralf* while the coarse fraction appeared embedded in a micromass with crystallitic b-fabric corresponding to carbonates in the *Calcixerept*. This resulted in a more compact fabric and less porosity in macroaggregates in the *Calcixerept*. Image analysis of thin sections was used to quantify and characterize the pore system of macroaggregates. Porosity (pores > 20 μm) was more than double (36.9% for 15.6%) within macroaggregates in the decarbonated soil, with more elongated pores. Although in both soils most pores were 20 to 150 μm in equivalent diameter, some porosity > 150 μm was observed only in macroaggregates from the decarbonated soil. These observations allow hypothesizing that the mechanisms responsible for aggregates stabilization and/or formation are different in the two soils, and that they result in different physical characteristics of soil aggregates. The implications of such differences on air and water flow rates within aggregates, and thus on the soil microbial activity and organic matter decomposition, as well as on soil erodibility, need to be studied and accounted for when evaluating the effect of soil management and other practices on soil quality in semi-arid Mediterranean agrosystems.

RESUMEN

La formación de agregados en el suelo es el resultado de la interacción entre los componentes orgánicos y minerales del suelo. Sin embargo, aunque es sabido que la naturaleza de la fracción mineral puede interferir en la agregación, su influencia en suelos semiáridos en regiones de clima mediterráneo es aún poco conocida. En este estudio se utilizaron técnicas de análisis micromorfológico para estudiar agregados formados en una incubación de 28 días de muestras del horizonte superficial Ap de dos suelos cultivados contiguos que difieren únicamente en su mineralogía (un Palexeralf descarbonatado con 21,5% de arcilla y un Typic Calcixerept rico en carbonatos con 20,9% de arcilla antes de la descarbonatación, que se reduce a 10,4% tras la eliminación de los carbonatos). Los dos suelos pertenecen al mismo campo de cultivo, por lo que han tenido un manejo similar durante décadas. En los dos suelos, se desagregaron completamente muestras frescas de suelo, para obtener las fracciones < 250 μm, que fueron incubadas con materia orgánica fresca para estimular la formación de agregados. Los macroagregados (> 2 mm) formados durante la incubación se cuantificaron y separaron los días 3, 7, 14, 21 y 28, y se utilizaron para la preparación de láminas delgadas. Estos macroagregados fueron, en los dos suelos, más abundantes el día 3 y su número decreció con el tiempo de incubación, lo que indica una dependencia entre su formación y la descomposición de la materia orgánica en los dos suelos. Estos macroagregados presentaron una mayor proporción de agregados estables pequeños en su interior en el suelo sin carbonatos. El análisis micromorfológico de las láminas delgadas reveló diferencias significativas en la fábrica y las características físicas de estos agregados, en los que la unión entre partículas primarias corresponde a la arcilla en el Palexeralf, mientras los elementos gruesos aparecen embebidos en una micromasa con fábrica de birefringencia cristalítica, correspondiente a la presencia de carbonatos en el Calcixerept. Como resultado, los agregados de este último suelo fueron más compactos y con menor porosidad. La cuantificación y caracterización de la porosidad de los agregados se realizó mediante análisis de imagen. La porosidad (> 20 μm) fue mayor (36,9% frente a 15,6%) en los macroagregados del suelo sin carbonatos, con una proporción mayor de poros elongados. Aunque en los dos suelos, la mayor parte de los poros de los macroagregados presentaron un diámetro medio equivalente entre 20 y 150 μm, en este suelo se observaron algunos poros mayores de 150 μm. Estas observaciones permiten suponer que los mecanismos responsables de la formación y/o estabilización de los macroagregados en los dos suelos podrían ser diferentes, resultando en las diferentes características físicas observadas en los agregados. Las consecuencias de estas diferencias en las tasas de flujo de agua y gases dentro de los agregados, y por lo tanto en la actividad biológica y la dinámica de la materia orgánica así como en la erosionabilidad del suelo, necesitan ser estudiadas y consideradas en la evaluación del efecto que el manejo del suelo y otras prácticas pueden tener en su calidad en las regiones semiáridas mediterráneas.

RESUMO

A formação de agregados no solo é o resultado da interação entre os componentes minerais e orgânicos do solo. No entanto, embora se saiba que a natureza da fração mineral pode interferir na agregação, a sua influência em solos semiáridos de regiões de clima mediterrânico é ainda pouco conhecida. Neste estudo, utilizaram-se técnicas de análise micromorfológica para estudar os agregados formados, recorrendo a uma incubação de 28 dias de amostras do horizonte superficial Ap de dois solos adjacentes cultivados que diferiam apenas na sua mineralogia (um Palexeralf descarbonatado com 21,5% de argila e um Calcixerept Típico rico em carbonatos com 20,9% de argila antes da descarbonatação, sofrendo uma redução para 10,4% após eliminação dos carbonatos). Os dois solos pertenciam ao mesmo campo de cultivo, pelo que estão sujeitos a uma gestão semelhante há décadas. Para obter as frações < 250 μm, amostras de solo fresco, foram totalmente desagregadas nos dois solos e incubadas com matéria orgânica fresca para estimular a formação de agregados. Os macroagregados (> 2 mm) formados durante a incubação foram retirados nos dias 3, 7, 14, 21 e 28 e usados para preparar lâminas finas. Os macroagregados predominaram nos dois solos no dia 3 e, em seguida, diminuíram com o tempo de incubação, o que é indicativo da dependência entre a decomposição de matéria orgânica do solo e a formação de macroagregados estáveis. A maior proporção de agregados de menores dimensões surgiu no solo descarbonatado. A análise micromorfológica das lâminas finas revelou diferenças significativas no tecido e características físicas destes macroagregados, sendo que a união entre partículas primárias corresponde à argila no Palexeralf enquanto que a fração de elementos grosseiros surgiu incorporada numa micromassa com malha cristalina correspondendo aos carbonatos no Calcixerept. Como consequência, os agregados deste último solo são mais compactos e apresentam menor porosidade. A quantificação e caracterização da porosidade dos agregados realizaram-se por análise de imagem. A porosidade (> 20 μm) era maior (36,9% versus 15,6%) nos macroagregados do solo sem carbonatos, com uma maior proporção de poros alongados. Ainda que nos dois solos, a maioria dos poros nos agregados apresentasse um diâmetro médio equivalente entre 20 e 150 μm, foram observados neste solo alguns poros maiores que 150 μm. Estas observações sugerem que os mecanismos responsáveis pela formação e/

ou estabilização de macroagregados nos dois solos poderá ser diferente, resultando em diferentes características físicas observadas nos agregados. As consequências dessas diferenças nas taxas de fluxo de água e gases dentro dos agregados e, portanto, a atividade biológica e a dinâmica da matéria orgânica, bem como a erodibilidade do solo, necessitam ser estudadas e consideradas na avaliação do efeito que a gestão do solo e outras práticas pode ter na sua qualidade nas regiões semiáridas mediterrânicas.

1. Introduction

Aggregation in soils is the result of the interaction of the soil organic components and soil minerals, which leads to soil aggregates formation and stabilization. These aggregates reciprocally contribute to organic matter protection and long-term organic C stabilization in soils (Angers and Chenu 1997; Six et al. 1999). The two major factors controlling the interaction of the organic and mineral fractions of the soil are the soil biological activity, which decomposes organic matter, and the soil physical-chemical properties, which determine the reactivity of the mineral phase and regulate the formation of organo-mineral complexes of different types (Six et al. 2004).

Much research has focused in the last years on the role of organic matter (OM) in aggregation, and on the inter-relationship between OM and its stabilization and the soil physical condition (e.g. Six et al. 1998; Wander 2004; von Lützow et al. 2006). The relevance of the soil mineral fraction in these processes is acknowledged in these studies. In fact, some authors (e.g. Six et al. 2002) have observed that the pathways of aggregate formation and stabilization can be different in temperate soils than in oxide-rich tropical soils. They associated this to different mineralogy in these soils.

However, scarce attention has been paid to the influence of the composition of soil minerals in aggregation. Most studies carried out so far are based in the comparison of aggregate dynamics in soils of contrasting mineralogy. In general it is observed that soils in which 2:1 clay minerals are dominant develop more aggregates with long-term stability than soils with 1:1 type minerals (Denef et al. 2002; Denef and Six 2005), due to their greater cation exchange capacity and specific surface, which favor strong interactions with OM and other clay minerals (Denef et al. 2004; Denef and Six 2006). The formation and stability of these aggregates seems however to be more dependent on OM concentration and dynamics than in soils rich in kaolinite-like clays. This has been associated to the fact that the latter are usually weathered soils with important concentrations of oxides, with variable charge, in which electrostatic interactions between 1:1 clays and oxides can lead to aggregate formation through mineral-mineral bonding (Denef et al. 2004; Denef et al. 2005). Moreover, in many of these studies soils from different climates are compared (Denef et al. 2002; Denef et al. 2004; Denef and Six 2005; Denef and Six 2006), which means that also the nature of the organic fraction involved in aggregation might be different.

In semi-arid land, such as large areas of the Mediterranean region where climate is the limiting factor for pedogenesis, differences in the mineral composition of soils can arise from different parent materials or from different degrees of pedogenesis. In addition to differences in clay mineralogy, many semi-arid Mediterranean soils are rich in lithogenic and/or secondary carbonates, while others are carbonate-free, at least in their upper horizons. Although carbonates are acknowledged to interfere with aggregation, their role in aggregation and in aggregates stabilization is still unclear. Fernández-Ugalde et al. (2011) have recently observed that carbonates, when naturally present, can promote longer stabilization rates of aggregates upon fresh organic matter additions than those observed in non-carbonated or

carbonate-depleted soil horizons. This suggests that an interaction between organic matter decomposition and carbonates may exist and affect aggregation in these soils. The mechanisms of this interaction are not completely known, but might be related to (i) to the promotion of mineral-mineral and mineral-organic interactions through cation bridges (carbonates as a source of calcium) and/or (ii) dissolution and precipitation processes of carbonates, which would contribute to the creation of permanent unions (precipitates) among soil particles (Baldock and Skjemstad 2000; Clough and Skjemstad 2000; Bronick and Lal 2005).

Regardless of the involved mechanisms, the result of the interference of carbonates in aggregation is a less strong relationship between organic matter decomposition and aggregation in the long term (Bouajila and Gallali 2008). As a consequence, greater stabilities of aggregates have been observed in soils with carbonates than in carbonate-free soils with similar organic matter contents and characteristics (Abiven et al. 2009). This suggests that the physical characteristics of carbonated soils may be different from those of soils not containing carbonates, since aggregates determine the architecture of the soil matrix. Thus, several basic physical properties such as porosity, the pore-size distribution, the water retention capacity, and water and air fluxes through the soil can be also different in semiarid soils depending on the composition of their mineral fraction.

The objective of this work was to determine the importance of the soil mineral composition (in particular, the presence or absence of carbonates) in short-term aggregation dynamics, by using micromorphological techniques to study individual soil aggregates formed in the very short term (3-28 days) in two contiguous soils differing only in their carbonates content. That for, we run a laboratory incubation experiment using samples from two soil units in an agricultural field (i.e. with identical historical agricultural management). Aggregates formation was controlled during the incubation period, and the characteristics and composition of aggregates from both soils were quantified.

2. Material and Methods

2.1. Soils characteristics, sampling and incubation set-up

Two soils were selected in an agricultural field in Rodezno (42º30'5"N, 2º51'12"W; La Rioja, Spain). The two soil units were contiguous in the area, and as a consequence, they have received identical historical agricultural management for decades. This includes dryland cropping of a rotation of wheat (*Triticum aestivum* L.), sunflower (*Helianthus annuus* L.) peas (*Pisum sativum* L.) and sugar beet (*Beta vulgaris* L.) or potato (*Solanum tuberosum* L.), with mouldboard plowing and conventional fertilization.

The two soils are a Typic Calcixerept (Soil Survey Staff 2006) with 20% of total carbonates (CALC), and a decarbonated Calcic Haploxerept (Soil Survey Staff 2006) (DECALC), corresponding to a Palexeralf where the A and Bt horizons have been mixed for decades by agricultural management. The basic characteristics of the Ap horizons used in this study were analyzed using standard methods (Carter 1993) and are shown in Table 1. Essentially, they differ in the presence of carbonates and clay minerals. Total clay content before decarbonation was similar and close to 20% in both soils, but it was reduced to 10% in CALC upon decarbonation. For details, see Fernández-Ugalde et al. (2011).

The mineralogy of these horizons was analyzed using X-ray diffractometry at the Laboratory of Geology of the Normal High School (ENS) in Paris, France. Approximately 100 mg of dry samples were re-suspended in 2.5 ml of deionized water. The suspensions were poured on glass slides to create oriented preparations that were subsequently analysed using X-ray diffraction (RIGAKU UltraX18HF). Intensities were collected at 0.05° step intervals from 4 to 30° using using Cu Kα radiation and a 1.5 s counting time per step. Except for carbonates, clay mineralogy was similar in both soils (Table 1). In particular, we measured the width at half height of illite peaks on all XRD patterns. The width of illite peaks did not change much between patterns and no trend with soil type was observed.

Table 1. Soil characteristics in the 0-20 cm depth. Average ± standard error (n=3 field replicates)

	Carbonate- rich soil (CALC)		Carbonate-depleted (DECALC)	
Soil type (SSS 2006)	*Typic Calcixerept*		*Calcic Haploxerept (Palexeralf)*	
Chemical properties				
Organic C (g kg⁻¹)	8.42 ± 0.09		7.37 ± 0.12	
C/N ratio	10.24 ± 0.08		9.19 ± 0.18	
pH (1:2.5 water)	8.58 ± 0.02		8.30 ± 0.12	
CaCO₃ (g kg⁻¹)	221 ± 5.5		< 50	
Extractable Fe (g kg⁻¹)	2.42 ± 0.03		4.55 ± 0.22	
Particle-Size Distribution (g kg⁻¹)				
	Before decarbonation	**After decarbonation**	**Before Fe extraction**	**After Fe extraction**
Sand (50-2000 μm)	425 ± 9.5	361 ± 6.5	446 ± 14.0	371 ± 14.9
Silt (2-50 μm)	366 ± 12.8	314 ± 6.2	338 ± 10.5	344 ± 11.6
Clay (< 2 μm)	209 ± 3.5	104 ± 7.5	215 ± 4.5	200 ± 1.1
Mineralogy of clay fraction	Illite, Kaolinite, Quartz		Illite, Kaolinite, Quartz, Carbonates	

Surface (0-20 cm) samples were collected as undisturbed blocks in three replicate points at each site. After collection, one part from each site was air-dried and sieved (2 mm diameter) for basic analyses (see above). The remaining part of each sample was kept at field moisture for the incubation experiment.

These field-moist samples were disaggregated by carefully forcing the entire soil mass through a 250 μm mesh. The completely disaggregated fraction < 250 μm was stored at 4 °C to avoid complete desiccation and to ensure the presence of an active microbial population within this fraction. Only the sand fraction (250-1000 μm) was oven-dried to avoid germination of seeds contained in this fraction during the incubation. The air-dry < 250 μm fraction and the oven-dry sand fraction (250-1000 μm) were then mixed with fresh maize straw (to obtain a concentration of 1.75 mg maize-C g⁻¹ soil). One hundred grams (dry mass basis) of the mixture were packed in steel cores closed by a nylon mess of 53 μm at

the bottom, to get a final density of 1.2 g cm⁻³. Cores were moistened to field capacity with a NH_4NO_3 solution (3.03 g L⁻¹ for DECALC and 3.31 g L⁻¹ for CALC) to keep the C/N ratio around 10 (Cosentino et al. 2006), and suspended inside a sealed 1-L glass jar with 20 mL of deionized water in a beaker at the base to minimize desiccation (Cosentino et al. 2006). The jars were incubated in aerobic conditions at 25 ºC in the dark, in a complete randomized block design (n=3) for 28 days. One set of three replicates per soil was sacrificed at days 0, 3, 7, 14, 21 and 28 for large aggregates isolation and fractionation, and for micromorphological analysis.

2.2. Aggregates fractionation and aggregates thin sections preparation

Stable large aggregates (> 2 mm) formed during the incubation were separated at each sampling date by wet sieving (Elliott 1986), quantified and dispersed to determine their content in smaller ag-

gregates That for, the incubated sample was first gently submerged in deionized water on top of a 2-mm sieve for 5 min, and then manually sieved moving the sieve up and down 3 cm with 50 cycles in 2 min. Stable aggregates (designed macroaggregates) retained in each sieve were oven-dried at 50 ºC and stored at room temperature.

Once weighed, macroaggregates were dispersed using a device adopted from Six et al. (2002) into sand grains (250-1000 μm), non-aggregated silt+clay particles (< 50 μm), and water-stable smaller aggregates (50-250 μm), designed as microaggregates hereafter. The reason for doing so was the fact that different aggregate dynamics in the two soils may lead to a different organization of soil aggregates, which are usually understood as a continuum in which larger aggregates contain smaller aggregates and non-aggregated particles (Tisdall and Oades 1982). In order to avoid the bias of different textures, microaggregates were completely dispersed by mixing 10 g with 25 ml of deionized water and sonicating the mixture in an ice bath for a total input of 400 J ml^{-1} using a digital sonifier (max. output=400 W, operating at 20 kHz, Branson model 450). After dispersion the recovered material was sieved in a 50 μm sieve to separate the sand (50-250 μm) from the silt- and clay-sized particles (< 50 μm).

Data for both macroaggregates and microaggregates are shown as sand-corrected mass proportions (i.e., the proportion of these aggregate classes in the total soil or macroaggregates mass after subtracting the proportion of sand particles of the same size than aggregates).

Simultaneously, thin sections 5.5 cm long, 4.5 cm wide, were prepared of macroaggregates from each incubation date and soil, according to the methods described in Murphy (1986). Because of their nature, no special drying methods were required in order to maintain their original structure. Three to five individual macroaggregates were selected and used to prepare these sections.

2.3. Thin sections and image analysis

Thin sections were used to analyze the fabric and characteristics of macroaggregates, using a Olympus BX51 petrographic microscope coupled with a digital camera. The description was done following the guidelines given by Stoops (2003).

Image analysis was used to determine parameters related to macroaggregates porosity. Photographs (1000 x 750 μm^2) were taken on three spots per aggregate on three different aggregates per soil and sampling date. Twin photographs (one under polarized light (PL) and another one using circular cross-polarized light (CPL) to avoid extinction phenomena in mineral grains) were taken at each spot.

On these photographs, image analysis was run using the free software Image Tool 3.0 (UTH-SCSA, University of Texas Health Center in San Antonio), as described in Marcelino et al. (2007), in various consecutive steps. First, the two images were converted from color to grayscale, and then the PL image was subtracted to the CPL image. This produces an image in which all pores (resine) are black because they are the only features being black under CPL and white under PL. Organic matter and opaque bodies, which are black or dark in both images, appear white or grey. In a similar way, all other elements displaying some degree of color in CPL or PL pictures appear grey in the subtracted image. Second, the subtracted image was used to obtain a black-and-white image in which only pores appear black (segmentation). This was done by determining a threshold value of grey intensity in the subtracted image above which all features corresponded to dark pores. The binary image was used to determine percent porosity and pores characteristics. The segmentation threshold, which is a crucial point for image analysis (Marcelino et al. 2007; Peth et al. 2008; Elyeznasni et al. 2012; Rasa et al. 2012) was determined manually for each image, to ensure appropriate separation of the pores volume and the soil matrix.

Percent porosity was calculated as the proportion of black (pores) and white (other) pixels in the image, following Ringrose-Voase (1994) and Marcelino et al. (2007). For each day and soil, the variance of the observed porosity was used to calculate de standard error associated to the analysis of porosity. Porosity and pore size-distribution calculated form thin sections using this method has been observed to correlate well with 3D determinations and with porosity estimated from water retention curves (Elyeznasni et al. 2012). Objects in the black-and-white picture (pores) not touching the image borders (Ringrose-Voase 1994) were further analyzed for their size and shape. For size, after excluding pores smaller than 20 μm (the thickness of the thin sections), they were classified into three categories (20-50 μm, 50-150 μm and > 150 μm in equivalent pore diameter) according to Greenland (1979, cit. Carter and Ball 1993), who determined the pore-size limit for water transportation or storage in 50 μm. The pores shape was characterized by the "pore shape factor" (PSF) or sphericity index (Shipitalo and Protz 1987; Ringrsoe-Voase 1991; Costantini et al. 2002), calculated as:

$$PSF = \frac{perimeter^2}{4\Pi \times area}$$

A value of PSF of 1 corresponds to a perfect circle, PSF values between 1 and 2 were considered as corresponding to rounded pores, between 2 and 5 to irregular shapes, and values greater than 5 were considered elongated pores. This classification has been observed to be valid in assessing porosity in other Mediterranean soils (Pini et al. 2009).

2.4. Statistical analysis

As stated above, all treatments were replicated threefold for statistical analyses. Previous to further analysis, data were tested for normality and Levene's test was used to verify homogeneity of variance. Then, data from the aggregate fractionation experiment and image analysis were first analyzed using one-way ANOVA with soil type (CALC and DECALC) as independent factor, and then with time for each soil type to determine the effect of time within each soil and fraction. Separation of mean values for different incubations dates was performed using Duncan's test with a significance level of P=0.05. The SPSS 17.0 software (SPSS Inc 2009, Chicago, IL, USA) was used for all statistical treatments.

3. Results and discussion

3.1. Macroaggregation in the two soils

The evolution in time of the proportion of macroaggregates formed in the two soils showed a peak between days 0 and 3, and a progressive decline with incubation time in both soils (Figure 1). The proportion of microaggregates within large aggregates (Figure 1) was however constant in time since the starting of the incubation, and smaller in CALC than in DECALC.

These results indicate that the dynamics of macroaggregation in the short-term was similar and related to OM decomposition (which is known to be fast after fresh organic matter addition, and to decline with time) in the two soils, as described in the hierarchical model of aggregation (Tisdall and Oades 1982; Six et al. 2004). The greater proportion of stable microaggregates within macroaggregates in DECALC than in CALC suggests that either the units from which these aggregates are built are different (i.e. more pre-existing microaggregates are incorporated into macroaggregates in DECALC), or either the nature of the macroaggregates formed were different among soils (more stable microaggregates would form within macroaggregates in the short-term in DECALC). Considering that according to the hierarchical model of aggregation, microaggregates within macroaggregates are formed as a consequence of fresh OM decomposition, which produces polysaccharides and other organic binding compounds (Six et al. 2004), an explanation for our results would be an interference of carbonates in OM
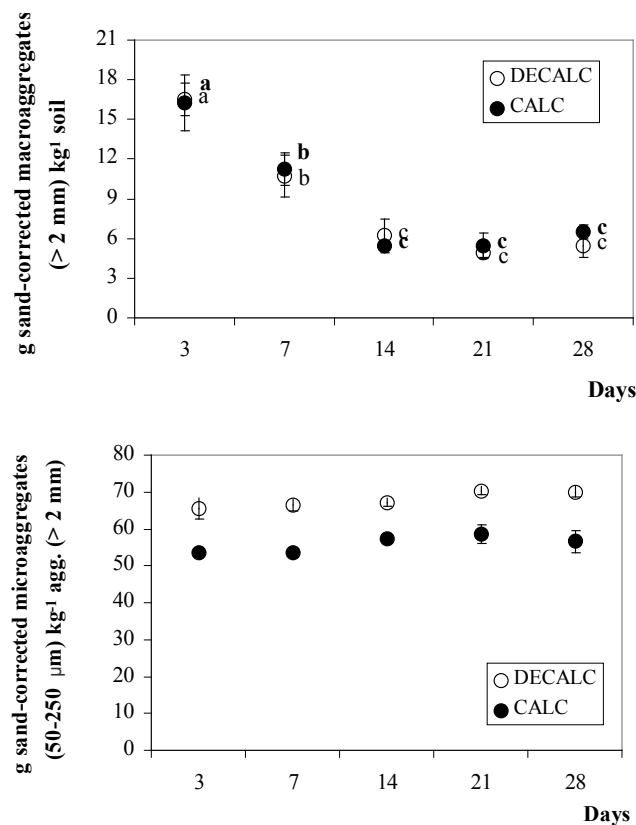
Figure 1. Evolution of the recovered mass of macroaggregates (> 2 mm, top), and microaggregates within them (50-250 μm, bottom) with incubation time in the carbonate rich (CALC) and the decarbonated (DECALC) soils. Bars represent the standard error (n=3). Different letters indicate, for each soil, different homogeneous Duncan groups (P<0.05).

decomposition, which would prevent the formation of new microaggregates within the newly-formed macroaggregates.

In relation to our hypothesis, this indicates first that although carbonates did not change the relationship between organic matter decomposition and macroaggregates formation in the short-term, they could interfere with OM decomposition within macroaggregates once formed and affect microaggregates formation. The creation of permanent unions among soil particles may be responsible for the stabilization of microaggregates formed or gathered together within macroaggregates in CALC (Baldock and Skjemstad 2000; Clough and Skjemstad 2000; Bronick and Lal 2005), but the lower content of microaggregates in CALC suggests that the presence of carbonates in this soil could not compensate for the lower amount of reactive clay minerals compared to DECALC (Table 1). In agreement with Kay (1998), clay abundance would be thus from this viewpoint the most important factor of microaggregates formation and stabilization within macroaggregates in the short-term in the studied soils.

3.2. Fabric and porosity of aggregates

Microscopic observation of the thin sections allowed determining the most important differences in the fabric and composition of the aggregates formed in the two soils. Table 2 summarizes the micromorphological characteristics of macroaggregates formed in CALC and DECALC. These results can be presented together for the five dates of sampling, because the major characteristics of macroaggregates did not

change with time in any of the two soils. As observed in Figure 2, macroaggregates formed in CALC were characterized by a more compact microstructure, expressed in a single to double-spaced c/f related distribution, and by a chitonic distribution in macroaggregates in DECALC. As a consequence, pores were scarcer and smaller in macroaggregates in CALC, and much more abundant and interconnected in DECALC (see below analysis of porosity), corresponding to a granular microstructure with packing voids, for an almost massive microstructure in CALC. The presence of carbonates resulted in a crystallitic b-fabric in macroaggregates in this soil. This type of fabric is caused by the presence of fine crystals of calcite (micrite and microsparite) precipitated in a clayey micromass (Durand et al. 2010). Larger crystals of calcite were also observed forming bridges between primary coarse particles, as well as some pedogenic crystals of calcite, very likely already present in the soil before sieving (Figure 2). In general, crystals of calcite were found in an irregular distribution in the soil matrix (Figure 2), which according to Durand et al (2010) can be understood as an evidence of recrystallization of carbonates. In contrast, speckled and grano-striated b-fabrics were observed in DECALC, where clay orientation around and among coarser grains was evident under polarized light (Figure 2).

Finally, as it corresponds to aggregates formed in the very short term following fresh organic matter addition, tissue and organ residues were abundant in both soils and for all incubation sampling dates.

These micromorphological observations confirmed that the agents of stabilization of macroaggregates were different in the two soils. While the mediation of clay minerals to form binds among coarser particles was evident in DECALC (Figure 2), the abundance of carbonates limited the observation of the role of clay minerals in macroaggregation in CALC. Although it is likely that such structures also existed in CALC, it can be thought from its lower clay minerals concentration (Table 1) that their presence and influence on aggregates stability would be smaller. Considering the lower proportions of stable microaggregates found in CALC macroaggregates in comparison to DECALC (Figure 1), the overabundance of carbonates and calcite crystals in CALC, which apparently resulted in a more massive structure, cannot be related to stronger or tighter unions of the constituents of aggregates than clay minerals alone. However, the physical constitution of these aggregates suggests that their mechanical, biological and physical-chemical characteristics were different than those formed in DECALC.

Table 2. Micromorphological characteristics of macroaggregates (> 2 mm) formed during the 28-day incubation

|	Carbonate-rich soil (CALC)	Carbonate-depleted (DECALC)
c/f related distribution	Single to double-spaced porphyric	Chitonic
Coarse material	Grains of quartz and calcite	Grains of quartz
Micromass b-fabric	Crystallitic	Speckled or grano-striated
Pores	Scarce, small vughs	Abundant, uniformly distributed packing voids
Microstructure	Almost massive	Vughy to granular
Other observations	- Abundant tissue and organ residues - Abundant micrite and larger calcite crystals	- Abundant tissue and organ residues
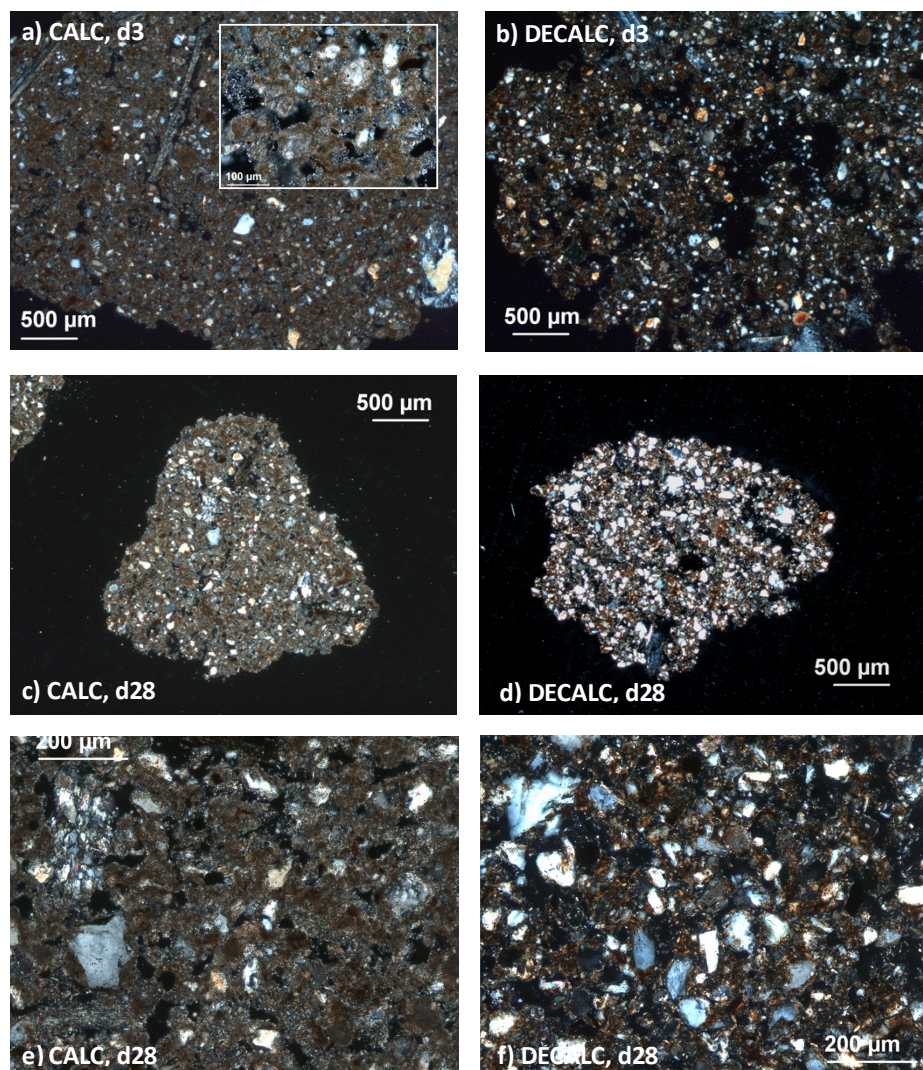
Figure 2. Morphology of macroaggregates (> 2 mm) in the carbonate rich (CALC, left) and the decarbonated (DECALC, right) soil at days 3 and 28 of incubation. Images taken under cross-polarized light. Inset in a) from the same aggregate with 8 times greater magnification.

The study of the porosity of macroaggregates in CALC and DECALC during the 28-day incubation allowed quantifying the observed differences in fabric, and revealed significant differences in the abundance and shape of pores. Porosity (> 20 µm) in macroaggregates in DECALC, which averaged 36.9 ± 1.14%, was more than two times that of CALC (15.6 ± 0.80%) all along the incubation (Figure 3). In addition, pores were on average more elongated (PSF > 5) in these macroaggregates in DECALC, as expressed by a greater proportion of this type of pores all along the incubation period (Figure 4). In relation to pores size, as measured by their equivalent diameter, pores 20-50 µm in size were the most abundant in macroaggregates in the two soils (81.4 ± 1.0% of total pores in CALC and 73.5 ± 0.97% in DECALC), followed by 50-150 µm
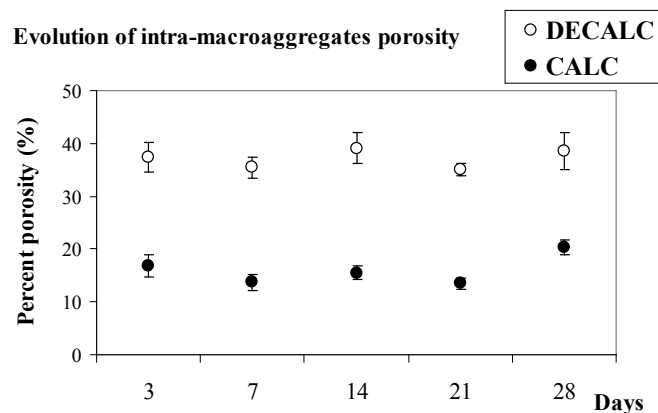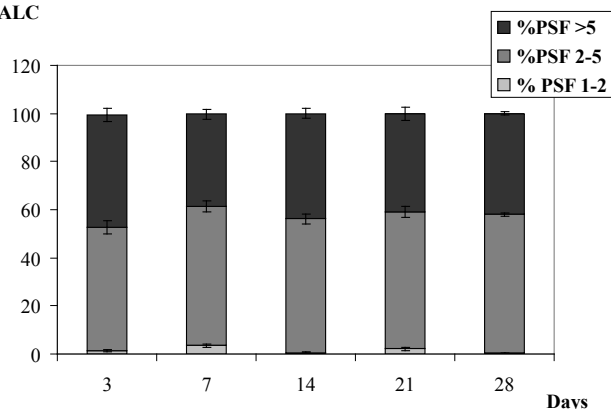
Figure 3. Percent porosity within macroaggregates (> 2 mm) formed in the carbonate rich (CALC) and the decarbonated (DECALC) soils. Bars represent the standard error (n=3).
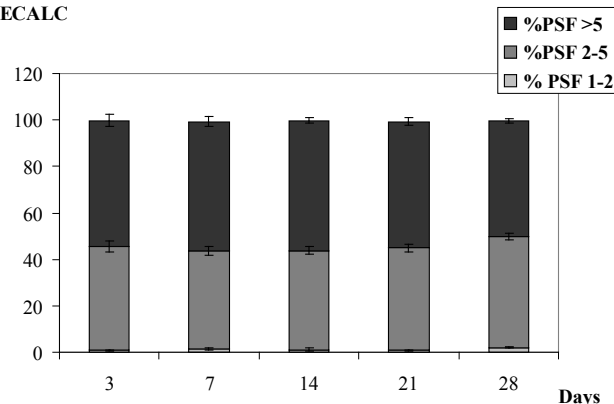

Figure 4. Distribution of pores according to the pore-shape factor (PSF) within macroaggregates (> 2 mm) formed along the incubation in the carbonate rich (CALC, left) and the decarbonated (DECALC, right) soils. Bars represent the standard error (n=3).

pores (17.0 ± 0.98% and 22.7 ± 0.94%, respectively). The only significant difference in pores size within macroaggregates among soils was observed for pores > 150 μm in equivalent diameter, which were proportionally more abundant in DECALC (2.9 ± 0.26%) than in CALC, where they were almost absent (0.6 ± 0.13%).

All in all, this means that the presence of carbonates induced changes in the physical archi-

tecture of recently formed macroaggregates, resulting in a less porous soil matrix, in which pores were more rounded as observed in 2D thin sections. Round and narrower pores have been described associated to a less efficient water transport and lower gas diffusion rates, which can have a strong impact on intra-aggregate microbial activity and biogeochemical processes (Peth et al. 2008). In contrast, Rasa et al. (2012) described abundant macroporosity and a greater

proportion of elongated pores, as observed in DECALC macroaggregates in relation to CALC (Figures 3 and 4), as responsible for favorable conditions for root and microbial growth, soil aeration and water infiltration. It has to be noted however that inter-aggregates porosity, which was not evaluated in this study, may be different from intra-aggregates porosity in the two soils, and compensate for the limitations observed in CALC.

From the viewpoint of aggregates stability, it can be hypothesized that the less interconnected pores observed in CALC could result in macroaggregates being more slaking-prone than in DECALC. This is consistent also with the smaller amount of stable microaggregates found within macroaggregates in CALC.

Finally, in relation to the evolution in time, no differences were observed in any of the parameters analyzed for porosity in macroaggregates in CALC or DECALC along the incubation period. This confirms the micromorphological observation that although the amount of macroaggregates decreased with time (Figure 1), their internal characteristics did not change, and were dependent on the nature of the mineral fraction.

4. Conclusions

By incubating two semi-arid Mediterranean soils differing in their carbonate and clay minerals content, and by studying the formation of macroaggregates in the short-term (28 days), we observed that in both soils macroaggregates formation was related to organic matter decomposition in the short-term. However, the presence of carbonates interfered with aggregation and resulted in a more massive intra-aggregate fabric, and in a smaller proportion of stable microaggregates within macroaggregates in the carbonate-rich soil. As a consequence, lower intra-aggregates porosity was observed in this soil. Pores were in addition smaller and more rounded pores than in the non-carbonated soil.

IN the latter the binding role of clay minerals was observed to be stronger, and resulted in a greater proportion of stable microaggregates within larger macroaggregates. These observations allow hypothesizing that the mechanisms responsible for aggregates formation and stabilization are different in the two soils. However, no evidences were found of carbonates inducing greater stability of macroaggregates by creating permanent bonds among soil particles.

In summary, the mineralogy of the soil, and especially of the finest fraction, did not interfere with the relationship between organic matter decomposition and macroaggregates formation in a short-term incubation. However, differences in mineralogy were observed to induce different characteristics to these macroaggregates, very likely related to the interference of carbonates with OM decomposition within macroaggregates and/or to the physical characteristics induced to macroaggregates by the presence of carbonates. Although the observed differences refer only to macroaggregates formed along an incubation experiment (which may differ from actual total soil porosity in the field), they can be related to the ability of these macroaggregates to store water, resist erosion, store organic matter, and support plant and microbial growth, compromising thus the soil physical quality. Since aggregation is at the heart of soil quality issues, further research is needed to establish whether the observed differences in aggregates formed during the short-term incubation are consistent with those in naturally formed aggregates. This would allow establishing the links between the characteristics of the soil matrix and soil properties in semi-arid agricultural soils.

5. Acknowledgments

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