Soil organic carbon along an altitudinal gradient in the Despeñaperros nature reserve, Southern Spain

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9 Abstract

10 Soil organic C (SOC) is extremely important in the global C cycle as C sequestration in nondisturbed soil ecosystems can be a C sink and mitigate greenhouse gas driven climate change. 11 12 Soil organic C changes in space and time are relevant to understand the soil system and its 13 role in the C cycle, and this is why the influence of topographic position on SOC should be studied. Seven soil profiles from a toposequence between 607 and 1168 masl were analyzed 14 15 in the Despeñaperros Natural Park (Córdoba, SW Spain). Depending on soil depth, one to three control sections (0-25, 25-50 and 75-cm) were sampled at each site. The SOC content in 16 17 studied soils is below 30 g kg⁻¹ and strongly decreases with depth. These results were related to the gravel content and to the bulk density. The SOC content from the topsoil (0-25 cm) 18 19 varied largely through the altitudinal gradient ranging between 27.3 and 39.9 g kg⁻¹. The SOC stock (SOCS) varied between 53.8 and 158.0 Mg ha⁻¹ in the studied area and was clearly 20 21 conditioned by the topographic position. Therefore, results suggest that elevation should be 22 included in SOCS models and estimations at local and regional scales.

23

24 1 Introduction

Soils are an important C reservoir (Barua and Haque, 2013; Yan-Gui et al., 2013). In fact, the primary terrestrial pool of organic carbon (OC) is soil, which accounts for more than 71% of the Earth's terrestrial OC pool (Lal, 2010). In addition, soils have the ability to store C for a long time (over the last 5000 years) (Brevik and Homburg, 2004). Soils play a crucial role in the overall C cycle, and small changes in the soil organic carbon stock (SOCS) could

- 1 significantly affect atmospheric carbon dioxide (CO₂) concentrations, and through that global
- 2 climate change. Within the C cycle, soils can be a source of greenhouse gases through CO₂
- 3 and methane (CH₄) emissions, or can be a sink for atmospheric CO_2 through C sequestration
- 4 in soil organic matter (OM) (Breuning-Madsen et al., 2009; Brevik, 2012).
- 5 Climate, soil use and soil management affect C variability, particularly in soils under
- 6 Mediterranean type of climate, characterized by low OC content, weak structure and readily
- 7 degradable soils (Hernanz et al., 2002). In temperate climates, recent studies show differences
- 8 in C sequestration rates in soils depending on use and management (Zinn et al., 2007), climate
- 9 and mineralogical composition (Wang et al., 2010), texture, slope and elevation (Hontoria et
- al., 2004), and tillage intensity and no-till duration (Umakant et al., 2010). Soil conservation
- 11 strategies are being seen as a strategy to increase soil OM content (Barbera et al., 2012; Batjes
- 12 et al., 2014; Jaiarree et al., 2014; Srinivasarao et al., 2014; Fialho and Zinn, 2014).
- 13 Several studies have been carried out to estimate differences in soil organic carbon (SOC)
- 14 dynamics in relation to soil properties, land uses and climate (Eshetu et al., 2004; Lemenih
- 15 and Itanna, 2004). Although the impact of topographic position on soil properties on SOC
- 16 content is widely recognized (Venterea et al., 2003; Fu et al., 2004; Brevik, 2013), relatively
- 17 few studies have been conducted to examine the role of topographic position (Ruiz-Sinoga et
- 18 al., 2012).

19 The spatial variation of soil properties may also be significantly influenced by aspect (which 20 may induce microclimate variations), physiography, parent material, and vegetation (Johnson 21 et al., 2000; Ollinger et al., 2002; Brevik, 2013). Ovales & Collins (1986) evaluated soil 22 variability due to pedogenic processes across landscapes in contrasting climatic environments 23 and concluded that topographic position and variations in soil properties were significantly 24 related. McKenzie and Austin (1993) and Gessler et al. (2000) found that variations of some 25 soil properties could be related to the slope steepness, length, curvature and the relative location within a toposequence. Both studies suggest that the assessment of the hillslope 26 sequence helps to understand variations of soil properties in order to establish relationships 27 28 among specific topographic positions and soil properties. Asadi et al. (2012) found that the 29 integrated effect of topography and land use determined soil properties. Topography is a relevant factor controlling soil erosion processes through the redistribution of soil particles 30

and soil OM (Cerdà and García Fayos, 1997; Ziadat and Taimeh, 2013).

Comentado [DBZ1]: I do not understand this. Do you mean variability in soil C content or variability in soil OC concentration?

Comentado [DBZ2]: According to Hernanz et al. (2002), this is not exact. Hernanz et al. (2002) refer to ,intensive tillage systems for rainfed crops⁴⁴. I suggest substituting "soils⁴⁵ in the first line of the paragraph with "cropped soils" or "intensively tillaged soils".

Comentado [DBZ3]: Recent studies on C sequestreation rates in Mediterranean soils are these:

•Muñoz-Rojas, M, Jordán, A., Zavala, L.M., De la Rosa, D., Abd-Elmabod, S.K., and Anaya-Romero, M.: Impact of land use and land cover changes on organic C stocks in Mediterranean soils (1956-2007). Land Degradation and Development, 2012. DOI: 10.1002/dr.2194.

 Muñoz-Rojas, M, Jordán, A., Zavala, L.M., De la Rosa, D., Abd-Elmabod, S.K., and Anaya-Romero, M: Organic carbon stocks in Mediterranean soil types under different land uses (Southern Spain). Solid Earth 3, 375-386, 2012.

Comentado [DBZ4]: A recent study on C sequestreation rates under climate change scenarios:

 Muñoz-Rojas, M., Jordán, A., Zavala, L.M., González-Peñaloza, F.A., De la Rosa, D., Anaya-Romero, M.: Modelling soil organic carbon stocks in global change scenarios: a CarboSOIL application. Biogeosciences 10, 8253-8268, 2013.

Comentado [DBZ5]: I am not in agreement. Ruiz-Sinoga et al. (2012) studied soils under different rainfall conditions (ranging from humid to semiarid Mediterranean type of climate). Elevation was not studied specifically in their research. So, I suggest substituting "have been conducted to examine the role of topographic position" with "have included the study of topographic position". In fact, this is a good place to include reference to your own work: •Lozano-García et al., 2014 (cited in the ref. list) •Fernánded-Romero et al., 2014 (cited in the ref. list)

Fernánded-Romero et al., 2014 (cited in the ref. list

And, probably, some of these recent papers:

•Chirinda, N, Elsgaard, L., Thomsen, I.K., Heckrath, G., Olesen, J.E.: Carbon dynamics in topsoil and subsoil along a cultivated toposequence. Catena 120, 20-28, 2014.
•Zhu, H., Wu, J., Guo, S., Huang, D., Zhu, Q., Ge, T., Lei, T.:Land

Valu, H., Wu, J., Juo, S., Huang, D., Zhu, Q., Ge, H. Lei, L. Land use and topographic position control soil organic C and N accumulation in eroded hilly watershed of the Loess Plateau. Catena 120, 64-72, 2014.

Comentado [DBZ6]: Add some specific recent references: •Ashley, G.M., Beverly, E.J., Sikes, N.E., Driese, S.G.: Paleosol diversity in the Olduvai Basin, Tanzania: Effects of geomorphology, parent material, depositional environment, and groundwater on soil development. Quaternary International 322-323, 66-77, 2014. •Bakhshandeh, S., Norouzi, M., Heidari, S., Bakhshandeh, S.: The role of parent material on soil properties in sloping areas under tea plantation in Lahijan, Iran. Carpathian Journal of Earth and Environmental Sciences 9, 159-170, 2014.

 Dingil, M., Öztekin, M.E., Şenol, S.: Definition of the physiographic units and land use capability classes of soils in mountainous areas via satellite imaging. Fresenius Environmental Bulletin 23 (3 A), 952-955, 2014.

•Gebrelibanos, T., Assen, M.: Effects of slope aspect and vegetation types on selected soil properties in a dryland Hirmi watershed and adjacent agro-ecosystem, northern highlands of Ethiopia. African Journal of Ecology, 52, 292-299, 2014.

 Kirkpatrick, J.B., Green, K., Bridle, K.L., Venn, S.E.: Patterns of variation in Australian alpine soils and their relationships to parent material, vegetation formation, climate and topography. Catena 121, 186-194, 2014.

 López-Vicente, M., Navas, A., Machín, J.: Effect of physiographic conditions on the spatial variation of seasonal topsoil moisture in Mediterranean soils. Australian Journal of Soil Research 47, 498-507, 2009.



The topographic factor has been traditionally included in the study of the spatial distribution 1 2 of soil properties (Fernández-Calviño et al., 2013; Haregeweyn et al., 2013; Ozgoz et al., 3 2013; Wang and Shao, 2013). Over time, many researchers have quantified the relationships 4 between topographic parameters and soil properties such as soil OM and physical properties 5 such as particle size distribution, bulk density and depth to specific horizon boundaries (McKenzie and Austin, 1993; Gessler et al., 1995; Gessler et al., 2000; Pachepsky et al., 6 7 2001; Ziadat, 2005). Soil OM content has been negatively correlated with the topographic 8 gradient (Ruhe and Walker, 1968), and slope gradient (Nizevimana and Bicki, 1992). 9 However, quantitative relationships between soil topography and soil physical-chemical 10 properties are not well established for a wide range of environments (Hattar et al., 2010). 11 Research along altitudinal gradients has shed light on the effects of climate on soil properties. Ruiz-Sinoga et al. (2012) found a strong relationship between soil OM and elevation, which 12

13 was due to reduced decomposition rates with lower temperatures. High erosion rates have 14 been found under dry climates and low altitudes in Israel (Cerdà, 1998a; Cerdà, 1998b), which support the idea of high OM losses due to soil erosion in dry areas. Similar results were 15 found by Ruiz-Sinoga and Martínez-Murillo (2009) in their study on the hydrological 16 response of soil along a climatological gradient in Andalucía, Spain. Ruiz-Sinoga and Diaz 17 18 (2010) found that the climatological (altitudinal) factors determined soil degradation rates in 19 the pluviometric gradient they studied in southern Spain. 20 Within the Despeñaperros nature reserve there is no information about the soil variability, and 21 little data is available related to the control topography exerts on soil properties (Lozano-

García and Parras-Alcántara, 2014). Therefore, the aims of this study are: (i) to quantify SOC contents and their vertical distribution in a natural forest area, (ii) to assess the SOCS differences in soils along an altitudinal gradient and (iii) their relationship with soil depth in a Mediterranean natural area.

26

27 2 Material and Methods

28 2.1 Study site

- 29 The Despeñaperros Natural Park (76.8 km²) is one of the best-preserved landscapes in
- 30 southern Europe. It is located within the Eastern Sierra Morena (province of Jaén,
- 31 southeastern Spain), at coordinates 38° 20' 38° 27' N, 3°27' 3°37'W. The study area is

Comentado [DBZ7]: I have deleted "SOC", and modified this statement, as it is nonsense talking of OM and SOC in this context

Comentado [DBZ8]: Again, I have carefully revised both papers and have not found any reference to elevation, except some table for description of study areas. But results are not correlated with elevation in any case. You can discuss about this in the discussion section, but, in both cases, soil properties were studied in relation to climate.

Revise also names, dashes (-) and diacritics (Martinez) according to the original sources.

Comentado [DBZ9]: Try to highlight the study area as a characterstic system, which includes specific soil use, vegetation and climate, representative of wide other areas in the Mediterranean. But results look not so interesting if they are restricted to the natural park.

characterized by warm dry summers and cool humid winters and climate is temperate semi-1 2 arid with continental features due to elevation. Average extreme temperatures range between -10 °C (winter) and 42 °C (summer), with mean temperature 15 °C. The moisture regime is dry 3 Mediterranean, with average annual rainfall is 800 mm. High temperatures and long drought 4 5 periods cause water deficits up to 350 mm annually. It is a mountainous area, with an altitudinal range of 540 m.a.s.l. in the Despeñaperros River 6 7 valley to 1250 m.a.s.l at Malabrigo Mountain. The relief is steep with slopes ranging from 3% 8 to 45%, and the parent materials are primarily slates and quartzites. Most abundant soils in the 9 area are Phaeozems (PH), Cambisols (CM), Regosols (RG) and Leptosols (LP), according to the classification by IUSS Working Group WRB (2006). Well-preserved Mediterranean 10 11 woodlands and scrublands occupy the study area and large game habitat is the main land use. 12

13 2.2 Soil sampling and analytical methods

- 14 Seven sites were selected along a topographic gradient in a south-facing slope in the
- 15 Despeñaperros Natural Park (Table 1). Soil samples were collected at each site following a
- 16 random sampling design according to FAO (2006). Each selected point was sampled using
- 17 soil control sections (SCS) at different depths (S1: 0-25, S2: 25-50 and S3: 50-75 cm). SCS
- 18 were used for a more uniform comparison between studied soils. Four replicates of each soil
- 19 sample were analyzed in laboratory (17 sampling points \times 1, 2 or 3 SCS \times 4 replicates).
- 20 Soil samples were air-dried at constant room temperature (25 °C) and sieved (2 mm) to
- 21 discard coarse particles. The analytical methods used in this study are described in Table 2.
- 22 Statistical analysis was performed using SPSS Inc. (2004). The physical and chemical soil
- 23 properties were analyzed statistically for each SCS of different soil groups (PH, CM, RG and
- 24 LP), including the average and standard deviation (SD). The statistical significance of the
- 25 differences in each variable between each sampling point (SCS) was tested using the
- 26 Anderson-Darling test at each control section for each soil type. Differences with p<0.05 were
- 27 considered statistically significant.

Comentado [DBZ11]: Not sure, just check: do you mean the statistical difference between data from each SCS and soil type? Mean values or unique?

Comentado [DBZ10]: I do not understand this. Revise.

28

Results and discussion 1 3

Soil properties 2 3.1

3 The Despeñaperros nature reserve soils are siliceous due to their parent materials (slate, 4 quartzite and sandstone). The studied soils were classified as Phaeozems, Cambisols, Regosols and Leptosols (IUSS-ISRIC-FAO, 2006) (Table 1). The soils are stony soils, acidic, 5 with low base concentrations, oligotrophic and with slightly unsaturated complex change and 6 7 located in areas of variable slopes ranging between 5% and 38%. Phaeozems are the most 8 developed soils in the study area. They are deep, dark, and well humidified with high 9 biological activity and high vegetation density on gentle slopes and shady side foothills. 10 Cambisols are developed and deep soils; however Leptosols are the least developed and 11 shallowest soils.

12 Phaeozems are the most pedogenically developed soils in the study area. They are found on gentle slopes (<3%), usually in shaded areas on Ordovician sandstones. The gravel content is 13 14 variable, ranging between 7% and 31%. Texturally they are sandy soils at the surface and

15 silty-clay-loam or silty-clay soils at depth, with a horizons sequence A0/A1/AB/Bt/C1. These

16 soils show luvic (lv) characteristics (luvic-Phaeozems (lv-PH)) and are >1 m in depth with pH

17 along the profile ranging from 6.3 to 5.6 at depth and about 4.3% OM content (Table 1 and 3).

18 Cambisols are less developed soils than luvic-Phaeozems, however, these soils are more 19 developed and deeper than Regosols and Leptosols. They appear in areas of variable slope (3-20 38%) and are >1 m in depth characterized by a cambic horizon (Bw) on Ordovician quartzites 21 (Table 1) with approximately 20% gravel content. At the surface they are sandy soils (<60% 22 sand content) with high clay content in the Bw horizon and increasing clay content with depth 23 (Table 3). The horizon sequences were A0/A1/AB/BW/BC/C1 or A0/A1/AB/BW. These soils 24 are characterized by low OM content at depth. Gallardo et al. (2000) showed that the low OM 25 content could be explained by the semiarid Mediterranean conditions. In addition, Parras-26 Alcántara et al. (2013a) found there is less OM and fewer mineral aggregates in sandy soils, thus favoring high levels of OM transformation. Because of this, Hontoria et al. (2004) 27 28 suggested that physical variables determine soil development in the driest areas of Spain to a 29 greater degree than management or climatic variables. The Cambisols topsoil has humic (hu) 30 characteristics, with >5% OM content (Table 3) due to plant debris accumulation in the A0 31 horizon. This OM is poorly structured and partially decomposed, thereby reducing the amount

Comentado [DBZ12]: I have a great problem with this section. It is OK, but:

Is this previous work by you and/or others? Then add a proper reference, simplify and move to Materials and methods. If it is your own work, carried out for this research, then you should modify the Materials and methods section, explaining: •Methods for description of the site characteristics at each soil profile or sampling site (slope, lithology, stones...) Methods for description of soil horizons or SCS: chemical and physical methods. I mean: for soil classification you need organic matter content, pH, carbonates, texture ... even soil colour and structure of aggregates! So, please, add this information. Comentado [DBZ13]: This information is in the description of

the study area, delete

Comentado [DBZ14]: I have changed it above. The correct citation is: IUSS Working Group WRB, 2006. Not IUSS_ISRIC-FAO, 2006.

Comentado [DBZ15]: Volume or weight?

and increasing the OM evolution degree with depth. In this line, Bech et al. (1983) reported
 that the free OM concentration in the surface horizon was higher than 90%, while humic and
 fulvic acid concentrations were less than 2% in soils with *Quercus ilex spp*. ballota
 vegetation. Free OM was reduced and humidification increased up to 30% in deeper layers.

Regosols can be found in steeply sloping areas (>8%) characterized by high water erosion and 5 subject to rejuvenation processes. We found eutric (eu), dystric (dy) and umbric (um) 6 7 Regosols (Table 1) on sandstone and quartzite parent materials with >25% gravel content in 8 surface layers that eventually disappeared in depth. These soils are sandy-loamy in surface 9 layers and silty-clay in deep layers, with different horizon sequences (A0/A1/AB/BC/C1, 10 A0/A1/AC/C1 and A1/AC/C1). Eutric-Regosols are deeper soils (>80 cm) that are loamy 11 with high gravel content (25.1-32.2%) at the surface decreasing with deep, acid pH (5.9) and 12 high OM content (6.7%) at the surface. The dystric-Regosols are stony soils that are shallow 13 (<40 cm), loamy at the surface and sandy at depth with high gravel content (>40%) at the 14 surface, acid pH (6.2) and high OM content (7.3%) in the surface horizon (Table 3). The 15 umbric-Regosols are also stony, they are deep soils (>70 cm) that are loamy with high gravel content (40%) in the surface decreasing to 11% at depth, acid pH (5.6) and high OM content 16 17 (6.5%) (Table 3).

Leptosols are the least developed soils of the study area. Lithic (li), mollic (mo) and eutric 18 19 (eu) Leptosols were identified (Table 1) formed in sandstones, quartzites and slates on 20 variable slopes (1.5-46%). Horizon sequences A1/AC/C1, A1/AC, and AC/C1 and A1 were 21 found. The gravel content was variable (>40% in the topographically elevated areas and 22 decreasing with depth) with high sand content (>50%) in the surface layers. One characteristic 23 of these soils is that the clay content increased with depth, reaching up to 30%. According to 24 Recio et al. (1986), the physical-chemical properties of the soils in the study area are due to 25 lithology, while their low edaphic development is conditioned by age (Porta et al., 2003). 26 According to Nerger et al. (2007) the alteration and pedogenesis processes taking place in 27 these soils usually occur on low slopes. The lithic-Leptosols are the least developed soils at 28 this study site, with thicknesses ranging between 10 and 15 cm in areas of steep slope. In flat 29 areas their low development is due to their extreme youth. These soils are loamy with a high 30 gravel content (>28%), acid pH and >4% OM content. Mollic-Leptosols are characterized by 31 mollic surface horizons (thick, well-structured, dark, high base saturation and high OM 32 content), on variable slopes (18.5%-38.5%). According to Corral-Fernández et al. (2013) **Comentado [DBZ16]:** Regosols on sandstones and quartzites from Sierra Morena with no rock fragments in depth? Hard to believe. At least, not in your manuscript.

Comentado [DBZ17]: I have not found such an statement here. Just check to be sure.

1 these soils are characterized by organic residue accumulation in the surface horizons; this OM

2 is poorly structured and partially decomposed at the surface with increasing decomposition

3 rate with depth. Umbric-Leptosols are characterized by high OM content, are shallow, and

4 either loamy with high stony content (>20% gravel content) or sandy (>55% sand content),

5 have low bulk density conditioned by the OM content, high porosity and acid pH (Table 3).

6

7 3.2 Distribution of soil organic carbon

Generally, soils in the study area are characterized by >3% OC content, making them part of the 45% of the mineral soils of Europe that have between 2 and 6% OC content (Rusco et al., 2001). In general, soil OM content decreased with depth at all topographic positions (A, B, C and D positions) (Table 4). However, this property cannot be observed in the lowest topographic positions (E, F and G positions) due to the low edaphic development (umbric-Leptosols, lithic-Leptosols and mollic-Leptosols) as only one SCS exists (S1: 0-25 cm) (Tables 1 and 4).

15 The soils in this study are characterized by high sand content at the surface (S1) varying between 59.2 and 34.2% for C and F positions respectively, and reduced sand content with 16 depth in all studied soils (Table 3). Therefore, this high sand content influenced the 17 18 development of OM, giving OM that is poorly structured and partially decomposed and 19 increasing OM development with depth due to sand content reduction and the clay content 20 increase; clay content reaches 45% in C: S3. In addition, the mineral medium may play an 21 important role in soil humidification processes, so we can explain low soil OM concentrations 22 with depth due in part to soil texture, because soil OM tends to decrease with depth in virtually all soils, regardless of textural changes. Clays over sands would have a decrease in 23 24 soil OM with depth also, and probably a more marked decrease. In addition, the formation of 25 aggregates made up of OM and the mineral fraction is reduced, thus favoring high OM levels 26 in sandy soils at depth (González and Candás, 2004). Furthermore, Gallardo et al. (2000) argued that the low concentrations of OM in depth can be explained by the climate 27 28 (Mediterranean semiarid). Similar results have been found by Corral-Fernández et al. (2013), 29 Parras-Alcántara et al. (2014) and Lozano-García and Parras-Alcántara (2013a) in the

30 Pedroches Valley, near the study area.

Comentado [DBZ18]: Is this necessary? Comentado [DBZ19]: Of course.

Comentado [DBZ20]: My dear friend, honestly, I think this is nonsense. Try to revise.

Comentado [DBZ21]: I do not understand.

Comentado [DBZ22]: ????

Comentado [DBZ23]: OM concentration usually decreases in depth. Perhaps you mean "relatively" low concentrations when compared to other soils (what soils?).

Another key issue is that the clay fraction increased with depth in the B and C positions 1 2 (reaching a clay content of as high as 45% (C: S3)) and its relation with soil OM at S2 (25-50 3 cm), which was characterized by high OM contents as compared to S3 (B:2.0/0.6%; C:1.8/0.06%) (Table 4). Burke et al. (1989) and Leifeld et al. (2005) have shown high OM 4 5 levels in soils with high clay content indicating clay stabilization mechanisms in the soil. This 6 effect can be observed in the B and C topographic positions, where an increase in clay content 7 was observed at depth as compared to the upper horizons (B:S1-17.2%/S2-22.1%; C:S1-8 16.1%/S2-35.7%). This OM increase may be due to carbon translocation mechanisms 9 (dissolved organic carbon), soil biological activity and/or the root depth effect (Sherstha et al., 10 2004).

Soil OM appears to be concentrated in the first 25 cm (S1) due to OM accumulation, where 11 the mineralization and immobilization C processes should be active. In these mineral soils, 12 13 the OM content in deeper layers generally follows a non-linear reduction and this relationship 14 may be expressed as an exponential function (Hiederer, 2009). This non-linear distribution 15 with depth was linked to the unequal OM concentrations that were found in the different SCS. In the surface layer (S1), OM was variable along the toposequence studied ranging between 16 39.9 and 27.3 g kg⁻¹ at the B and F positions, respectively (Table 4). In this regard, it is 17 important to point out that the S1 layer can reach over 60% of the total soil organic carbon (T-18 19 SOC) values documented, corresponding to 60, 64.4 and 63% for the B, C and D positions 20 respectively as compared to the rest of the soil profile (S2 or S2+S3). Batjes (1996) states that 21 for the 0 to 100 cm depth approximately 50% of soil organic carbon (SOC) appears in the first 22 30 cm of the soil. Jobbágy and Jackson (2000) showed that 50% of SOC is concentrated in the 23 first 20 cm in forest soils to 1-m depth. Civeira et al. (2012), showed that SOC in the upper 30 24 cm of soils in Argentina is much higher than in the 30-100 cm interval. Data provided by 25 these authors and the results obtained in this study may be comparable because in this study 26 we used a 75 cm depth and the mentioned authors used a 1m depth. Also, we used SCS with 27 25 cm increments and they used SCS with 30 and 20 cm increments, therefore, there are not 28 significant differences between our research procedures and the procedures used by Batjes 29 (1996), Jobbagy and Jackson (2000) and Civeira et al. (2012) to investigate SOC distribution 30 with depth. Furthermore, Jobbágy and Jackson (2000) indicated that changes in SOC were 31 conditioned by vegetation type (which determines the vertical distribution of roots) and to a lesser extent the effect of climate and clay content. Despite this, climatic conditions can be a 32

33 determining factor in the SOC concentrations for surface horizons, whereas clay content may

Comentado [DBZ24]: This sentence is not complete. ...And its relation wit soil OM at S2, which was characterized by ... what?

Comentado [DBZ25]: ???? In soils or in depth?

Comentado [DBZ26]: Yeas, but these statements are too general. I still do not see a clear explanation of the influence of clay and OM contents here. In fact, my opinion is that

Comentado [DBZ27]: Delete.

Comentado [DBZ28]: Accumulation of OM cannot be produced by active mineralization.

Comentado [DBZ29]: Delete.

Comentado [DBZ30]: Delete.

- 1 be the most important element in deeper horizons. At the regional-global scale SOC increases
- 2 with precipitation and decreases with temperature (Post et al., 1982).
- 3 Results of T-SOC analysis in the studied area did not show great along the toposequence. In
- 4 this regard, T-SOC depended on the degree of development of the soil that appeared at each
- 5 topographical position. The T-SOC was highest at the B (66.5 g kg⁻¹), D (58.1 g kg⁻¹) and C
- 6 (52.3 g kg⁻¹) positions, corresponding to Cambisols-Regosols-Leptosols, Regosols, and
- 7 Phaeozems-Cambisols-Regosols respectively. Leptosols showed the lowest T-SOC content
- 8 with 27.3 g kg⁻¹, 31.9 g kg⁻¹, 32.7 g kg⁻¹ and 38.1 g kg⁻¹ at the F, G, E and A topographic
- 9 positions, respectively. Similarly, >60% of SOC concentrated in the S1 layer of deeper soils
- 10 (B, C and D).

11 Precipitation and temperature varied through the studied toposequence, where precipitation

12 increasing and temperature decreasing with increasing elevation. T-SOC content was not

- 13 affected by climatic variations, but depended on the soil development in each landscape
- 14 position. Reduced T-SOC contents were observed at the lowest topographic positions, where
- soils were shallower. This is in agreement with Power and Schlesinger (2002) who concluded
- 16 that topographic position affects T-SOC, due to low OM decomposition rates under low
- 17 temperatures.
- 18

19 **3.3 Soil organic carbon stocks**

- 20 **SOCS** in the study area showed a reduction with depth in all topographic positions (Table 4).
- 21 This SOCS reduction along the profile is linked to OM reduction with depth, this reduction in
- 22 SOCS also depended on the gravel content and the bulk density (Table 3).
- 23 When the upper SCS was analyzed we observed high SOCS values as high as 91.1 Mg ha⁻¹ in
- the elevated topographic positions (highest value at the B position). The lowest SOCS values
- 25 were found at the G position (53.8 Mg ha^{-1}), the lowest site in the toposequence. This trend of
- decreasing SOCS with decreasing elevation is constant except at the A and E positions. This
- 27 was caused by the soil type, mollic-Leptosols at the A position and umbric-Leptosols at the E
- 28 position. Both are poorly developed soils with high OM content in the surface horizon).
- 29 We observed that at the D and B topographic positions between 53.8 and 58% of SOCS,
- 30 respectively, occurred in the S1 SCS. This constituted 63% and 60% of T-SOC in these

Comentado [DBZ31]: Clay contributes to stabilize organic matter by protecting physically of microbial activity and reducing C outputs. But you should state that this effect is important under homogeneous climate conditions (as those in your research area).

Comentado [DBZ32]: Why? Explain. Precipitation contributes to maximize inputs and temperature accelerates mineralization.

Comentado [DBZ33]: Delete.

Comentado [DBZ34]: "Cambisols-Regosols-Leptosols associations"?

Comentado [DBZ35]: Obvious. In fact, OM is calculated from OC, and SOCS from OC. The interesting question here: why SOCS decreases?

Comentado [DBZ36]: I suggest, but not sure: "High SOCS values were found in the upper SCS of the higher topographic positions, specially at the B position".

Comentado [DBZ37]: This is circular logic. Soil type cannot be the cause of SOCS trends, when soil type is (partly) the consequence of soil organic C content. Although mollic and umbric horizons in the lowest sites imply a relatively high organic matter content, shallow depth, effective soil volume (without gravels), bulk density and others are the cause of low SOCS. You can mention real soil depth here (which should be between 0 and 25 cm). I suggest deleting this statement and modifying the following one.

Comentado [DBZ38]: Revise and re-word this sentence: between X and Y, respectively? SOCS (Mg ha⁻¹) or OM content (%)? Use the same number of decimal digits (58.0?). I have not found these values in tables.

Comentado [DBZ39]: 58.1 (D) and 66.6 g kg⁻¹ (B) according to Table 4?

topographic positions. This shows that the gravel content and bulk density affects the SOCS 1 2 in the surface horizons of the toposequence studied, and, therefore, SOCS decreases when SOC increases. In the most developed soil, similar SOC and SOCS concentrations (B: 60%-3 SOC; 58%-SOCS) were observed in the S1 layer, conditioned by bulk density and gravel 4 5 content. In addition, SOCS decreased in depth conditioned by reduction of gravel content and increasing bulk density. This is not in agreement with Tsui et al. (2013) and Minasny et al. 6 7 (2006), who suggested a negative relation between bulk density and depth as a consequence 8 of high OM content at the surface, linked to low clay concentrations (Li et al., 2010). In this 9 sense, we observed that high SOCS depended on the SOC concentration and the clay content. 10 However, the SOC concentration affected the SOCS to a lesser degree so that in S2 (25-50 cm) we found >10% of SOCS related to SOC (C position). 11 In contrast, low SOCS can be found in S3 (50-75 cm) except at the B topographic position (19 12 13 Mg ha⁻¹). This situation could be due to the fact that pedological horizons were generally 14 different than the SCS divisions (S1: 0-25 cm; S2: 25-50 and S3: 50-75 cm) (Hiederer, 2009); 15 in other words, the SCS divisions often led to the mixing of two or more soil horizons (depending on thickness horizon) in any given SCS division. 16 In all studied soils, the clay content increased with depth. This clay content increase is 17 associated to higher values of SOC (B: S2 and C: S2). In this line, we can explain high SOCS 18 19 concentrations in clayey soils caused by clay stabilization mechanisms on SOC, this effect 20 can observed at the A topographic position which has higher clay content with respect to the B and D positions. However, a SOCS increase can be observed. This is the case at the D and 21 C topographical positions with SOCS values of 52.1 and 50.1 Mg ha⁻¹ respectively in the S2 22 23 sampling layer (Table 4), showing a correlation between S1 and S2, due to carbon 24 translocation processes as dissolved organic carbon, bioturbation and/or deep rooting 25 (Sherstha et al., 2004).

26 3.4 Soil organic carbon stocks (SOCS) along the altitudinal gradient

The SOCS results along the toposequence were also studied. It is important to point out that SOCS (T-SOCS) were influenced by topographical position in the toposequence

29 analyzed. T-SOCS increased linearly with elevation from G (607 m.a.s.l.) to B site (1009

- 30 m.a.s.l.), with the exception of the highest topographic position, A (1168 m.a.s.l.), with a
- 31 linear regression relationship (Figure 1). Similar results were found by Ganuza and

Comentado [DBZ40]: I have modified the sentence for better syntax, but SOC and SOCS are two different variables and cannot be "similar" or "different". They cannot be compared, trends can..

Comentado [DBZ41]: Delete. Comentado [DBZ42]: 19.1?

Comentado [DBZ43]: This statement is correct, but gives no explanation.

Comentado [DBZ44]: Not sure. Probably just part of the A horizon (or an A2, A/B, A/C...) is included in S2.

Comentado [DBZ45]: This is possible, and may be due to low pH and high rainfall, but would imply the existence of a spodic horizon. I think that rainfall in D and C are not enough to produce this.

Comentado [DBZ46]: I suggest "bioturbation" instead of "soil fauna".

Comentado [DBZ47]: Personally, I think that abbreviations should not be used in titles.

Almendros (2003), Leifeld et al. (2005) and Fernández-Romero et al. (2014). These studies 1 showed that the T-SOCS increased with elevation. However, Avilés-Hernández et al. (2009) 2 found that T-SOCS from forest soils decreased with elevation in a toposequence in Mexico 3 4 due to variations in the OM decomposition rate as a result of the different vegetation types 5 found in the different topographic positions; and Lozano-García and Parras-Alcántara (2014) found that T-SOCS decreased with elevation in a traditional Mediterranean olive grove due to 6 7 erosion. With respect to the A position in this study, the lower T-SOCS (72.9 Mg ha⁻¹) values 8 with respect to the rest of the studied toposequence may be due to soil loss caused by erosion 9 processes in soils with a low level of development. Similar results have been found by Parras-10 Alcántara et al. (2004) and Durán-Zuazo et al. (2013). Parras-Alcántara et al. (2004) explained their findings as a consequence of high soil erosion rates, caused by high erosivity 11 12 of rainfall, high erosionability, steep slopes, low vegetation cover and the lack of conservation practices in the studied area. Durán-Zuazo et al. (2013) explained this effect by low 13 14 vegetation densities in the upper parts of mountain areas that can cause high erosion with strong water runoff. Martínez-Mena et al. (2008) have emphasized the effects of erosion on 15 soil OM loss, especially under semi-arid conditions. In this context, a low vegetation ratio can 16 17 accelerate OM decomposition, weakening soil aggregates (Balesdent et al., 2000; Paustian et al., 2000). Cerdà (2000) indicated that this effect (OM decomposition and aggregate 18 19 destruction) could occur regardless of climatic conditions. 20 As can be seen in Table 4, T-SOCS decrease was not homogeneous. In some cases, rapid 21 changes were found, while in other situations gradual changes were noted. Abrupt changes in 22 T-SOCS occurred between the B/C and D/E topographic positions, showing T-SOCS 23 differences of 38 Mg ha⁻¹ and 44 Mg ha⁻¹ respectively. Gradual changes in T-SOCS occurred between the C/D, E/F and F/G topographic positions with variations of 3 Mg ha⁻¹, 13 Mg ha⁻¹ 24 25 and 6 Mg ha⁻¹ respectively. Many authors have concluded that the SOCS reduction can be 26 explained by soil physical properties - mainly texture (Corral-Fernández et al., 2013; Parras-

Alcántara et al., 2013b). The studied soils are sandy at the surface, with clay increasing with depth (soils that have S2 and/or S3 SCS), therefore, OM stabilizing mechanisms are produced, reducing the aggregate formation between SOC and mineral fraction at depth. As a result, the SOCS content is lower with sandy soils (Nieto et al., 2013). González and Candás

31 (2004) and Parras-Alcántara et al. (2013a) obtained similar results, the first in sandy-loamy

32 soils and the second in Mediterranean clayey soils. In addition, low SOC levels are

33 conditioned by the climatic characteristics of southern Europe (Gallardo et al., 2000).

Comentado [DBZ48]: Delete.

Comentado [DBZ49]: Yes, but: is it a coherent explanation in your study area? Comentado [DBZ50]: Changed to OM. Soil C is correct, but soil OM is better in this context. Comentado [DBZ51]: ???? Cover?

Comentado [DBZ52]: Delete.





2 Conclusions

3 Soils found in the Despeñaperros nature reserve include Phaeozems, Cambisols, Regosols and

4 Leptosols. Phaeozems are the deepest and most developed soils, and Leptosols are the least

5 developed and shallowest soils. These soils are characterized by low OM content with depth

6 due to the semiarid Mediterranean conditions and the high sand content. The studied soils are

7 characterized by organic residue accumulation in the surface horizons.

8 The SOC content decreased with depth at all topographic positions and the clay fraction

9 increased with depth. The mineral medium played an important role in soil humidification

10 processes. In addition, the SOC in the S2 layers is characterized by high SOC values with

11 respect to the S3 layers indicating clay stabilization mechanisms in the soil. We can explain 12 this increase due to carbon translocation mechanisms (dissolved organic carbon), soil

13 biological activity and/or the root depth effect.

With respect to T-SOC content, there is not a large difference between T-SOC along the 14 15 toposequence. The T-SOC of these soils depends on the degree of development of the soils found at each topographic position. We can observe a T-SOC reduction at the lowest 16 17 topographic positions for less developed soils and a T-SOC increase at the highest 18 topographic positions in the more developed soils. SOCS in the study zone show a reduction 19 with depth in all topographic positions. This SOCS reduction along the profile is linked to 20 OM and gravel content reduction and an increase in bulk density with depth. The T-SOCS 21 increased with altitude, due to the higher turnover of organic material (plants) and the lower 22 decomposition rate due to lower temperatures.

23

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26

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Comentado [DBZ56]: Conclusions should be revised after changes in the rest of the text.

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- 15
- 16

1 Table 1. Soil groups of the study area at each of the seven topographic positions with properties. The key refers to the reference soil groups of the IUSS

2 Working Group WRB (2006) with lists of qualifiers.

Topographic position	m.a.s.l.ª	Slope %	Parent material	Vegetation series	Soil groups	Qualifiers	n ^b
A	1168	15.3	Quartzite - Sandstone	Maritime pine (<i>Pinus pinaster</i>) Holm oak (<i>Quercus ilex</i>) Gum rockrose (<i>Cistus ladanifer</i>)	Leptosols - LP	Mollic - mo	2
В	1009	16.5	Quartzite - Sandstone	Holm oak (<i>Quercus ilex</i>) Cork oak (<i>Quercus suber</i>) Strawberry tree (<i>Arbutus unedo</i>) Gum rockrose (<i>Cistus ladanifer</i>)	Regosols - RG Leptosols - LP Cambisols - CM	Eutric - eu Mollic - mo Humic - hu	3
С	945	20.8	Quartzite - Sandstone	Stone pine (Pinus pinea) Mastic (Pistacia lentiscus)	Cambisols - CM Regosols - RG Phaeozems - PH	Humic - hu Dystric - dy Luvic - lv	3
D	865	5.5	Quartzite	Portuguese oak (<i>Quercus faginea</i>) Strawberry tree (<i>Arbutus unedo</i>) Gum rockrose (<i>Cistus ladanifer</i>)	Regosols - RG	Umbric - um	2
E	778	10.7	Quartzite - Slates	Holm oak (<i>Quercus ilex</i>) Strawberry tree (<i>Arbutus unedo</i>) Gum rockrose (<i>Cistus ladanifer</i>)	Leptosols - LP	Umbric - um	3
F	695	12.0	Quartzite	Cork oak (<i>Quercus suber</i>) Holm oak (<i>Quercus ilex</i>) Strawberry tree (<i>Arbutus unedo</i>) Gum rockrose (<i>Cistus ladanifer</i>)	Leptosols - LP	Litic - li	2
G	607	18.5	Slates	Holm oak (<i>Quercus ilex</i>) Mastic (<i>Pistacia lentiscus</i>)	Leptosols - LP	Mollic - mo	2

3 ^a Metres above sea level; ^b Sample size

Comentado [DBZ59]: Series? Series of what?

I suggest using only Latin names.

Table 2. Methods used in field measurements, laboratory analysis and to make calculations from study data.

Parameters	Method
Field measurements	
Bulk density (Mg m ⁻³)	Cylindrical core sampler [*] (Blake and Hartge, 1986)
Laboratory analysis	
Particle size distribution	Robinson pipette method (USDA, 2004)**
$pH - H_2O$	Volumetric with Bernard calcimeter (Duchaufour, 1975)
Organic C (%)	Walkley and Black method (Nelson and Sommers, 1982)
Parameters calculated from study data	
SOC stock (Mg ha ⁻¹)	(SOC concentration×BD×d×(1- δ_{2mm} %)×0.1)*** (IPCC, 2003)
Total SOC stock (Mg ha ⁻¹)	Σ_{horizons} SOC Stock _{horizon} (IPCC, 2003)

3 * 3 cm diameter, 10 cm length and 70.65 cm³ volume.

4 ** Prior to determining the particle size distribution, samples were treated with H_2O_2 (6%) to remove organic matter (OM). Particles larger than 2 mm were 5 determined by wet sieving and smaller particles were classified according to USDA standards (2004).

6 *** Where SOC is the organic carbon content (g Kg⁻¹), d the thickness of the soil layer (cm), δ2mm is the fractional percentage (%) of soil mineral particles >2 7 mm in size in the soil, and BD the soil bulk density (Mg m⁻³).

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Topographic position	m.a.s.l. m	SCS	Depth cm	Gravel %	Sand %	Silt %	Clay %	B.D. Mg m ⁻³	O.M. g kg ⁻¹	pH H ₂ O
А	1168	S 1	0-25	33.1±13.8 aA	56.5±1.1 aA	22.3±3.0 aA	21.2±4.1 aA	1.1±0.19 aA	64.5±8.9 aA	6.3±0.7 aA
		S2	25-50	7.0±3.1 bA	39.3±0.81 bA	30.7±4.2 aA	30.0±6.1 aA	1.5±0.21 bA	0.99±0.21 bA	5.3±0.5 bA
В	1009	S 1	0-25	17.0±10.0 aB	52.9±29.8 aA	29.9±30.6 aA	17.2±5.3 aA	1.1±0.10 aA	68.6±5.2 aA	5.9±0.4 aA
		S 2	25-50	27.1±6.4 bB	58.7±20.1 aB	19.1±12.2 bB	22.1±8.0 aB	1.3±0.12 aB	35.3±3.4 bB	5.6±0.7 aA
		S 3	50-75	14.3±16.9 aA	41.6±18.1 bA	25.7±15.2 aA	32.6±2.9 bA	1.5±0.12 bA	10.5±2.8 cA	5.7±0.5 aA
С	945	S 1	0-25	34.0±5.5 aA	59.2±7.2 aA	24.7±3.1 aA	16.1±6.2 aA	1.2±0.10 aA	58.0±9.5 aA	5.9±0.8 aA
		S 2	25-50	14.4±7.2 bC	36.1±12.2 bA	28.2±2.5 aA	35.7±14.1 bA	1.3±0.06 aB	30.9±6.3 bB	5.5±0.4 aA
		S 3	50-75	14.9±11.9 bA	24.4±15.9 cB	30.4±9.8 aA	45.2±16.2 cB	1.5±0.05 aA	0.99±0.12 cB	5.2±0.6 aA
D	865	S 1	0-25	39.9±6.2 aA	47.6±19.3 aB	38.1±7.5 aB	14.3±2.1 aA	1.1±0.09 aA	62.9±10.4 aA	5.6±1.0 aA
		S 2	25-50	24.0±4.5 bB	46.6±18.2 aC	36.2±7.9 aA	17.2±5.4 aB	1.3±0.10 aB	35.9±7.6 bB	5.7±0.8 aA
		S 3	50-75	11.9±10.2 cA	30.9±11.1 bB	47.1±5.4 bB	22.0±6.8 aC	1.5±0.13 bA	1.0±0.30 cB	4.5±0.4 bB
Е	778	S 1	0-25	25.5±6.8 aC	52.2±7.2 aA	30.2±5.1 aA	17.6±2.4 aA	1.2±0.13 aA	56.3±8.9 aA	5.7±0.7 aA
F	695	S 1	0-25	28.2±7.4 aC	34.2±5.3 aC	41.0±9.8 aB	24.8±2.8 aA	1.2±0.14 aA	46.9±7.4 aB	6.3±0.5 aA
G	607	S 1	0-25	42.9±19.3 aD	54.9±4.1 aA	27.7±2.5 aA	17.3±6.6 aA	1.3±0.13 aB	54.9±9.2 aB	6.2±0.7 aA
.: Metres abov	e sea leve	el; SCS	: Soil co	ntrol section; BI	D: Bulk density;	O.M.: Organic	matter.			
lard deviation.										

1 Table 3. Properties of the soils evaluated (average \pm SD*) in the Despeñaperros Nature Reserve.

m.a.s 2

3 *Stan

Numbers followed by different lower case letters within the same column have significant differences (P<0.05) at different depths, considering the same topographic position. Numbers followed by different capital letters within the same column have significant differences (P<0.05) considering the same SCS at 4 5

different topographic position. 6

Comentado [DBZ60]: Check this: it is written ,within the same column" in both cases. Capitals for columns and low-case for rows or viceversa?

Comentado [DBZ61]: It is OK, but I suggest moving this to the caption.

1	Table 4. Soil organic carbon (SOC) content and soil organic carbon stock (SOCS) (average ± SD*) in the Despeñaperros Nature Reserve.	
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Topographic	m.a.s.l.	200	SOC	T-SOC	SOCS	T-SOCS
position	m	202	g kg ⁻¹	g kg-1	Mg ha ⁻¹	Mg ha ⁻¹
А	1168	S 1	37.5±16.8 aA	38.1±8.4 A	70.8±33.5 aA	72.9±17.0 A
		S2	0.58±0.09 bA		2.1±0.57 bA	
_						
В	1009	S1	39.9±10.3 aA	66.6±8.2 B	91.1±13.2 aB	158.0±15.8 B
		S 2	20.5±6.4 bB		49.8±14.9 bB	
		S 3	6.1±7.8 cA		19.1±19.2 cA	
C	045	C 1	22719601	52 2 5 0 C	67 4 0 7 0 4	110 2 10 0 C
C	945	51	35.7 ± 0.0 aA	52.5±5.9 C	07.4 ± 9.7 aA	119.5±10.9 C
		52	18.0 ± 9.1 DB		50.1±22.4 0B	
		22	0.58±0.09 cB		1.8±0.26 cB	
Л	865	S 1	36 6+7 9 24	58 1+5 7 C	62 1+8 9 a A	116 1+8 6 C
D	005	\$2	20.0 ± 7.9 mR 20.9 ± 9.0 hR	50.1±5.7 C	$52.1\pm0.7 \text{ hB}$	110.1±0.0 C
		S2 S3	0.57 ± 0.09 cB		1.9 ± 0.30 cB	
		55	0.57±0.07 CD		1.)±0.30 CD	
Е	778	S 1	32.7+13.2 aA	32.7+13.2 A	72.6+25.0 aA	72.6+0.65 A
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F	695	S 1	27.3±15.1 aB	27.3±15.1 A	59.3±27.3 aC	59.3±27.3 A
G	607	S 1	31.9±13.1 aB	31.9±13.1 A	53.8±18.3 aC	53.8±18.3 A

m.a.s.l.: Metres above sea level; SCS: Soil control section; SOC: Soil organic carbon; T-SOC: Total SOC; SOCS: Soil organic carbon stock; T-SOCS: Total

SOCS.

*Standard deviation.

Numbers followed by different lower case letters within the same column have significant differences (P<0.05) at different depths, considering the same topographic position. Numbers followed by different capital letters within the same column have significant differences (P<0.05) considering the same SCS at different topographic position.

Comentado [DBZ63]: Column/column?

