Universidad de Córdoba

Departamento de Ingeniería forestal
Sistemas y Recursos Forestales y Agrosilvopastorales. Energías Renovables.
Instituto de Agricultura Sostenible – CSIC.

Tesis Doctoral:

Carbono orgánico en suelos agrícolas y agroforestales: Efecto de las diferentes prácticas de gestión

Memoria redactada para optar al grado de Doctor con Mención Internacional por la Universidad de Córdoba, por el Ingeniero en Agronomía:

José Lizardo Reyna Bowen

Supervisores: ***Pilar Fernández Rebollo y José Alfonso Gómez Calero***

Oct. 1, 2020

TITULO: *Organic carbon in agricultural and agroforestry soils: Effect of different management practices*

AUTOR: *José Lizardo Reyna Bowen*

© Edita: UCOPress. 2021
Campus de Rabanales
Ctra. Nacional IV, Km. 396 A
14071 Córdoba

https://www.uco.es/ucopress/index.php/es/
ucopress@uco.es

University of Córdoba
TECHNICAL SCHOOL OF AGRICULTURAL AND FORESTRY ENGINEERING

Doctoral Thesis

Organic carbon in agricultural and agroforestry soils: Effect of different management practices

Memoria redactada para optar al grado de Doctor con Mención Internacional por la Universidad de Córdoba, por el Ingeniero en Agronomía:

José Lizardo Reina Bowen

VºBº	VºBº
Pilar Fernández Rebollo ETSIAM, UCO	José Alfonso Gómez Calero Instituto de Agricultura Sostenible, CSIC

Oct. 1, 2020

José Lizardo Reyna Bowen
ORGANIC CARBON in AGRICULTURAL AND AGROFORESTRY soils: Effect of different MANAGEMENT PRACTICES
Doctoral Thesis, Oct. 1, 2020
Supervisors: Pilar Fernández Rebollo and José Alfonso Gómez Calero

University of Córdoba
Technical School of Agricultural and Forestry Engineering
Department of Forestry Engineering
Campus of Rabanales, Madrid-Cádiz Road Km. 396, Spain
14014 Córdoba

Acknowledgement

Este trabajo se ha realizado en el marco del programa de doctorado INGENIERÍA AGRARIA, ALIMENTARIA, FORESTAL Y DE DESARROLLO RURAL SOSTENIBLE, en la línea sistemas y recursos forestales y agrosilvopastorales. Energías renovables.

Agradezco al Dpto. de Ingeniería Forestal de la ETSIAM, UCO y al Instituto de Agricultura Sostenible (IAS) del CSIC por haberme permitido realizar los trabajos en sus instalaciones y laboratorios. A su vez, también agradezco a los proyectos de investigación del gobierno Andaluz sobre cubiertas vegetales INTCOVER P12-AGR-0931 (Andalusian Government). Proyecto INIA “La Seca de la encina y el alcornoque en la dehesa. Seguimiento temporal de su impacto y alternativas de control: biofumigantes, enmiendas y búsqueda de resistencias” (RTA2014-00063-C04-03). Prorecto de la comisión Europea sobre (SHui, Soil Hydrology research platform underpinning innovation to manage water scarcity in European and Chinese cropping systems European Commission Grant Agreement number: 773903) sobre uso sostenible de agua y suelo en sistemas agrarios de la Union Europea y China. Estos proyectos financiaron parte de los trabajos incluidos en esta tesis.

A la Universidad de Córdoba. UCO, Universidad de Agricultura de Cracovia y el programa Eramus Plus, por haberme ayudado con la financiación de las estancias en el extrajero.

Escuela Superior Politécnica Agropecuaria de Manabí. (ESPAM), por la finaciación de los proyectos de suelo en el Ecuador. Preyecto codigo: 393343.

Quiero dar las gracias en especial a Liza Krylova, sin ella no creo haber podido terminar esta meta. Ella Siempre en las buenas y malas ha estado ahí y a toda mi familia, que a pesar de la distancia siempre han estado pendiente de cada detalle que he vivido en España.

Dar las gracias a mis supervisores Pilar Fernández y José Alfonso Gómez, por haber tenido la paciencia conmigo. Gracias por todos esos consejos para mejorar cada día en este duro proceso de conseguir el doctorado.

A mis compañeros de oficina Maite y Ramón que me recibieron con sus buenas energías desde el primer día que llegue. A Alma por su ayuda con sus tips de controlar el insomnio y sus conversaciones profundas que me ayudaron mucho. A todos los demás del grupo de investigación que a diario compartíamos buenos momentos y especialmente en los desayunos.

Gracias a Azahara que me enseñó con mucha paciencia los trabajos del laboratorio y con la ayuda de Gema, Manolo y Clemente en trabajo de campo.

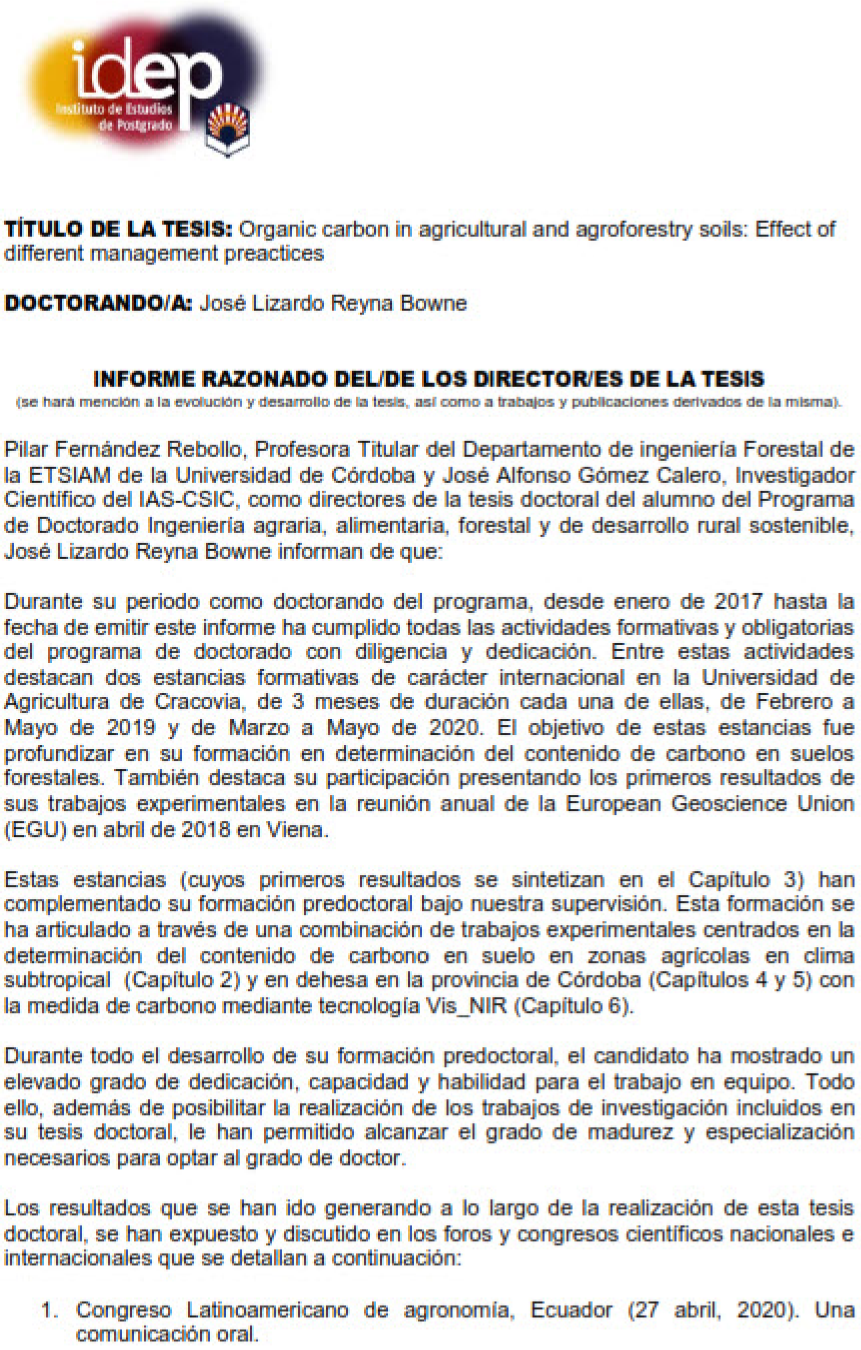
Francisco Sánchez Tortosa, José Guerrero, por la confianza y motivación a seguir este camino.

Thanks so much Krylova's family (Irina, Sergey, Sasha and Babuska), they are a wonderful family.

Y por último, y no por eso menos importante agradezco a mis buenos amigos que siempre han estado en este camino y los que he ido conociendo a lo largo de todo este proceso del doctorado. Arthur van den Pol, Andrea Prichard, Rebbeka Balser, Juan Alava, Hernan Veliz, Karolina Bartocha, Agata Grebocka, Pavlina Koukolova, Anna Biesiadecka, Ewa Błońska.

Dedicated in memory to June, 2014.

Supervisor's Reports



TÍTULO DE LA TESIS: Organic carbon in agricultural and agroforestry soils: Effect of different management preactices

DOCTORANDO/A: José Lizardo Reyna Bowne

INFORME RAZONADO DEL/DE LOS DIRECTOR/ES DE LA TESIS

(se hará mención a la evolución y desarrollo de la tesis, así como a trabajos y publicaciones derivados de la misma).

Pilar Fernández Rebollo, Profesora Titular del Departamento de ingeniería Forestal de la ETSIAM de la Universidad de Córdoba y José Alfonso Gómez Calero, Investigador Científico del IAS-CSIC, como directores de la tesis doctoral del alumno del Programa de Doctorado Ingeniería agraria, alimentaria, forestal y de desarrollo rural sostenible, José Lizardo Reyna Bowne informan de que:

Durante su periodo como doctorando del programa, desde enero de 2017 hasta la fecha de emitir este informe ha cumplido todas las actividades formativas y obligatorias del programa de doctorado con diligencia y dedicación. Entre estas actividades destacan dos estancias formativas de carácter internacional en la Universidad de Agricultura de Cracovia, de 3 meses de duración cada una de ellas, de Febrero a Mayo de 2019 y de Marzo a Mayo de 2020. El objetivo de estas estancias fue profundizar en su formación en determinación del contenido de carbono en suelos forestales. También destaca su participación presentando los primeros resultados de sus trabajos experimentales en la reunión anual de la European Geoscience Union (EGU) en abril de 2018 en Viena.

Estas estancias (cuyos primeros resultados se sintetizan en el Capítulo 3) han complementado su formación predoctoral bajo nuestra supervisión. Esta formación se ha articulado a través de una combinación de trabajos experimentales centrados en la determinación del contenido de carbono en suelo en zonas agrícolas en clima subtropical (Capítulo 2) y en dehesa en la provincia de Córdoba (Capítulos 4 y 5) con la medida de carbono mediante tecnología Vis_NIR (Capítulo 6).

Durante todo el desarrollo de su formación predoctoral, el candidato ha mostrado un elevado grado de dedicación, capacidad y habilidad para el trabajo en equipo. Todo ello, además de posibilitar la realización de los trabajos de investigación incluidos en su tesis doctoral, le han permitido alcanzar el grado de madurez y especialización necesarios para optar al grado de doctor.

Los resultados que se han ido generando a lo largo de la realización de esta tesis doctoral, se han expuesto y discutido en los foros y congresos científicos nacionales e internacionales que se detallan a continuación:

1. Congreso Latinoamericano de agronomía, Ecuador (27 abril, 2020). Una comunicación oral.

2. 2ª Convención científica en la Universidad Técnica de Manabí, Manabí, Ecuador (5 septiembre 2019). Una comunicación oral.
3. 8th International Conference for Young Researchers: Multidirectional Research in Agriculture, Forestry and Technology, Cracovia, Polonia (25 abril, 2019). Una comunicación en poster.
4. 58 Reunión Científica de la Sociedad Española para el Estudio de los Pastos, Sevilla, España (8 Abril, 2019). Dos comunicaciones orales.
5. European Geoscience Union (EGU) General Assembly 2018, Viena, Austria (11 abril, 2018). Dos comunicaciones en poster.
6. VII encuentro de estudiantes de doctorado, Universidad de Córdoba, Córdoba, España (18 octubre 2017). Una comunicación oral.
7. 1ª Convención científica, Universidad Técnica de Manabí, Manabí, Ecuador (18 octubre, 2017). Una comunicación oral.

y han dado lugar a las siguientes publicaciones:

1. Lizardo Reyna-Bowen , José A. Gómez, María T. Hidalgo, Ramón Leal, Jesús Fernández-Habas, Pilar Fernádez-Rebollo (2020). Stock of soil organic carbon and functional fractions in response to grazing intensities in permanent pasture of dehesa system. Geoderma (under review).
   Índice de impacto JCR: 4.336 (2018). Área: ciencia del suelo. Cuartil: Q1

2. Lizardo Reyna-Bowen, Pilar Fernádez-Rebollo, Jesús Fernández-Habas, José A. Gómez (2020). The influence of tree and soil management on soil organic carbon stock and pools in dehesa systems. Catena 190: 104511
   Índice de impacto JCR: 3.851 (año 2018). Área: Ciencia del suelo. Cuartil: Q1
   Link:https://www.sciencedirect.com/science/article/pii/S0341816220300606?dgcid=coauthor

3. Reyna-Bowen Lizardo, Lasota Jarosław, Vera-Montenegro Lenin, Vera-Montenegro Baly, Błońska, Ewa. (2019) Distribution and Factors Influencing Organic Carbon Stock in Mountain Soils in Babia Góra National Park, Poland. MDPI, Applied Science 9:3070
   Índice de impacto JCR: 2.217 (año 2018). Área: Multidisciplinar. Cuartil: Q3
   Link: https://www.mdpi.com/2076-3417/9/15/3070

4. Reyna-Bowen Lizardo, Vera-Montenegro Lenin, Reyna, Lizardo (2018). Soil-Organic-Carbon Concentration and Storage under Different Land Uses in the Carrizal-Chone Valley in Ecuador. MDPI, Applied Science 9: 45
   Índice de impacto JCR: 2.217 (año 2018): Área: Multidisciplinar. Cuartil: Q3
   Link: https://www.mdpi.com/2076-3417/9/1/45

Por todo ello, se autoriza la presentación de la tesis doctoral.

Córdoba, 15 de Mayo de 2020

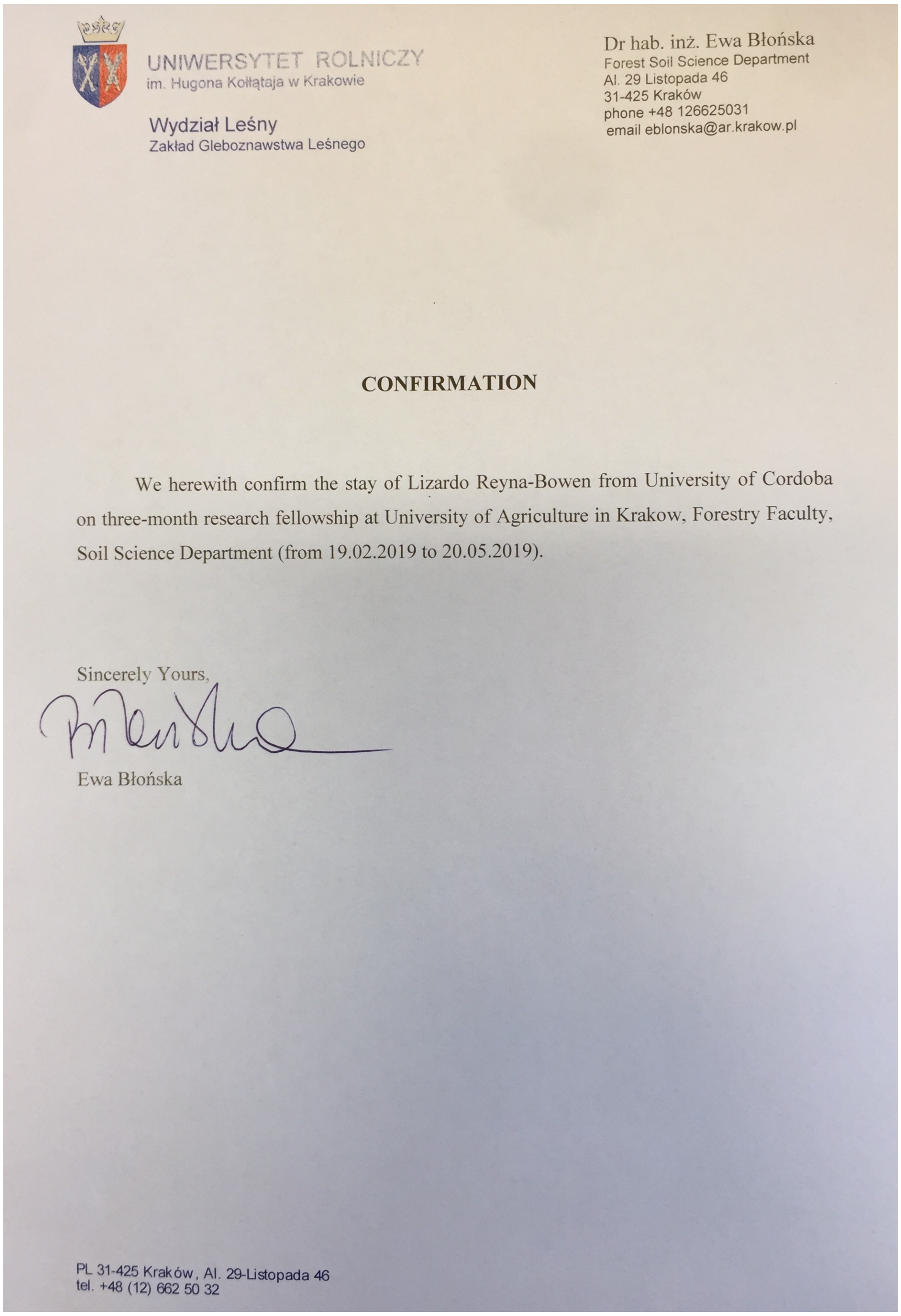
Firma del/de los director/es

Pilar

Fdo.:Pilar Fernández Rebollo
ETSIAM, UCO

Fdo.: José Alfonso Gómez Calero
IAS, CSIC

International Stay



UNIWERSYTET ROLNICZY
im. Hugona Kołłątaja w Krakowie

Wydział Leśny
Zakład Gleboznawstwa Leśnego

Dr hab. inż. Ewa Błońska
Forest Soil Science Department
Al. 29 Listopada 46
31-425 Kraków
phone +48 126625031
email eblonska@ar.krakow.pl

CONFIRMATION

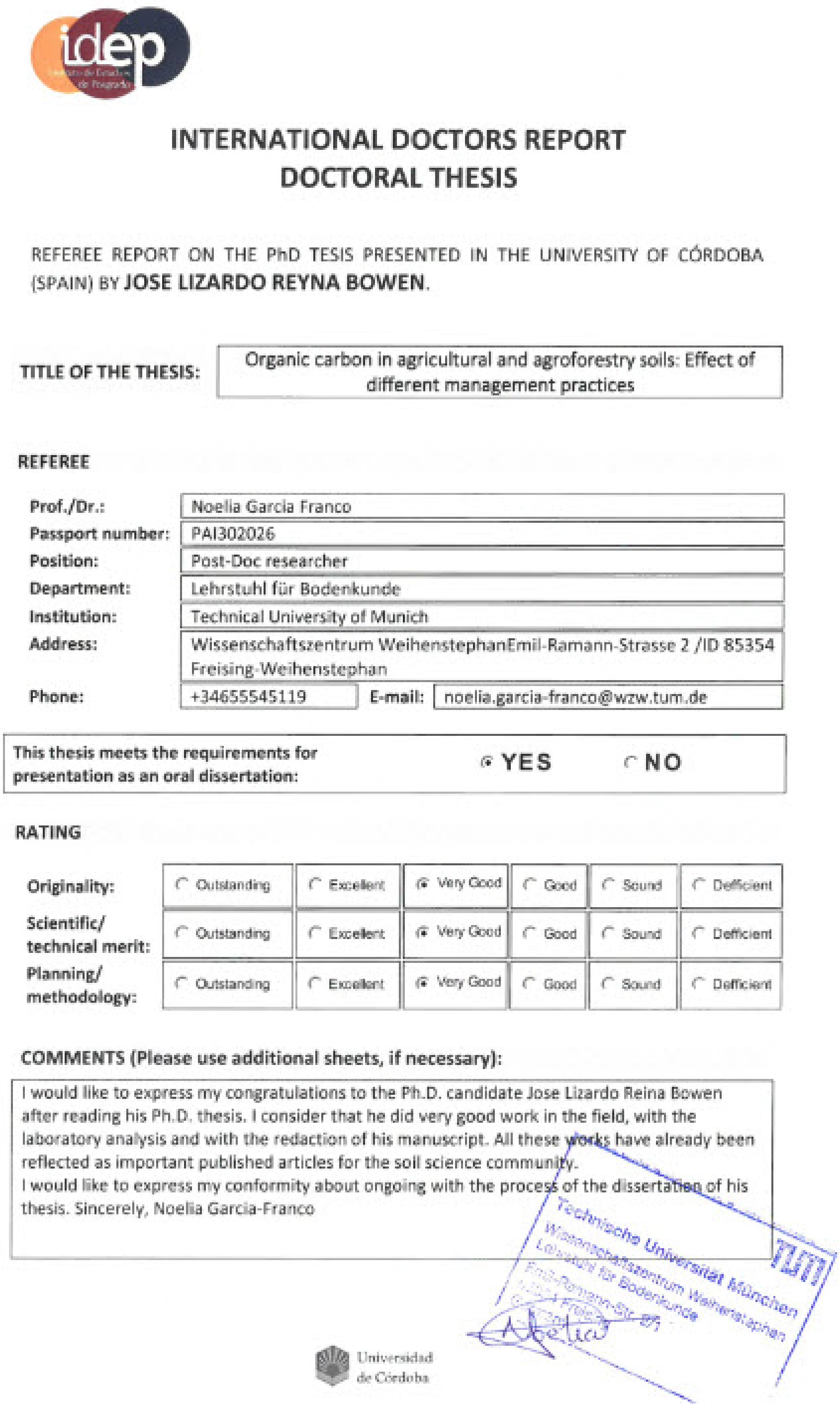
We herewith confirm the stay of Lizardo Reyna-Bowen from University of Cordoba on three-month research fellowship at University of Agriculture in Krakow, Forestry Faculty, Soil Science Department (from 19.02.2019 to 20.05.2019).

Sincerely Yours,

Ewa Błońska

PL 31-425 Kraków, Al. 29-Listopada 46
tel. +48 (12) 662 50 32

External Reviewers



INTERNATIONAL DOCTORS REPORT
DOCTORAL THESIS

REFEREE REPORT ON THE PhD TESIS PRESENTED IN THE UNIVERSITY OF CÓRDOBA (SPAIN) BY **JOSE LIZARDO REYNA BOWEN**.

TITLE OF THE THESIS: Organic carbon in agricultural and agroforestry soils: Effect of different management practices

REFEREE

Prof./Dr.: Noelia Garcia Franco
Passport number: PAI302026
Position: Post-Doc researcher
Department: Lehrstuhl für Bodenkunde
Institution: Technical University of Munich
Address: Wissenschaftszentrum WeihenstephanEmil-Ramann-Strasse 2 /ID 85354 Freising-Weihenstephan
Phone: +34655545119 **E-mail:** noelia.garcia-franco@wzw.tum.de

This thesis meets the requirements for presentation as an oral dissertation: ● **YES** ○ **NO**

RATING

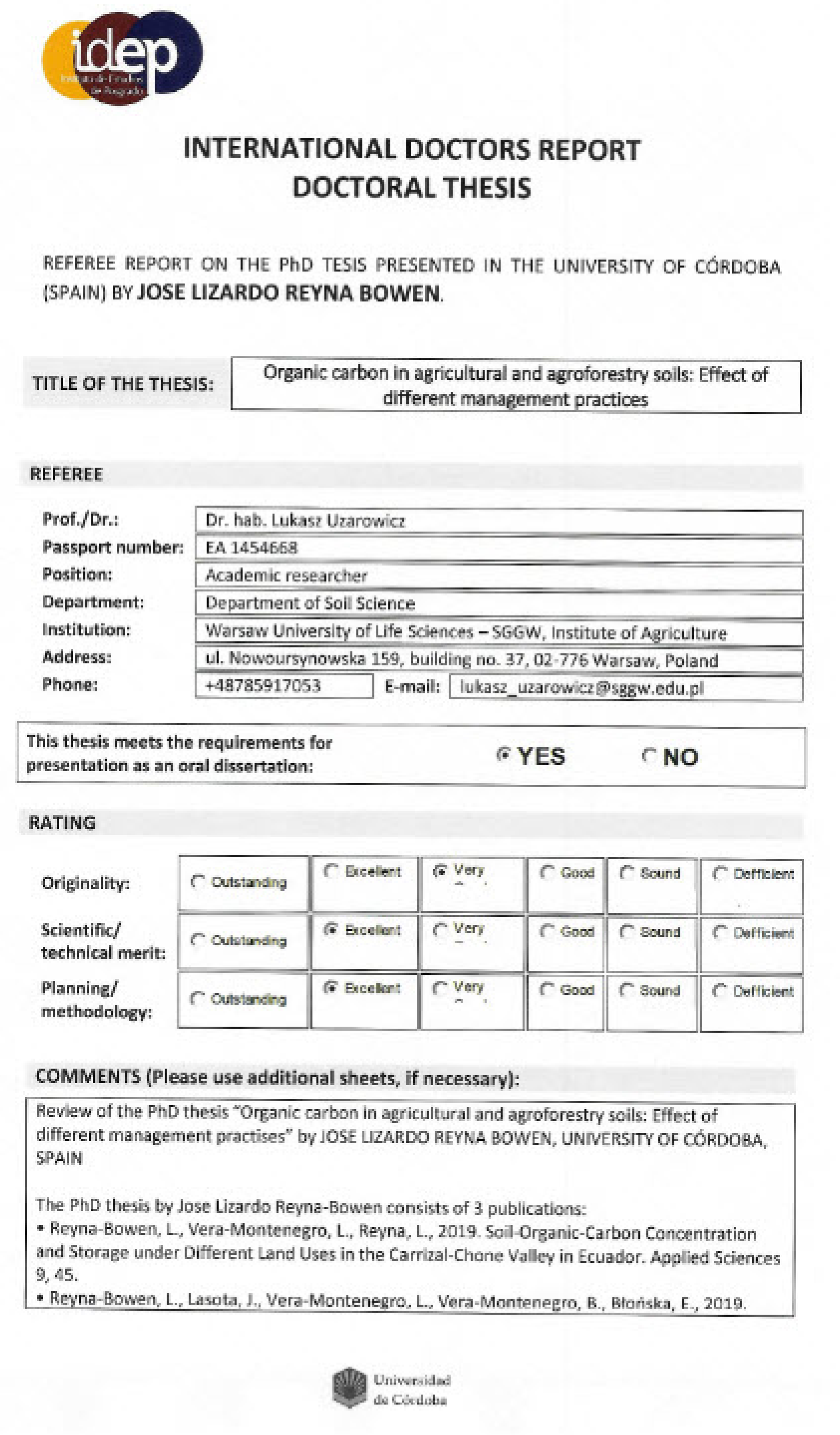
Originality:	○ Outstanding	○ Excellent	● Very Good	○ Good	○ Sound	○ Defficient
Scientific/ technical merit:	○ Outstanding	○ Excellent	● Very Good	○ Good	○ Sound	○ Defficient
Planning/ methodology:	○ Outstanding	○ Excellent	● Very Good	○ Good	○ Sound	○ Defficient

COMMENTS (Please use additional sheets, if necessary):

I would like to express my congratulations to the Ph.D. candidate Jose Lizardo Reina Bowen after reading his Ph.D. thesis. I consider that he did very good work in the field, with the laboratory analysis and with the redaction of his manuscript. All these works have already been reflected as important published articles for the soil science community.
I would like to express my conformity about ongoing with the process of the dissertation of his thesis. Sincerely, Noelia Garcia-Franco

Technische Universität München
Wissenschaftszentrum Weihenstephan
Lehrstuhl für Bodenkunde
Emil-Ramann-Str. 2

Universidad de Córdoba



INTERNATIONAL DOCTORS REPORT
DOCTORAL THESIS

REFEREE REPORT ON THE PhD TESIS PRESENTED IN THE UNIVERSITY OF CÓRDOBA (SPAIN) BY **JOSE LIZARDO REYNA BOWEN**.

TITLE OF THE THESIS: Organic carbon in agricultural and agroforestry soils: Effect of different management practices

REFEREE

Prof./Dr.: Dr. hab. Lukasz Uzarowicz
Passport number: EA 1454668
Position: Academic researcher
Department: Department of Soil Science
Institution: Warsaw University of Life Sciences – SGGW, Institute of Agriculture
Address: ul. Nowoursynowska 159, building no. 37, 02-776 Warsaw, Poland
Phone: +48785917053 **E-mail:** lukasz_uzarowicz@sggw.edu.pl

This thesis meets the requirements for presentation as an oral dissertation: ● **YES** ○ **NO**

RATING

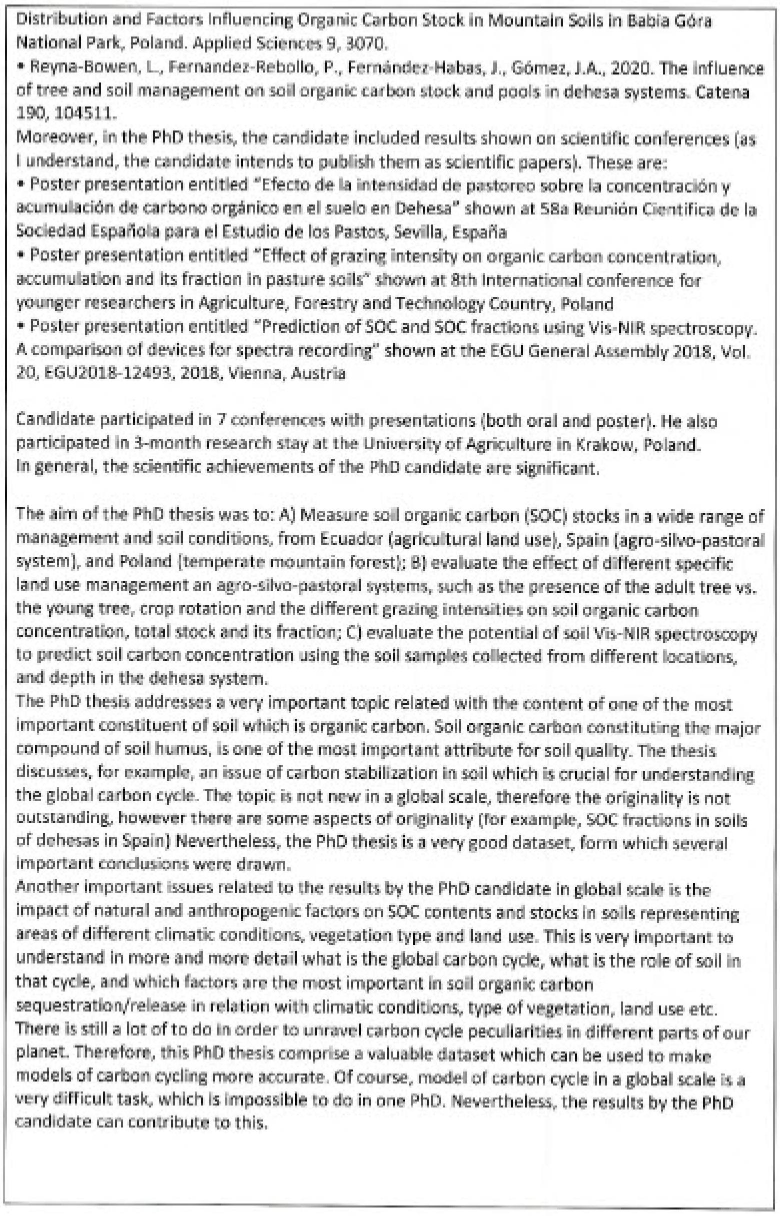
Originality:	○ Outstanding	○ Excellent	● Very	○ Good	○ Sound	○ Defficient
Scientific/ technical merit:	○ Outstanding	● Excellent	○ Very	○ Good	○ Sound	○ Defficient
Planning/ methodology:	○ Outstanding	● Excellent	○ Very	○ Good	○ Sound	○ Defficient

COMMENTS (Please use additional sheets, if necessary):

Review of the PhD thesis "Organic carbon in agricultural and agroforestry soils: Effect of different management practises" by JOSE LIZARDO REYNA BOWEN, UNIVERSITY OF CÓRDOBA, SPAIN

The PhD thesis by Jose Lizardo Reyna-Bowen consists of 3 publications:
- Reyna-Bowen, L., Vera-Montenegro, L., Reyna, L., 2019. Soil-Organic-Carbon Concentration and Storage under Different Land Uses in the Carrizal-Chone Valley in Ecuador. Applied Sciences 9, 45.
- Reyna-Bowen, L., Lasota, J., Vera-Montenegro, L., Vera-Montenegro, B., Błońska, E., 2019.

Universidad de Córdoba



Distribution and Factors Influencing Organic Carbon Stock in Mountain Soils in Babia Góra National Park, Poland. Applied Sciences 9, 3070.

- Reyna-Bowen, L., Fernandez-Rebollo, P., Fernández-Habas, J., Gómez, J.A., 2020. The influence of tree and soil management on soil organic carbon stock and pools in dehesa systems. Catena 190, 104511.

Moreover, in the PhD thesis, the candidate included results shown on scientific conferences (as I understand, the candidate intends to publish them as scientific papers). These are:

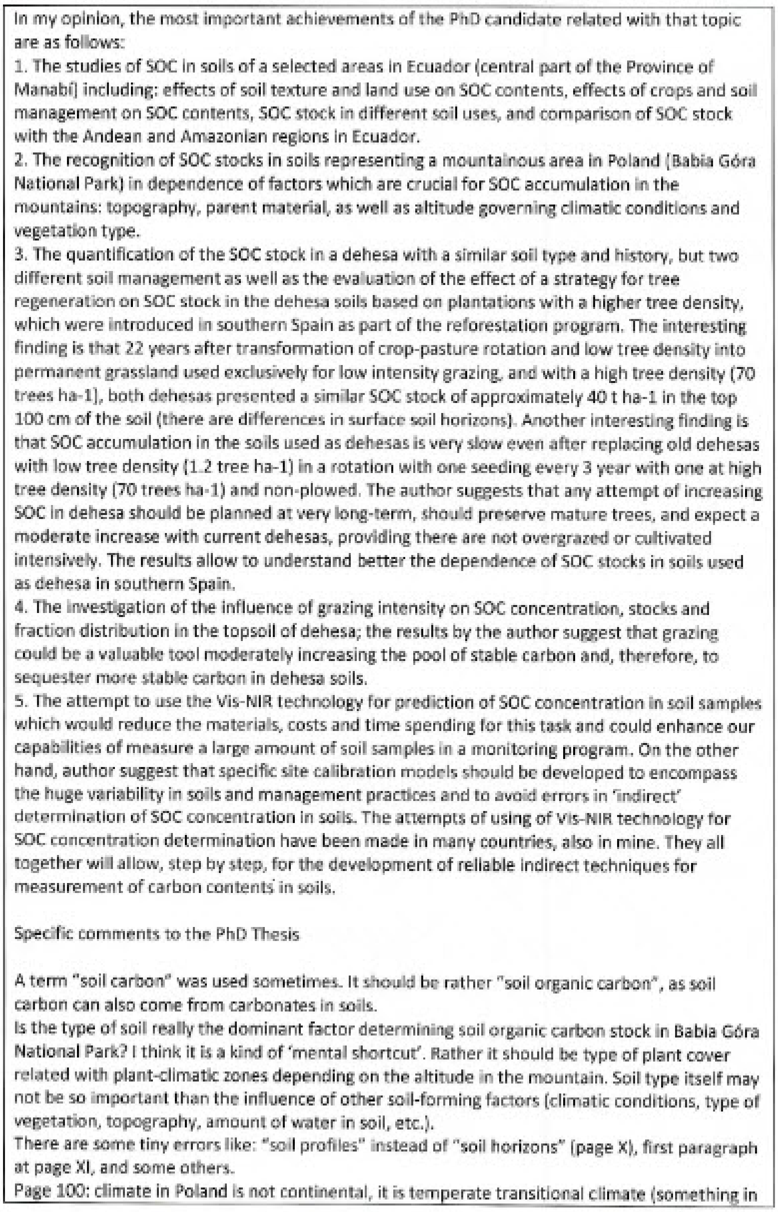
- Poster presentation entitled "Efecto de la intensidad de pastoreo sobre la concentración y acumulación de carbono orgánico en el suelo en Dehesa" shown at 58a Reunión Cientifica de la Sociedad Española para el Estudio de los Pastos, Sevilla, España
- Poster presentation entitled "Effect of grazing intensity on organic carbon concentration, accumulation and its fraction in pasture soils" shown at 8th International conference for younger researchers in Agriculture, Forestry and Technology Country, Poland
- Poster presentation entitled "Prediction of SOC and SOC fractions using Vis-NIR spectroscopy. A comparison of devices for spectra recording" shown at the EGU General Assembly 2018, Vol. 20, EGU2018-12493, 2018, Vienna, Austria

Candidate participated in 7 conferences with presentations (both oral and poster). He also participated in 3-month research stay at the University of Agriculture in Krakow, Poland.
In general, the scientific achievements of the PhD candidate are significant.

The aim of the PhD thesis was to: A) Measure soil organic carbon (SOC) stocks in a wide range of management and soil conditions, from Ecuador (agricultural land use), Spain (agro-silvo-pastoral system), and Poland (temperate mountain forest); B) evaluate the effect of different specific land use management an agro-silvo-pastoral systems, such as the presence of the adult tree vs. the young tree, crop rotation and the different grazing intensities on soil organic carbon concentration, total stock and its fraction; C) evaluate the potential of soil Vis-NIR spectroscopy to predict soil carbon concentration using the soil samples collected from different locations, and depth in the dehesa system.

The PhD thesis addresses a very important topic related with the content of one of the most important constituent of soil which is organic carbon. Soil organic carbon constituting the major compound of soil humus, is one of the most important attribute for soil quality. The thesis discusses, for example, an issue of carbon stabilization in soil which is crucial for understanding the global carbon cycle. The topic is not new in a global scale, therefore the originality is not outstanding, however there are some aspects of originality (for example, SOC fractions in soils of dehesas in Spain) Nevertheless, the PhD thesis is a very good dataset, form which several important conclusions were drawn.

Another important issues related to the results by the PhD candidate in global scale is the impact of natural and anthropogenic factors on SOC contents and stocks in soils representing areas of different climatic conditions, vegetation type and land use. This is very important to understand in more and more detail what is the global carbon cycle, what is the role of soil in that cycle, and which factors are the most important in soil organic carbon sequestration/release in relation with climatic conditions, type of vegetation, land use etc. There is still a lot of to do in order to unravel carbon cycle peculliarities in different parts of our planet. Therefore, this PhD thesis comprise a valuable dataset which can be used to make models of carbon cycling more accurate. Of course, model of carbon cycle in a global scale is a very difficult task, which is impossible to do in one PhD. Nevertheless, the results by the PhD candidate can contribute to this.

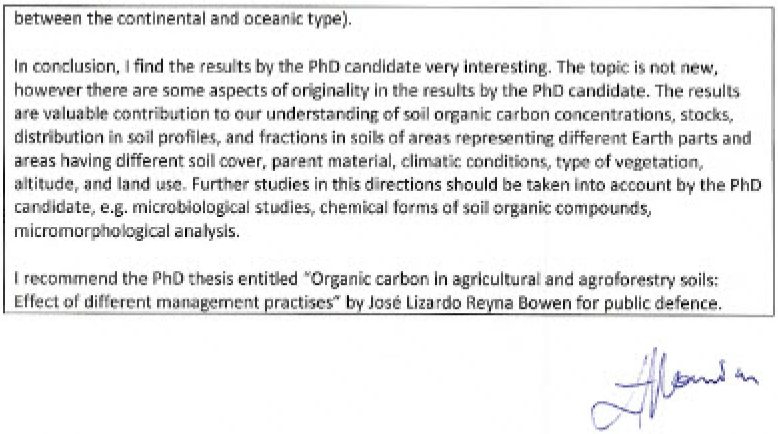


In my opinion, the most important achievements of the PhD candidate related with that topic are as follows:

1. The studies of SOC in soils of a selected areas in Ecuador (central part of the Province of Manabí) including: effects of soil texture and land use on SOC contents, effects of crops and soil management on SOC contents, SOC stock in different soil uses, and comparison of SOC stock with the Andean and Amazonian regions in Ecuador.
2. The recognition of SOC stocks in soils representing a mountainous area in Poland (Babia Góra National Park) in dependence of factors which are crucial for SOC accumulation in the mountains: topography, parent material, as well as altitude governing climatic conditions and vegetation type.
3. The quantification of the SOC stock in a dehesa with a similar soil type and history, but two different soil management as well as the evaluation of the effect of a strategy for tree regeneration on SOC stock in the dehesa soils based on plantations with a higher tree density, which were introduced in southern Spain as part of the reforestation program. The interesting finding is that 22 years after transformation of crop-pasture rotation and low tree density into permanent grassland used exclusively for low intensity grazing, and with a high tree density (70 trees ha-1], both dehesas presented a similar SOC stock of approximately 40 t ha-1 in the top 100 cm of the soil (there are differences in surface soil horizons). Another interesting finding is that SOC accumulation in the soils used as dehesas is very slow even after replacing old dehesas with low tree density (1.2 tree ha-1) in a rotation with one seeding every 3 year with one at high tree density (70 trees ha-1) and non-plowed. The author suggests that any attempt of increasing SOC in dehesa should be planned at very long-term, should preserve mature trees, and expect a moderate increase with current dehesas, providing there are not overgrazed or cultivated intensively. The results allow to understand better the dependence of SOC stocks in soils used as dehesa in southern Spain.
4. The investigation of the influence of grazing intensity on SOC concentration, stocks and fraction distribution in the topsoil of dehesa; the results by the author suggest that grazing could be a valuable tool moderately increasing the pool of stable carbon and, therefore, to sequester more stable carbon in dehesa soils.
5. The attempt to use the Vis-NIR technology for prediction of SOC concentration in soil samples which would reduce the materials, costs and time spending for this task and could enhance our capabilities of measure a large amount of soil samples in a monitoring program. On the other hand, author suggest that specific site calibration models should be developed to encompass the huge variability in soils and management practices and to avoid errors in 'indirect' determination of SOC concentration in soils. The attempts of using of Vis-NIR technology for SOC concentration determination have been made in many countries, also in mine. They all together will allow, step by step, for the development of reliable indirect techniques for measurement of carbon contents in soils.

Specific comments to the PhD Thesis

A term "soil carbon" was used sometimes. It should be rather "soil organic carbon", as soil carbon can also come from carbonates in soils.
Is the type of soil really the dominant factor determining soil organic carbon stock in Babia Góra National Park? I think it is a kind of 'mental shortcut'. Rather it should be type of plant cover related with plant-climatic zones depending on the altitude in the mountain. Soil type itself may not be so important than the influence of other soil-forming factors (climatic conditions, type of vegetation, topography, amount of water in soil, etc.).
There are some tiny errors like: "soil profiles" instead of "soil horizons" (page X), first paragraph at page XI, and some others.
Page 100: climate in Poland is not continental, it is temperate transitional climate (something in



between the continental and oceanic type).

In conclusion, I find the results by the PhD candidate very interesting. The topic is not new, however there are some aspects of originality in the results by the PhD candidate. The results are valuable contribution to our understanding of soil organic carbon concentrations, stocks, distribution in soil profiles, and fractions in soils of areas representing different Earth parts and areas having different soil cover, parent material, climatic conditions, type of vegetation, altitude, and land use. Further studies in this directions should be taken into account by the PhD candidate, e.g. microbiological studies, chemical forms of soil organic compounds, micromorphological analysis.

I recommend the PhD thesis entitled "Organic carbon in agricultural and agroforestry soils: Effect of different management practises" by José Lizardo Reyna Bowen for public defence.

Abstract

Soil is a global resource that has the capacity to contain large amounts of organic carbon. In fact, soils contain more carbon than plants and the atmosphere combined. However, in recent decades human activities such as land-use change, deforestation, biomass burning, and environmental pollution have accelerated the release of terrestrial carbon into the atmosphere, increasing the greenhouse effect. The study of soil organic carbon cycle was recognized in the last decades as a necessary step for controlling future increases in atmospheric CO_2, as well as necessary to simultaneously ensure the sustainability agricultural activities. A better comprehension of the he dynamics of soil organic carbon (SOC) in different agricultural systems will allow an improvement of soil quality and soil organic carbon storage under different climate and soil conditions. However, despite of decade's long research on this subject, there is still the need for a better appraisal of soil carbon dynamics in specific agricultural systems based on robust in field empirical studies. So, relevant contributions to a better understanding of the impact of land use on the global carbon cycle is of great importance.

The present research, framed in the context of a PhD specialization on soil carbon in agricultural areas, is aimed to generate new information on the effect of different factors (climate, land use, management, altitude, and soil type) that influence the sequestration and accumulation of organic carbon along the profile in the soil in different agricultural and forest systems across contrasting edaphoclimatic conditions. This research includes not only new quantitative information on soil organic carbon, but also innovative studies on its distribution among different soil carbon compartments and on the use of near infrared spectroscopy (NIR) on soil organic carbon determination.

The first study (Chapter 2) is an analysis of the effect of different agricultural uses in a subtropical climate, in the area of the Carrizal River valley in the province of Manabí Ecuador, based on the analysis of 64 soil profiles. In each profile samples were taken in the soil profile horizons to obtain the concentration of organic carbon up to a maximum depth of 150 cm in different agricultural management (permanent, intensive rotation and abandoned crops), In this study twenty-one different agricultural uses were identified. As expected, the highest concentrations of soil

organic carbon happened in the A horizon, which has an average thickness of 40 cm. A trend towards a higher carbon sequestration potential was observed in the grass, intercropping like cocoa with banana and corn area management with an average value of 1.7% C, much higher than the area under mechanized agriculture, which presented lower carbon concentration, with an average value of 0.26% C. Regarding the total soil organic carbon stock, the first horizon accumulated more carbon compared to the other (B and C) soil profiles, with an average value of 41.32±20.97 t C ha^{-1} and 15.06±15.61 t C ha^{-1}, respectively.

The second study (Chapter 3) evaluated the effect of forest management in a temperate climate. For this study, soil samples were taken in a managed environment of forest species (*Alnus incana, Fagus sylvatica, Picea abies* and Mixed: stands containing *beech* and *spruce*) in an elevation range from <900 m a.s.l. to >1100 m a.s.l. from the Babia Góra National Park in southern Poland. Sampling points were taken up to a maximum depth of 100 cm. The results in this study revealed that the SOC reserves in the mountain soils of the Babi Góra National Park are characterized by their great variability (from 50.10 t ha^{-1} to 905.20 t ha^{-1}). In the conditions of this study, the type of soil is the dominant factor determining soil organic carbon stock, which coupled with topographic factors influence soil and vegetation conditions. This explains such diversity in the accumulation of soil organic carbon in different mountain soils in the areas. The largest carbon stock was recorded in histosols (>550 t C ha^{-1}), which are located in the lower part of the national park.

The third block of the research focused on two field studies in one of the most important agroforestry systems across the Mediterranean, dehesa. The first study (Chapter 4) is located in a dehesa in Hinojosa del Duque in Córdoba, Spain: Dehesa is an agro-silvo-pastoral system which combines open land and low density trees (holm oaks). In this first study we investigated two adjacent dehesas on the same soil type but different characteristics. One was a pastureland with young holm oaks (planted in 1995 with a density of 70 trees ha^{-1} at 12 m x 12 m spacing. The area had been grazed by Merino sheep since 2000, at a grazing rate of 3 sheep per hectare. The second, adjacent area is a cultivated pasture with mature oaks with a minimum age of 90-100 years widely spaced (1.2 trees ha^{-1}). Every three years, a mixture of peas and oats is grown for hay. Tillage is used for the preparation of this seeding except in the immediate vicinity (about 0.3-0.4 m) of the tree trunk. The first dehesa at higher tree density was part of this second dehesa, and so both had the same characteristics until year 1995. Both dehesas were sampled simultaneously in 2017. Sampling points were taken under and outside the canopy projection up to a maximum depth of 100 cm divided into 8 sections (0-2 cm, 2-5 cm, 5-10 cm, 10-20 cm, 20-40 cm, 40-60 cm, 60-80 cm, and 80- 100 cm). The results showed that a change in dehesa type from an old low density dehesa combining pasture with seeding every 3 years to a one only pastured with increased tree growth (70 trees ha), showed no significant differences in carbon concentration after 22 years' sicen implanting the more dense dehesa. A clear stratification of carbon was observed in the soil profile, particularly in the top 10 cm of the soil, as well as an effect of the adult tree which resulted in a higher concentration under the tree canopy in the middle soil depth section (20-40 cm) in the mature dehesa. Significant difference in carbon stock was only observed in the top 0-2 cm (5.86±0.56 t ha1 vs 3.24±0.37 t ha1, been higher in the newly planted dehesa. To our knowledge this is the first study evaluating in dehesa the distribution of soil organic carbon into this four (unprotected and physically, chemically and biochemically protected) fractions. Our results showed how most of the carbon in the two dehesas was stored in the unprotected fraction, been its relative contribution higher in the top 0-2 cm o the pastured dehesa and in the below canopy area of the mature trees in the cropped dehesa. This indicates that much of the fraction contained in these soils is particularly vulnerable to hypothetical changes to less sustainable managements.

The second study in dehesa (Chapter 5) was located in the municipality of Pozoblanco in the north of the province of Cordoba. In this area three areas of continuous extensive grazing for more than 50 years with cattle, sheep, and pigs were identified, and three areas with different intensity were studied. These areas were: I) Intensive grazing management. II) moderate grazing management and III) no grazing (area excluded for more than 20 years). Sampling points were taken at each of the three areas up to a maximum depth of 30 cm divided into 5 sections (0-2 cm, 2-5 cm, 5-10 cm, 10-20 cm, and 20-30 cm). Concentrations at different grazing intensities showed, as expected, higher carbon concentrations at the surface soil layer (0-2 cm) average of 1.59±0.44%, decreasing to 0.48±0.15% in the deeper section of the soil profile at 20-30 cm. Contradicting our initial hypothesis, no differences in soil organic carbon concentration were detected among the three areas with different grazing intensities, The total carbon stock was analyzed in the whole soil profile (0-30 cm), indicating non significant differences among the two grazed areas, average value of 27 t ha^{-1}, or the area without grazing 26 t ha^{-1}. As in the previous dehesa, the dominant fraction was the unprotected carbon. However, in this case the relative differences in the soil organic carbon concentration between the unprotected fraction and the physically and the chemically protected fractions was larger than in the first dehesa, particularly because the protected fractions tended to show a higher concentration than in the dehesa studied in Chapter 4.

Using the empirical results from the study of the second dehesa, we developed a spectral library and predictive equations of concentration of soil organic carbon using Vis-NIR (Chapter 6) from this dataset. The accuracy of the SOC predictive models was very good, with R^2 higher than 0.95 and residual predictive deviation (RPD) higher than 4.54, respectively. Refinement of VIS-NIR techniques, such as the one discussed in Chapter 6, could increase our ability to provide more affordable and robust technologies to measure large numbers of samples with the required accuracy, although it is less clear how to address other important sources of variability, such as soil depth, soil type, bulk density, and rock content. To reduce this uncertainty will be of great relevance to continue performing detailed experiments to better quantify on the effect of land use and cropping systems on soil organic carbon content, such as those described in chapters 3, 5 and 5. To date, these experiments are irreplaceable to test specific hypothesis relevant at local level (like the time to increase soil organic carbon stock after planting at higher density, Chapter 4), but also to create a corpus of available data which could improve, or lead to new ones, conceptual or numerical simulation models that can systematize our understanding of the soil organic carbon cycle and eventually reduce the need for large-scale sampling to verify the evolution of soil organic carbon in agricultural systems.

Resumen

El suelo es un recurso mundial que tiene la capacidad de contener grandes cantidades de carbono orgánico. De hecho, los suelos contienen más carbono que las plantas y la atmósfera juntas. Sin embargo, en los últimos decenios, las actividades humanas, como el cambio de uso de la tierra, la deforestación, la quema de biomasa y la contaminación ambiental, han acelerado la liberación de carbono terrestre en la atmósfera, aumentando el efecto invernadero. El estudio del ciclo del carbono orgánico del suelo ha sido reconocido en las últimas décadas como un paso necesario para controlar los futuros incrementos del CO_2 atmosférico, y también para asegurar la sostenibilidad de la producción agrícola. Una mejor comprensión de la dinámica del carbono orgánico del suelo (SOC) en los diferentes sistemas agrícolas permitirá mejorar la calidad del suelo y el almacenamiento de carbono orgánico del suelo en diferentes condiciones edafoclimáticas. Sin embargo, a pesar de décadas de investigaciones sobre este asunto, sigue siendo necesaria una mejor evaluación de la din[́a]mica del carbono del suelo en sistemas agrícolas específicos, basada en estudios empíricos de campo sólidos. Una mejor comprensión del impacto del uso de la tierra en el ciclo mundial del carbono es de gran relevancia.

La presente investigación, enmarcada en el contexto de una especilización de doctorado sobre el carbono del suelo en las zonas agrícolas, tiene por objeto generar nueva información sobre el efecto de los diferentes factores (clima, uso de la tierra, ordenación, altitud y tipo de suelo) que influyen en el secuestro y la acumulación de carbono orgánico a lo largo del perfil en el suelo en diferentes sistemas agrícolas y forestales condiciones edafoclimáticas muy diversas. Esta investigación incluye no sólo nueva información cuantitativa sobre el carbono orgánico del suelo, sino también estudios innovadores sobre su distribución entre diferentes compartimentos y sobre el uso de la espectroscopia del infrarrojo cercano (NIR) en la determinación del carbono orgánico del suelo.

El primer estudio (Capítulo 2) es un análisis del efecto de los diferentes usos agrícolas en un clima subtropical, en la zona del valle del río Carrizal en la provincia de Manabí, Ecuador basado en el análisis de 64 perfiles de suelo. En cada perfil se tomaron muestras en los horizontes de los perfiles de suelo para obtener la concentración de carbono orgánico hasta una profundidad máxima de 150 cm en diferentes manejos

agrícolas (permanentes, rotación intensiva y cultivos abandonados),. En esta zona se identificaron veintiún usos agrícolas diferentes. Como era de esperar, las mayores concentraciones de carbono orgánico en el suelo se produjeron en el horizonte A, que tiene un espesor medio de 40 cm. Se observó una tendencia hacia un mayor potencial de secuestro de carbono en zonas pastos, cultivo intercalado como cacao con plátano y maíz con un valor promedio de 1.7% C, mucho mayor que las zons de agricultura mecanizada que presentó una menor concentración de carbono con un valor promedio de 0. 26% C. El contenido total de carbono, el primer horizonte (A) fue mucho mayor en comparación con los otros perfiles de suelo (B y C), con un valor medio de 41,32±20,97 t C ha^{1} y 15,06±15,61 t C ha^{1}, respectivamente.

El segundo estudio (Capítulo 3) evaluó el efecto de la ordenación forestal en un clima templado. Para ello, se tomaron muestras de suelo en un entorno de gestión de especies forestales (*Alnus incana, Fagus sylvatica, Picea abies*, y Mixto: rodales que contienen *hayas* y *abetos*) en un rango de elevación de <900 m s.n.m. a >1100 m s.n.m.. del Parque Nacional de Babia Góra en el sur de Polonia. El suelo se muestreó hasta una profundidad máxima de 100 cm. Los resultados de este estudio en Polonia revelaron que las reservas SOC en los suelos de montaña del Parque Nacional de Babi Góra se caracterizan por su gran variabilidad (de 50,10 t ha^{1} a 905,20 t ha^{1}). En las condiciones de este estudio, el tipo de suelo es fue el factor dominante que determina el contenido total de carbono orgánico del suelo, que junto con los factores topográficos determina las condiciones del suelo y la vegetación. Esto explica tal diversidad en la acumulación de carbono orgánico del suelo en diferentes suelos de montaña en las zonas La mayor reserva de carbono se registró en los histosoles (>550 t C ha^{1}), que están situados en la parte baja del parque nacional.

El tercer bloque de la investigación se centró en dos estudios de campo en uno de los sistemas agroforestales más importantes del Mediterráneo, la dehesa. El primer estudio (Capítulo 4), se investigó una dehesa en Hinojosa del Duque en Córdoba, España: La dehesa es un sistema agro-silvo-pastoril que combina zona de cultivo y/o pastoreo con árboles a baja densidad (encinas). En este estudio localizamos dos dehesas adyacentes en el mismo tipo de suelo pero de características diferentes. Una era una dehesa con encinas jóvenes (plantadas en 1995 con una densidad de 70 árboles ha^{1} a 12 m x 12 m de distancia. La zona había sido pastoreada por ovejas merinas desde el año 2000, a una tasa de pastoreo de 3 ovejas por hectárea. La segunda zona, adyacente a la primera, es un pastizal cultivado con robles maduros con una edad mínima de 90-100 años ampliamente espaciados (1,2 árboles ha^{1}). Cada tres años se cultiva una mezcla de guisantes y avena para el heno. La parcela se labra para la preparación del terreno para siembra excepto el suelo en las inmediaciones (alrededor de 0,3-0,4 m) del tronco del árbol. La primera dehesa con mayor densidad de árboles formaba parte de esta segunda dehesa, por lo que ambas tuvieron las mismas características hasta el año 1995. Ambas dehesas fueron muestreadas simultáneamente en 2017. Los puntos de muestreo se tomaron bajo y fuera del dosel vegetal hasta una profundidad máxima de 100 cm divididos en 8 secciones (0-2 cm, 2-5 cm, 5-10 cm, 10-20 cm, 20-40 cm, 40-60 cm, 60-80 cm y 80- 100 cm). Los resultados mostraron que un cambio en el tipo de dehesa de una antigua dehesa de baja densidad que combinaba el pastoreo con la siembra cada 3 años a una dehesa única con un mayor crecimiento de los árboles (70 árboles ha), no resultó en diferencias significativas en la concentración de carbono después de 22 años de pecado implantando la dehesa más densa. Se observó una clara estratificación del carbono en el perfil del suelo, en particular en los 10 cm superiores del suelo, así como un efecto del árbol adulto que dio lugar a una mayor concentración de carbono bajo el dosel de los árboles en la profundidad intermedia (20-40 cm) en la dehesa madura. Sólo se observó una diferencia significativa en la reserva de carbono en los 0-2 cm superiores (5,86±0,56 t ha^{1} vs 3,24±0,37 t ha^{1}, siendo mayor en la dehesa recién plantada. Hasta donde sabemos, este es el primer estudio que ha evaluado en dehesa la distribución del carbono orgánico del suelo estas cuatro fracciones (desprotegida, física, química y bioquímicamente protegidas). Nuestros resultados mostraron cómo la mayor parte del carbono en las dos dehesas se almacenaba en la fracción no protegida, siendo su relevancia relativa particularmente alta en la profundidad superior de 0-2 cm de la dehesa sólo pastoreada y en la zona de la copa de los árboles maduros en la dehesa cultivada. Esto indica que gran parte de la fracción contenida en estos suelos es particularmente vulnerable a hipotéticos futuros cambios en los manejos menos sostenibles.

El segundo estudio en dehesa (Capítulo 5) se efectuó en el municipio de Pozoblanco, al norte de la provincia de Córdoba. En esta zona se identificón una dehesa que de manera continuada se ha pastoreada desde hace más de 50 años de manera extensiva extensivo con ganado vacuno, ovino y porcino. En la misma se delimitaron tres zonas con diferente densidad de pastoreo. Estas zonas fueron:. I) Manejo de pastos intensivos. II) Manejo moderado del pastoreo y III) no pastoreo (área excluida durante más de 20 años). Se tomaron puntos de muestreo en cada zona hasta una profundidad máxima de 30 cm divididos en 5 secciones (0-2 cm, 2-5 cm, 5-10 cm, 10-20 cm y 20-30 cm). Los resultados mostraron, como era de esperar, mayores concentraciones de carbono en la superficie (0-2 cm) 1,59±0,44% disminuyendo a 0,48±0,15% en la última sección del perfil del suelo a 20-30 cm. Contra nuestra hipótesis de partida no se detectaron diferencias en concentración de carbono en el suelo entre las tres zonas. Se analizó la cantidad total de carbono en todo el perfil del suelo (0-30 cm), indicando diferencias no significativas entre las dos áreas de pastoreo, valor promedio de 27 t ha^{1}, o el área sin pastoreo 26 t ha^{1}. Al igual que en la dehesa estudiada en el Capítulo 4, la fracción dominante fue el carbono no protegido. Sin embargo, en este caso las diferencias relativas en la concentración de carbono orgánico del suelo entre la fracción no protegida y las fracciones física y químicamente protegidas fué mayor que en la primera dehesa, particularmente debido a que las fracciones protegidas tendían a mostrar una mayor concentración de carbono orgánico que en la dehesa estudida anteriormente en el Capítulo 4.

Utilizando los resultados experimentales de este último estudio„ desarrollamos una biblioteca espectral y para desarrollar ecuaciones predictivas de concentración de carbono orgánico utilizando Vis-NIR (Capítulo 6) para este set de datos. La precisión de los modelos SOC fue muy buena, con R^2 mayor de 0.95 y la desviación predictiva residual (RPD) superior a 4,54. El perfeccionamiento de las técnicas Vis-NIR, como la que se analiza en el Capítulo 6, podría aumentar nuestra capacidad de proporcionar tecnologías más asequibles y robustas para medir un gran número de muestras con la precisión necesaria, aunque no resulta claro cómo abordar otras fuentes importantes de variabilidad, como son la profundidad del perfil y el tipo de suelo, la densidad aparente y el contenido de material grueso superior a 2mm. Para reducir esta incertidumbre será de gran relevancia continuar realizando experimentos bien diseñados para cuantificar mejor el efecto del uso de la tierra y los sistemas de cultivo en el contenido de carbono orgánico del suelo, como los descritos en los capítulos 3, 4 y 5. Estos experimentos son irreemplazables para validar hipótesis relevantes a nivel local (como el momento de aumentar las reservas de carbono orgánico del suelo después de la plantacién a una mayor densidad, Capítulo 4), pero también para crear un corpus de información disponible que podría mejorar, o conducir a nuevos, modelos de simulación conceptual o numérica que pueden sistematizar nuestra comprensión del ciclo del carbono orgónico del suelo y eventualmente reducir la necesidad de muestreo a gran escala para verificar la evolución del carbono orgánico del suelo en los sistemas agrícolas.

Contents

List of Figures

List of Tables

Chapter 1

Introduction

Soil is a global resource that has the capacity to contain large amounts of organic carbon in the biosphere, containing more carbon than plants and the atmosphere combined [Leavitt, 1998, Lal, 2004]. In the 1990s, carbon reservoirs regulating the carbon (C) cycle were quantified to have approximately 750 Pg C in the atmosphere, and 2,200 Pg C in terrestrial ecosystems [Hobbs, 1996]. In 2006, the carbon pool may have decreased to 2,157 Pg C in terrestrial systems [Zimov, 2006]. Furthermore, in the last decades, human activities such as land-use change, deforestation, biomass burning, and environmental pollution released terrestrial carbon into the atmosphere, increasing the greenhouse effect [Hobbs, 1996, Nie et al., 2013]. Many in the scientific community find these rapid changes to be alarming. In an effort to regulate the global carbon cycle, scientists have proposed controlling land use to capture more atmospheric carbon and store it in soil. Soil carbon is influenced not only by human activities, but by other life terrestrial life forms, and in turn, the carbon cycle. The carbon stored in soil originates mainly from the decomposition of plant and animal biomass, root-exudatesm, and dead microbial biomass. In fact, labile organic carbon is the primary and main source of energy for microorganisms [Heimann and Reichstein, 2008, Bongiorno et al., 2019]. Considering the complexity of terrestrial systems, the carbon dynamics in the world's soils are still poorly understood [Batjes, 2014].

Soil organic carbon (SOC) dynamics allow us to explain the relationship between climate change and soil quality. Understanding the impact of land use on the global carbon cycle is of great importance. The study of soil organic carbon is emerging as a valuable tool to control future increases in atmospheric CO2 while ensuring agricultural sustainability and security [Jenny, 1980, Lal, 2004]. The abundance of carbon in soil depends on many factors such as geology, vegetation, organic compounds, and climate [Batjes, 2014]. In this context, the climate is considered to be the main factor driving storage of SOC [Cameron et al., 2013]. Warm temperatures and high precipitation rates are associated with high organic carbon content due to increased biomass production [Cameron et al., 2013]. The largest accumulations of SOC at world level are distributed around the equator line, between latitudes 25 degrees north and south. The climatic conditions allow tropical soils to contain a quarter of the world's storing a total of 471 Pg of organic carbon reserves [Pan et al., 2011, Köchy et al., 2015, Huang et al., 2018, Vitharana et al., 2019]. Soil carbon in the tropics may be very labile, since rapid biomass production may keep most of the carbon from reaching below the superficial layers.

Tropical systems contribute much of the Earth's biological diversity [Myers, 2000]. However, tropical systems are being disrupted by large-scale land use changes [Laurance, 2007]. Tropical systems will face even greater pressures in the future, especially from the expansion of agriculture [Gibbs et al., 2010]. In addition, agriculture is expanding faster in the last 50 years to cover the food demand [Grassini et al., 2013]. Countries with tropical climates as Venezuela, Colombia, Peru, and Ecuador, in particular, have the highest annual deforestation rates in South America [FAO, 2015]. According to Bahr et al. [2014], farmers use fire to convert primary forest to land for agriculture and pasture. Up to 40% of the economy in Ecuador is concentrated in agriculture for national use or exportation [Quintana et al., 2019]. Besides natural biodiversity, there is a great variety of agricultural crops in this tropical climate. Agriculture in the coastal area of Ecuador can be quite diverse with up to 21 different crop managements. The main crops include permanent crops (*Theobroma cacao* L, *Coffea sp*) and rotation crops (*Zea mays, Arachis hypogaea, Phaseolus vulgaris, Citrullus lanatus, Cucumis melo*) [Reyna-Bowen et al., 2018]. The concentration of organic carbon in the agricultural soils of the coastal region fluctuates between 0.60% and 1.85%. [Barrezueta-Unda and Paz-González, 2017, Reyna-Bowen et al., 2018]. On the other hand, it can be seen in other types of climate and regions in the world as an example on the European continent how climatic conditions affect the sequestration of organic carbon in the soil.

Soil carbon dynamics are quite different in other climates. In Europe, we must consider less biomass production and biodiversity, as well as, greater atmospheric carbon emissions. For example, soils store approximately 1.5 more carbon than trees in European forests. It has been estimated that the biosphere absorbs about 10% of the total European atmospheric carbon emissions [Cameron et al., 2013]. The importance of the forest soil on carbon storage is expected to increase in the future. In this context, Jones et al. [2005] found a variation in organic carbon concentration from 0.01% to more than 35% around Europe. The boreal region contains higher concentrations of organic carbon compared to temperate and Mediterranean zones. In Europe's temperate zones, soil carbon may vary greatly depending on climate, precipitation, and forest species. This was apparent in central Poland, where values of organic carbon concentration varied from 1.15±0.29% in Betula pendula and 1.29±0.20% in *Pinus sylvestris*, to 1.95±0.43% in *Robinia pseudoacacia* [Rawlik et al., 2019]. Despite the mild climate and adequacy for agriculture in the Mediterranean region, Europe's lowest concentrations of organic carbon are found (0.1% to 2%) here, and it has been confirmed in other works [Cameron et al., 2013](Figure 1.1).

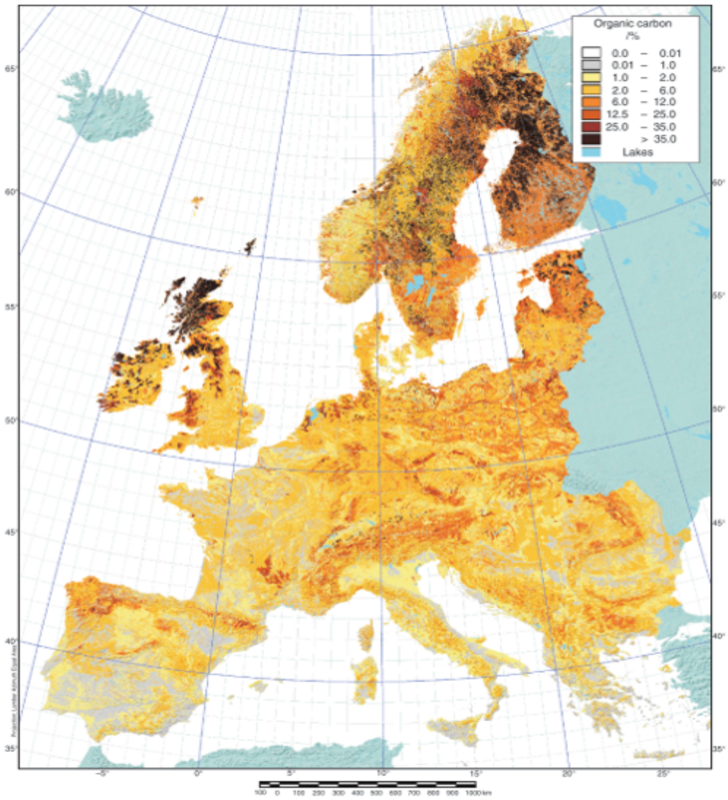


Fig. 1.1: Organic carbon content (%) in the surface horizon of soils in Europe [Jones et al., 2005].

In Mediterranean regions such as southwestern Spain, Portugal and Sardinia in Italy, agro-silvo-pastoral systems dominated by evergreen oaks (*Quercus spp. Quercus ilex* L. or *Quercus suber* L.) with traditional multipurpose management systems are widespread and have attracted much attention in recent years [Cappai et al., 2017]. These systems, which combine trees with crops, pasture or shrubs, have extensive livestock rearing (sheep, Iberian pig, cows) as their main economic activity [Hunt, 2002, Moreno and Pulido, 2009, Cappai et al., 2017]. These traditional agro-silvo-pastoral systems in southwestern Europe in Spain are known as "dehesas" and are included in the Habitats Directive of the European Union for their environmental value [Campos et al., 2013]. Dehesas cover around 4 million ha of land in the southwestern provinces of Spain, with 1,237,000 ha in Extremadura, 946,482 ha in Andalucía, 751,544 ha in Castilla- La Mancha, and 467,759 ha in Castilla y León [Andalucia, 2017].

From a landscape point of view, the Spanish dehesa has a similar vegetation structure than savannah [Joffre et al., 1988]. In addition, it is of great ecological and socio-economic importance, providing various products and services including carbon sequestration. Dehesas sequester much of the organic carbon in the soil. However, the average organic carbon concentration in Spain is less than 1%. [Martín et al., 2016]. Furthermore, the dehesa has a potential for organic carbon soil sequestration by the formation of plant biomass, which is estimated to be between 0.29 and 15.21 Mg ha^1 per year [Nair, 20008]. However, the ability to maintain and accumulate carbon can be modified by management practices associated with woody crops and grazing, as well as other factors [Campos et al., 2013]. As occur in other agricultural systems, woody plantations have a strong potential for sequestering organic carbon in a dehesa. The tree (oak in this case) has an important influence on the horizontal and vertical distribution of carbon content in soil, the extent of the roots affects the organic carbon content below the tree and outside the tree crown [Simón et al., 2012]. In Southern Spain, The highest soil organic carbon stock in soils from dehesas (42.3 t ha^1) is found in the vicinity of the trees, and it is being almost twice that of tree-less grasslands (23.9 t ha^1) [Pulido-Fernández et al., 2013]. On the other hand, grazing has a great potential to sequester CO2 from the atmosphere as stable organic carbon in the soil influenced by various factors such as grazing intensity, climate, and animal type [Pulido et al., 2017, Abdalla et al., 2018].

In this context, Ferreiro-Domínguez et al. [2016], suggests that the presence of animal plays an important role in the sequestration of organic carbon in the soil by direct or indirect modification of pH, bulk density, and soil fractions, mentioning that the highest concentrations were found in moderate grazing areas, while high grazing intensities or no grazing did not have the same effect on accumulation in tons per hectare up to 1 m deep. Abdalla et al. [2018], stated that the impact of extensive grazing on soil organic carbon storage depend on the grazing intensity and climate. Also, they noted that in areas with warm, moist climate grazed at different intensities (high, moderate and low) increased the level of organic carbon storage (+7%). Ferreiro-Domínguez et al. [2016] measured soil organic carbon storage in an area of Spain with an Atlantic climate (warm and moist) grazed by mature sheep and found that grazing with a light stocking rate (moderate with 4 and 8 sheep ha^{-1}) increased carbon storage in the top 1 m of soil depth when compared ungrazed land (abandoned). Likewise, grazing also has an effect on carbon concentration and its fractionation.

Although the quantification of total soil organic carbon allows us to compare different regions and evaluate the current status of the carbon balance, we may get more detailed information by evaluating the subdivisions of soil carbon. These organic fractions of soil carbon help describe and evaluate the processes of carbon stabilization and decomposition and the effects of land use management. Several classification systems exist, and one of the most widely used is that proposed by Six et al. [2002], which provides insight into the dynamics of carbon, including four main mechanisms of soil organic matter (SOM) stabilization: (1) unprotected, (2) physical protection, (3) chemical protection and (4) biochemical protection.

The unprotected carbon is associated to the fresh vegetation residual and it can be found on the soil surface. However, the unprotected carbon can also be found in deeper soil layers. The contents of particulate organic matter (POM particles with size $>250\ \mu m$) or light fraction (LF particles with size $<250\ \mu m$) have made it possible to detect early effects of management practices [Galantini, 2006]. Physically protected (FP) carbon is present within soil microaggregates (particles with size $53 - 250\ \mu m$). FP carbon in soil microaggregates is also sensitive to changes in land use given that land use can affect soil structure and nutrient availability for vegetation [Berhongaray, 2012]. Chemical protected carbon is associated to silt and clay soil particles (particles with size $>53\ \mu m$). These particles protect organic carbon from decomposition by soil microorganisms. It should be taken into account that there is a relationship between physically protected carbon and chemically protected carbon, because the content of clay and silt can modify the structure of the soil and in turn the content of FP carbon [Feller and Beare, 1997]. Continuing on the explanation, the biochemical protected carbon depends on the organic material of the origin either from vegetation or animal. The biochemical carbon will be modified according to the biochemical, physico-chemical characteristics of the plants of origin. Likewise, if the inputs are of animal origin, it will depend on the type of livestock or wildlife there are on the land (Sheep, pig, cow) [Six et al., 2002]. There can be different mechanisms of stabilization and these mechanisms are related to environmental conditions which makes a greater unawareness of how they affect soil organic carbon. The process to obtain the physical, chemical and biochemical separations is a more arduous work. In addition, the analysis of the organic carbon content in the soil, involves a very elaborate work in a traditional soil laboratory. The high economic cost of the process of separating the fractions would also be added to this process. However, there is another alternative that is growing rapidly, it is Diffuse Reflectance Spectroscopy (DFS).

Diffuse reflectance spectroscopy, due to its ease, speed of sample handling, and cost-effectiveness, is as a possible alternative to conventional soil analysis [Kusumo et al., 2018a]. The use of Near Infrared Visible Spectroscopy (VIS-NIR) is based on the electromagnetic radiation radiated on the ground surface and reflected at different wavelengths [van der Meer, 2018]. One of the first works observed the influence of organic matter in certain spectral ranges, between 1400 - 1900 mn, due to water and clay content [Bowers and Hanks, 1965]. Soil samples associated with the wavelength of the electromagnetic spectrum may be responsible for predicting organic and inorganic soil components, in this case it would be for organic carbon [Rossel et al., 2006]. Likewise, other soil properties can also be estimated from the same spectra. The most commonly used algorithm for calibrating spectra and predicting soil properties is Partial Least Square Regression (PLSR) [Gomez et al., 2008]. PLSR combines principal component (PCA) and multiple regression analysis [Andrade-Garda et al., 2013]. The main objective of PLSR is to predict or analyze a group of dependent variables from a group of independent variables. In order to achieve an acceptable estimate of organic carbon, it is necessary to have a large and diverse data base for the calibration of the mathematical model to be used. This calibration is based on using up to 20% of samples for the validation of the result obtained with 80% of the database [Liu et al., 2019]. However, most of the results have shown that the best calibrations are those shown in groups located in the same environment [Askari et al., 2018]. Therefore, to have a more accurate prediction with calibrations, learning algorithms are being developed. The learning algorithms allow the adaptation and location of isolated groups and generate their own equation [Morellos et al., 2016].

With all the above mentioned, soil carbon management will be an increasingly important strategy in the coming decades for preserving or improving the stocks, as a natural solution to mitigate climate change. Monitoring of organic carbon levels in the soil at different depths would be necessary to quantify the contribution of the different natural and Anthropocene systems to CO2 sequestration.

Within this context this thesis has the general objective: “Quantify” the stock of organic carbon and its distribution in soil profile in some agricultural, agro-silvo-pastoral systems, and temperate forests by reducing the uncertainty of key estimates in Ecuador, Poland and Spain.

Specifics objectives:

A) Measure stocks in a wide range of management and soil conditions, from Ecuador (Agricultural land use), Spain (Agro-silvo-pastoral system), and Poland (Temperate Forest), (Chapters 2,3,4 and 5).

B) To evaluate the effect of different specific land use management an agro-silvo-pastoral systems, such as the presence of the adult tree vs. the young tree, crop rotation and the different grazing intensities on soil organic carbon concentration, total stock and its fraction, (Chapters 4 and 5).

C) To evaluate the potential of soil Vis-NIR spectroscopy to predict soil carbon concentration using the soil samples collected from different locations, and depth in the dehesa system, (Chapter 6).

Chapter 2

SOC Concentration and Storage under Different Land Uses in the Carrizal-Chone Valley in Ecuador

This chapter was published in the journal Applied Sciences.

Title: "SOC Concentration and Storage under Different Land Uses in the Carrizal-Chone Valley in Ecuador"
Journal: APPLIED SCIENCE
Editorial: MPDI
(Click in the Link): https://www.mdpi.com/2076-3417/9/1/45

Authors: **Lizardo Reyna-Bowen**[1,2], Lenin Vera-Montenegro[3], Mauricio Reyna Bowen[4]

[1]Forestry Department, University of Córdoba, Rabanales University Campus, Ctra. Madrid-Cádiz Km. 396, 14014 Córdoba.

[2]Institute for Sustainable Agriculture-CSIC, 14080, Córdoba, Spain.

[3]Facultad de Ingeniería Agrícola, Escuela Superior Politécnica Agropecuaria de Manabí MFL, Calceta 130602, Ecuador.

[4]Facultad de Ingeniería Agrícola, Universidad Técnica de Manabí, Lodana 130401, Ecuador

2.1 Abstract

Soil organic carbon (SOC) is an important indicator of soil quality—an elevated percentage of SOC indicates very high-quality soil, physically as well as chemically. As such, the principal objective of the present study was to determine the concentration of SOC at different depths as well as its accumulation through the entire soil profile. The Carrizal-Chone system (SCCH) area was stratified by agricultural use. Sixty-three soil samples were taken from different depths up to a maximum of 150 cm. The physical and chemical properties of the soil were determined. SOC was determined by the Walkley & Black method. The following results are highlighted: (1) 21 different varieties of soil management were identified, (2) the largest area was livestock grazing land, which had the greatest concentration of SOC, (3) the type of soil with the greatest SOC sequestration capacity was silty clay loam, (4) the area cultivated with corn presented the highest accumulation of total carbon, and (5) the highest concentration of SOC was found in the top 40 cm, with a tendency to decrease with depth. It is concluded that soil management influences the concentration and accumulation of SOC in the topsoil layers and the entire soil profile.

keyword: Sequestration, organic matter, land-used, agricultural-conventional, Agricultural

2.2 Introduction

Sustainable agro-ecosystems tend to balance land exploitation for human needs, such as food, fiber and wood, with long-term conservation of natural resources. Sustainable intensification (SI) is defined as a process or system where the agricultural yield is increased or additional non-agricultural land is converted without adverse environmental impact [Pretty and Bharucha, 2014]. Some benefits of this system, including productivity, decrease in erosion, conservation of soil moisture, greater soil biological activity, and reduction in production costs, have been previously described [Holland, 2004]. The global carbon balance is maintained when plant growth creates ideal conditions for the decomposition of organic matter, while live roots contribute to respiration, according to [Schlesinger and Andrews, 2000]. However, soil scientists are alarmed by the current status of global warming and positive carbon balance in the Earth's atmosphere. This may be resolved by increasing soil organic carbon (SOC) sequestration and identifying areas and soils with the greatest potential to undergo this process by spatial quantification of SOC, not only in the topsoil layer but also in the deeper ones [MEERSMANS et al., 2009, Rumpel and Kögel-Knabner, 2010].

The province of Manabí has the largest concentration of agricultural land in Ecuador, 766.744 ha including natural and cultivated pastures (INEC 2017), and largest territory has slopes of greater than 12 % [Reyna-Bowen, 2017]. The main water reservoir in the province, which has a capacity of 450 Mm^3, is regulated by the La Esperanza dam. Its content is intended for human consumption and irrigation [Reina, 2015]. The current landscape in the study area, the Carrizal-Chone valley, is dominated by a conventional system of cultivated pastures, forestry and crops, such as banana, cacao, coffee, and citrus, among others. The physical and chemical properties of soil have been improved through the management of grazing [Reina, 2015]. However, the impact of these systems on soil carbon sequestration have not been evaluated.

Proper soil management practices benefit the sequestration of organic carbon, which has an essential role in soil properties [Bhavya V. and Shivanna, 2018]. However, the intensification of conventional agriculture has meant that soils are losing the capacity to sequester carbon, causing the soil to become impoverished and to lose its diversity over time [Nicholls and Altieri, 2012]. Agroforestry systems aid the recuperation of degraded soils. Some soil factors, such as soil structure and aggregate stability, may improve porosity, decrease erosion, and increase soil productivity [Bronick and Lal, 2005]. It is important to describe these and other factors that improve the capacity of soil to store carbon. Organic carbon concentration and stock may vary by type of land use, as demonstrated by [Bhavya V. and Shivanna, 2018], who found higher carbon accumulation in perennial and woody plantations compared to those of short cycle and rotation. SOC storage and dynamics have long been known to depend on the region, soil forming factors, climate, parent material, organisms, relief, time, and soil management [Jenny, 1980, Muñoz-Rojas et al., 2012]. Therefore, the aim of this study was to compare the influences of different land management types on soil organic carbon.

2.3 Materials and Methods

2.3.1 Area description

The work was carried out in the Carrizal-Chone System project (SCCH) area of interest, located in the central part of the Province of Manabí near Chone and

Tosagua counties. The study area is situated at 0°51′46″ S, 80°08′61″ W, and between 19 and 80 m.a.s.l. (Figure 2.1).

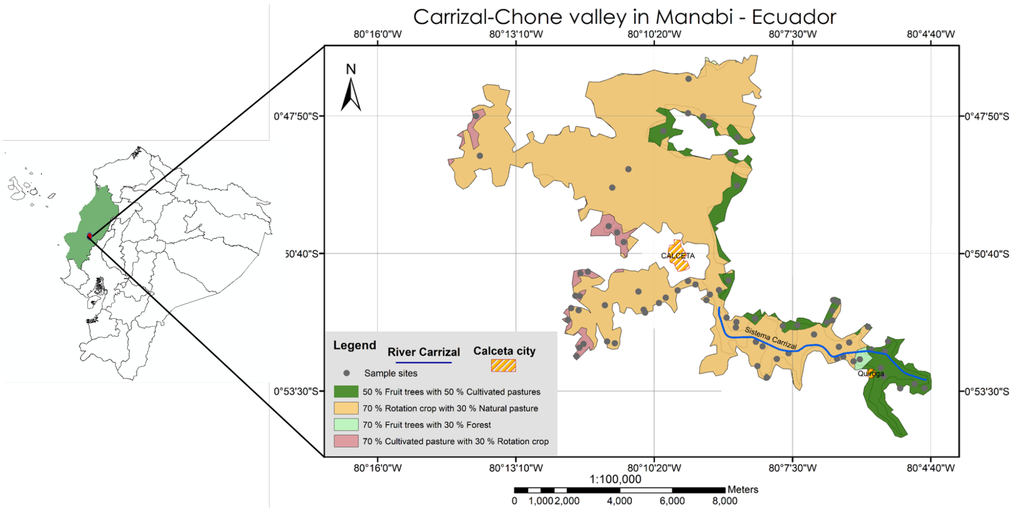


Fig. 2.1: Area of study with the general land use in the Carrizal-Chone System (SCCH), Manabí, Ecuador.

The average annual temperature of the area is 25.6 °C, the average annual potential evapotranspiration is 1365.2 mm, and the average annual precipitation is 838.7 mm, with a dry period occurring from June to December and a rainy period from January to May. The SCCH zone is composed of 50 % natural pastures, 25 % agricultural crops, 10 % artificial pastures, 4 % secondary forests, and 11 % fallow, scrub, and others.

The slopes of the study area are less than 30 %. The soil types are generally sandy silts of high plasticity, silty sands, and clay of high and low plasticity.

Agriculture is the dominant land use, occupying the 75 % of the territory [Castro et al., 2016].

2.3.2 Soil Profile Description and Sampling for Bulk Density and SOC

The sampling was carried out between March and August, 2015, following the profile description method by [Schoeneberger and Staff, 2012]. Sixty-four pits were created in different areas representing different soil management types around the valley. The soil color was determined by the Munsell classification [Charts, 2010]. Soil samples were taken manually in each horizon, having been previously removed from the grass and mulch surface. The mean of horizon (Hz) thickness of the 218 sites was 40 cm. The top horizons thickness varied between 15 and 60 cm depending on the soil type.

The samples were passed through a 2 mm sieve and homogenized, and stoniness was determined as (%) in mass. The samples were dried to a constant mass at 40 °C for 72 h. The SOC concentration was determined in accordance with Walkley [1947] Equation 2.1. The stock of soil organic carbon for each soil depth interval (SOCstock) and for the whole soil profile, were calculated in accordance with [IPCC, 2003] Equation 2.2:

$$SOC = M * \frac{V_1 - V_2}{W} * 0.30 * CF \tag{2.1}$$

where M is the molarity of the $FeSO_4$ solution (from a blank titration), V_1 is the volume (mL) of $FeSO_4$ required in the blank titration, V_2 is the volume (mL) of $FeSO_4$ required in actual titrations, W is the weight (g) of the oven-dried soil sample, and CF is the correction factor.

$$SOC_{stock} = 10.000 SOC_i * BD_i * d * (1 - \delta) \tag{2.2}$$

where SOCstock i is the total soil organic carbon in a given layer (t ha^{-1}). SOCi is the organic carbon concentration (g g^{-1}), BD_i is bulk density (Mg m^{-3}), d is the thickness of the depth interval (m), δ is the fraction (0 - 1) of gravel larger than 2 mm in the soil, and n is the number of soil layers. So, Equation 2.3 gives the total soil organic carbon, SOCstock (t ha^{-1}) of the whole soil profile.

$$T - SOCstock = \sum_{i=1}^{i \ldots n} SOC_{stocki} \tag{2.3}$$

Undisturbed soil samples were taken with a hand soil sampler for the determination of bulk density (BD). These samples were taken from every pit in each horizon, totalling 218 samples. The samples were dried to a constant mass at 105 °C for 48 h. The bulk density was calculated by dividing the dry mass by the volume of the cylinder 98.2 cm^3 [Doran and Mielke, 1984].

2.3.3 Statistic analysis

The statistical analysis was performed using InfoStat software version 2018. The effect of each land use on SOC and stocks was analyzed using a Kruskal–Walis non-parametric test, and T-SOCstock was analyzed with an ANOVA test. A test of data normality was done to verify the model assumptions. The data analysis was made by land use groups as follow: abandoned (A), permanent cultivation (PC), rotation crop (RC), grazing (G), natural bush (N).

2.4 Results

Two different soil types were identified according to United States Department of Agriculture-Natural Resources Conservation Service (USDA-NRCS) soil taxonomy: (a) Udic, Fluventic Hapludoit and/or Tropofluvient, which are deep soils of variable texture (dominant loam), and (b) Ustic, Vertic Ustropept and/or Ustret which have hills with a slope of between 12 % and 40 %. Most soils are deeper than 50 m throughout the valley; only few sites are less than 15 cm in depth.

2.4.1 Bulk density

No significant differences in bulk density (BD) ($P = 0.858$) were found between depths. When samples were grouped by land use, the BD showed a non-significant tendency to be different between land uses. The values of each horizon (Hz) were averaged as follows: Hz 1) 1.25±0.16 g cm^{-3}, Hz 2) 1.19±0.12 g cm^{-3}, Hz 3) 1.21±0.15 g cm^{-3}, Hz 4) 1.22±0.11 g cm^{-3} and Hz 5) 1.19±0.10 g cm^{-3}.

2.4.2 Soil Organic Carbon Concentration in Soil Profile

In the SOC analysis, data was grouped by soil texture, soil management, and soil depth. The soil texture with the highest SOC concentration was loam-silt loam (1.57 %), followed by clayey soil (1.07 %). Soils with larger particles, such as sand, showed lower SOC values (Figure 2.2). There were higher SOC values in the first horizons where the greatest presence of roots is found.

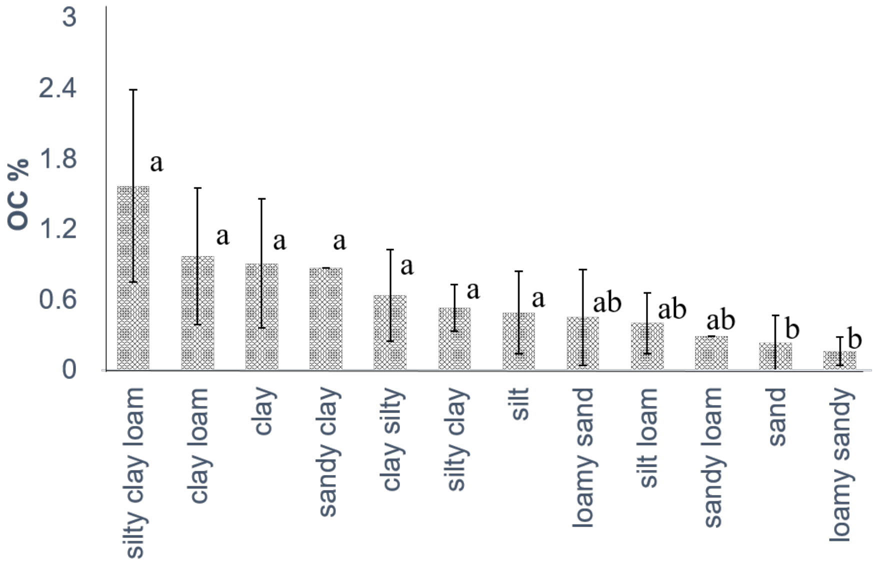


Fig. 2.2: Distribution of the soil organic carbon (SOC) concentration. Different letters indicate significant differences grouped by soil texture according to the Kruskal–Wallis test, ($p < 0.05$).

When management according to land use was grouped, there were no significant differences in SOC concentration (P = 0.347). The group with the highest concentration value was found in natural shrubs (N), with 0.62 % organic carbon compared to grazing (G) with 0.57 %. The abandoned (A) group had the lowest concentration of organic carbon with an average value of 0.26 % SOC. This grouping shows that the study area has a high frequency of grazing (G) for livestock (Table 2.1).

Tab. 2.1: SOC concentration in (%), grouping by land use: abandoned (A), permanent cultivation (PC), rotation crop (RC), grazing (G), natural bush (N) according to the Kruskal–Wallis test, (P < 0.05).

Group	n	Average	S.D.	P-value
A	4	0.26	0.1	0.347
PC	26	0.54	0.42	
RC	50	0.57	0.39	
G	135	0.57	0.44	
N	3	0.62	0.12	

The analysis of data by depth factor showed no differences in SOC concentration, (p-value = 0.0588). The thicknesses of all horizons were averaged and then analyzed with the Kruskal–Wallis test (Table 2.2). There was a significant difference between horizons Hz (p-Value < 0.0001). Figure 2.3 shows that the surface horizon had the highest SOC concentration (Hz1) (0.87 %), with a decrease in the second horizon (Hz2) (0.41 %), and in the last horizon (Hz5) (0.36 %).

Tab. 2.2: Average thickness in each horizon (Hz); standard deviation (SD).

Hz	*n*	Thickness (m)	SD
1	64	0.41	0.17
2	61	0.38	0.14
3	56	0.41	0.15
4	32	0.38	0.13
5	5	0.38	0.16

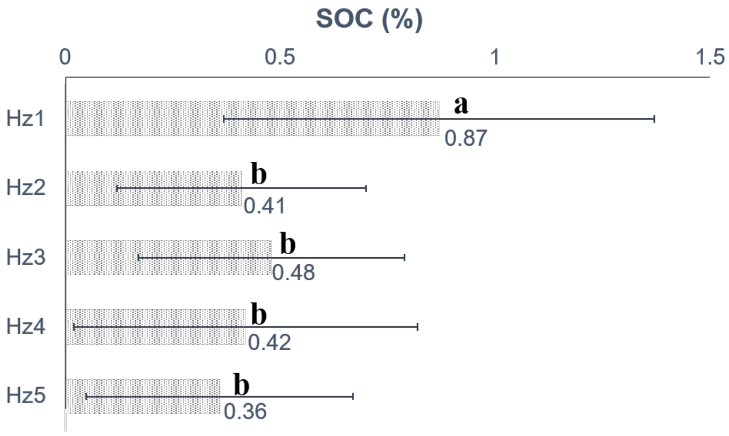


Fig. 2.3: Soil organic carbon concentration (SOC) in %. Different letters indicate significant differences according to the Kruskal–Wallis test ($p < 0.05$). Horizon (Hz).

2.4.3 SOC Concentration in Hz 1

An average depth of 41 cm was found for the surface horizon Hz 1). The analysis was done with the type of texture factor, without taking land use as an influence or interaction. Silty clay loam soil (1.57 %), clay loam (1.29 %) and clay (1.14 %) had the highest SOC concentration percentages, with significant differences for (p = 0.001) silty clay, silt and silt loam textural soils (Figure 2.4).

2.4.4 SOC Concentration in Different Land Uses

We determined the significant differences between 21 different soil management types ($p > 0.999$) using the Kruskal–Wallis test (Figure 2.5). The highest percentages of organic carbon were found in areas cultivated with banana and cocoa 1.80 % and 1.68 %, respectively. The percentages of organic carbon for soil used for maize and cacao were 1.33 % and 1.07 %, respectively—these were the management types with the highest organic carbon concentrations in the surface horizon. The lowest values were found in soils used for lemon cultivation and in the area of land plowed for the preparation of rotation crop use with 0.35 % organic carbon in both cases.

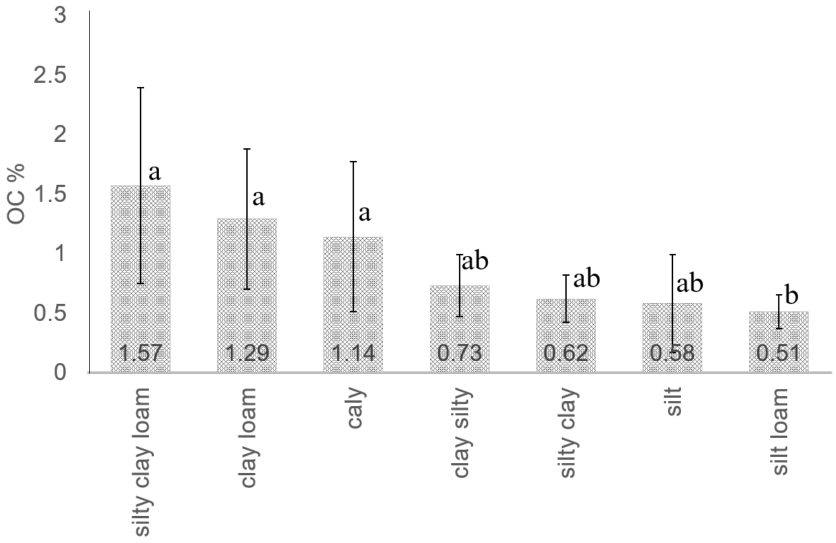


Fig. 2.4: Soil organic carbon (SOC) concentration (%) for the surface horizon (Hz1). Different letters indicate significant differences based on soil texture according to the Kruskal–Wallis test, (P $<$ 0.05).

These organic carbon concentration values were determined from the surface 41 cm of soil the average depth of the surface horizon in each profile studied.

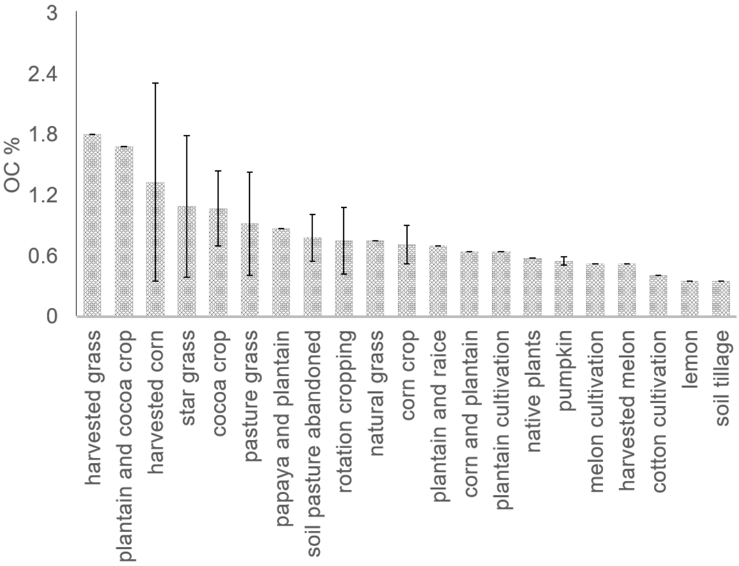


Fig. 2.5: Soil organic carbon (SOC) concentration in% in the surface horizon (Hz1) only. Different letters indicate significant differences based on soil land use according to the Kruskal–Wallis test, ($p < 0.05$).

2.4.5 SOCstock Soil vs. Management

The SOCstock analysis showed that the surface horizon was different from the rest horizons in all profiles; the accumulation was (41±21 t ha^{-1}) (Table 2.3).

SOCstock was analyzed by land use groups. No significant differences were found (P = 0.160) between natural (44.6±10.5 t $^{-1}$), grazing (26.9±22.2 t ha^{-1}), rotation crop (26.8±18.5 t ha^{-1}), permanent crops (24±18.8 t ha^{-1}), and abandoned (11.8±4.3 t ha^{-1}) areas. Therefore, the greatest SOCstock among the horizons was in the surface horizon.

Tab. 2.3: Distribution of soil organic carbon stock (SOCstock t ha^{-1}) by horizon (Hz). Different letters indicate significant differences according to the Kruskal–Wallis test ($P < 0.05$).

Hz	**n**	**SOCstock t ha^{-1}**	**S.D.**	**H**	**P-value**
1	64	41.32 a	20.97	55.86	<0.0001
2	61	18.85 b	16.84		
3	56	23.79 b	19.71		
4	32	17.81 b	13.2		
5	5	14.88 b	12.71		

2.4.6 T-SOCstock

Silt loam the texture had the greatest SOCstock in the top soil horizon with 59 t ha^{-1}. The texture with the least SOCstock was silt texture had 17 t ha^{-1} (Table 2.4).

Tab. 2.4: Distribution of soil organic carbon stock (SOCstock, t ha^{-1}) in Hz 1 only. Different letters indicate significant differences according to the Kruskal–Wallis test ($P < 0.05$).

Soil Texture	**n**	**SOCstock (t ha^{-1})**	**S.D.**	**H**	**P-value**
silt loam	5	58.6 a	29.67	17.73	0.0069
clay	18	48.34 a	18.04		
silty clay loam	2	43.65 ab	25.53		
clay loam	20	40.28 ab	17.94		
clay silty	12	37.51 ab	22.87		
silty clay	5	20.88 b	4.1		
silt	2	16.95 b	14.07		

T-SOCstock varied across the different uses. However, abandoned soil had the highest T-SOCstock with 177 t ha^{-1}, followed by native plants with 134 t ha^{-1}, and soil tillage had the lowest accumulation with 47 t ha^{-1}. Although the differences between land uses were notable, there were no statistically significant differences.

2.5 Discussion

2.5.1 Effects of Soil Texture and Land Use on SOC

In this study, silty clay loam soils (1.57 %) had the highest SOC values, followed by clay loam (0.97 %). Similarly, an SOC concentration of 1.85 % was found in Los Rios province in Ecuador under woody plantations with cacao CNN51 and National cacao [Barrezueta, 2018].

According to Barrezueta-Unda and Paz-González [2017], the soils of the El Oro province could have higher or more frequent fertilization than the soils in Manabí, since there is a strong demand for agronomic products in this province. On the other hand, the concentrations vary with depth, with more concentrated SOC in the top soil layer (above 30 cm), but there are no significant differences across the whole profile. The SOC concentration does not depend only on the crop type, but also on the soil type. In other regions of Ecuador, the SOC concentration varies due to the elevation, climate, or texture of the soil. Similar work on SOC in the Andean zone of Ecuador showed a high SOC concentration due to the climate (constant humidity in soil) and the permanent vegetation in the Andean páramos that helps to sequester organic carbon over the long term [Henry et al., 2013].

2.5.2 Effects of Crops and Soil Management on SOC

The SOC concentration was affected by changes in land use and various factors such as the climatic conditions, soil texture, site preparation and management, vegetation type, history of land use, etc [Deng et al., 2016]. In this study, the highest concentration of carbon in soil was found under cultivated pastures; the abundance of roots under this crop could explain the major concentration of SOC. Likewise, the permanent plantations of plantain and cacao have an abundance of roots, but the area is not totally covered by vegetation; thus, the carbon concentration is lower than in the grass area. However, the values found are not out of range for this type of soil, use and management. Another factor that influences SOC is the rainfall. Manabí has a dry tropical climate and the rain is concentrated in winter; the significant amount of precipitation in February and March leads to a loss of fertile soil due to water erosion.

2.5.3 SOCstock in Different Soil Uses

According to Hernández et al. [2013], the accumulation of organic carbon at a depth of 20 cm varies between pastures and overgrazing with 37 t ha^{-1} and 49 t ha^{-1}, respectively, in land cultivated with cacao 41 t ha^{-1} and 60 t ha^{-1} (depending on age of trees and management) in the short cycle corn group with 43 t ha^{-1} of stock.

Compared to the present work in the same study area, the T-SOCstock in the surface horizon (around 41 cm thickness) was shown to vary among cultivated grass (50.9 t ha^{-1}), cacao crop (43.8 t ha^{-1}), corn (47.7 t ha^{-1}), and the average of rotation crop (42.8 t ha^{-1}). The highest SOCstock was found in abandoned grass harvested corn (74.9 t ha^{-1}) and melon crop (61.7 t ha^{-1}). These results show a tendency for an increase in SOCstock with an adequate management of the plantations.

In the Ecuadorian Amazon, the SOCstock varies at a depth of 30 cm as follows: 49.44 t ha^{-1} in agroforestry systems and 36.75 t ha^{-1} in Dali Grass without three [Bravo, 2017, Jadan et al., 2015]. At a 10 cm depth in cacao cultivation areas, the agroforestry system can reach 69 t ha^{-1}.

This shows that the average accumulation of SOC in the surface horizon is low, compared with the average of other regions. This can be related to rainfall the Manabí area is drier compared to the Andean and Amazonian regions.

2.6 Conclusions

Soils that contain high percentage of sand tend to have a lower organic carbon concentration than loamy and clayey soils.

The management of the first layer of soil is fundamental for the accumulation of SOC. The potential of organic carbon sequestration is increased throughout the profile with the management of soil and water resources.

Extending the cacao area could increase the potential concentration and accumulation of total organic carbon throughout the soil profile, benefiting soil, the environment, and system productivity.

Chapter 3

Distribution and Factors Influencing Organic Carbon Stock in Mountain Soils in Babia Góra National Park, Poland

This chapter was published in the journal Applied Sciences.

Title: "Distribution and Factors Influencing Organic Carbon Stock in Mountain Soils in Babia Góra National Park, Poland"
Journal: APPLIED SCIENCES
Editorial: MPDI
(Click in the Link): https://www.mdpi.com/2076-3417/9/15/3070

Authors: **Lizardo Reyna-Bowen**[1,2], Jarosław Lasota[3], Lenin Vera-Montenegro[4], Baly Vera-Montenegro[4], Ewa Błońska[3]

[1]Forestry Department, University of Córdoba, Rabanales University Campus, Ctra. Madrid-Cádiz Km. 396, 14014 Córdoba.

[2]Institute for Sustainable Agriculture-CSIC, 14080, Córdoba, Spain.

[3]Faculty of Forestry, Department of Forest, Engineering, University of Agriculture in Kraków, Al. 29 Listopada 46, 31-425 Kraków, Poland

[4]Facultad de Ingeniería Agrícola, Escuela Superior Politécnica Agropecuaria de Manabí MFL, Calceta 130602, Ecuador.

3.1 Abstract

The objective of this study was to determine the soil organic carbon stock (T-SOC stock) in different mountain soils in the Babia Góra National Park (BNP). Environmental factors, such as the topography, parent material, and vegetation, were examined for their effect on carbon stock. Fifty-nine study plots in different BNP locations with diverse vegetation were selected for the study. In each study plot, organic carbon stock was calculated, and its relationships with different site factors were determined. The results reveal that the SOC stocks in the mountain soils of the BNP are characterized by high variability (from 50.10 to 905.20 t ha^{-1}). The general linear model (GLM) analysis indicates that the soil type is an important factor of soil organic carbon stock. Topographical factors influence soil conditions and vegetation, which results in a diversity in carbon accumulation in different mountain soils in the BNP. The highest carbon stock was recorded in histosols (>550 t C ha^{-1}), which are located in the lower part of the BNP in the valleys and flat mountain areas.

keyword: mountain soils; SOC; topography

3.2 Introduction

Forest ecosystems contain the highest organic carbon stock among terrestrial ecosystems [Pan et al., 2011, Vanguelova et al., 2013]. Forest systems cover more than 4.1 × 10^9 hectares of the Earth's land area [Dixon et al., 1994]. In these ecosystems, organic carbon accumulates in the biomass of trees, shrubs, and herbaceous plants, as well as in the soil horizons that form the soil profile. It is estimated that the carbon contained in soil constitutes 75% of the total organic carbon stock stored in terrestrial ecosystems, and it is twice the amount of carbon stock in the atmosphere [Dixon et al., 1994, Six et al., 2002]. Soil carbon (C) stock is influenced by several environmental factors, such as the topography, slope, exposure, elevation, climate, parent material, and vegetation [Tsui et al., 2004, Zhang et al., 2011, Bardelli et al., 2017, Łajczak and Spyt, 2018]. Site properties (altitude, exposition, slope) influence the soil conditions and soil properties. Above all, vegetation affects soil parameters because it supplies organic matter that varies in quantity and quality [Ayres et al., 2009].

Mountain areas are characterized by climatic and topographic factors that are highly variable, and this variation results in the diversification of vegetation and, as a consequence, differences in soil properties among locations. Depending on the definition, mountain areas cover roughly 22–27% of the Earth's total land area [Grabherr, 2011]. According to Egli [2016], mountain soils are highly dynamic systems that are sensitive to environmental changes. Soil fertility and productivity depend on soil organic matter (SOM), which is a reservoir of nutrients and plays an important role in cycling nutrients and improving the physical, chemical, and biological properties of soils [Steiner et al., 2007, Bhattacharyya et al., 2009]. The amount and quality of soil organic matter depend on forest land management. The amount of SOM in forest soils is determined by the balance between soil organic matter input by on the one hand, and the release of C during decomposition on the other hand [Jandl et al., 2007]. Changing the land use from natural forest to plantations affects the C stored in the soil [Haghdoost et al., 2013]. According to Liao et al. [2010], ecosystem C stock in plant and soil pools was 284 Mg C ha^{-1} in natural forests and decreased by 28% in plantations. In the study in [Manojlovic, 2011], the land-use system and altitude were shown to be important factors in the regulation of SOM decomposition by altering the natural soil characteristics. Unmanaged or old-growth forests are important for carbon sequestration [Krug et al., 2012].

The mechanisms responsible for carbon stabilization in soil have received much interest recently because they are crucial for understanding the global carbon cycle. Knowledge about the factors that shape the accumulation of organic carbon in the soil is important for understanding the carbon cycle in mountain forest ecosystems. The primary objective of this study was to quantify the soil organic carbon stock (T-SOCstock) in sites with different conditions in the Babia Góra National Park (BNP). We predicted that topographical factors influence the soil conditions and vegetation, leading to a diversity of carbon accumulation in the mountain soils of the BNP.

3.3 Materials and Methods

3.3.1 Study sites

The study was carried out in the Babia Góra National Park (BNP) ($49°34'$ N and $19°31'$ E) in southern Poland (Figure 3.1). The BNP occupies an area of 3391.55 ha, and its altitude ranges from approx. 700 m a.s.l. to the summit of Babia Góra at 1725 m a.s.l. The climate is cool and humid: at the timberline level of Babia Góra Mt., the mean annual temperature is about 2 °C, and the annual sum of precipitation is slightly over 1400 mm [Obrebska Starkel, 2004]. The growing season in the lowest portion of the Babia Góra National Park (700 m a.s.l.) is 202 days, and it is shorter in the higher climatic and vegetation zones (140 days at 1100 m a.s.l. and 105 days at the highest point).

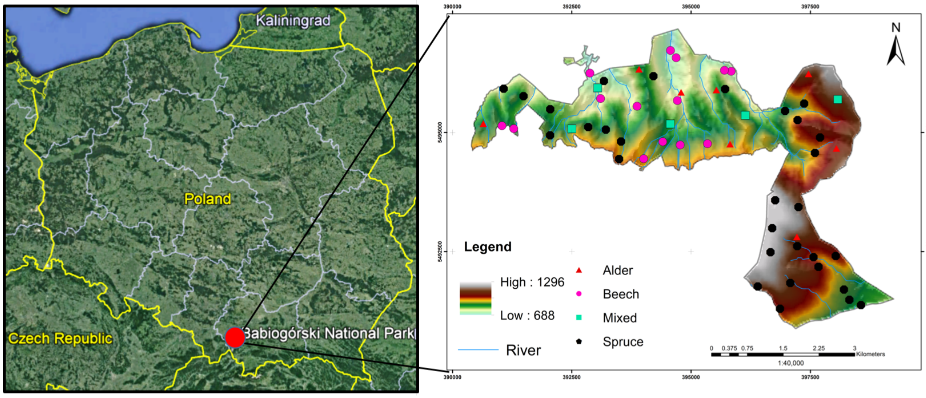


Fig. 3.1: The study area, Babia Góra National Park in Poland.

The Babia Góra massif comprises Upper Cretaceous–Paleogene flysch sediments, with Magura sandstone in the uppermost part. The latter is represented by thick layers of sandstone with thin intercalations of mudstone and shale. Quaternary sediments also constitute part of the study area [Alexandrowicz, 2004]. A considerable majority of the Babia Góra National Park is predominated by hyperdystric cambisols (36.8%) and, to a lesser extent, epidystric cambisols (13.6%), albic cambisols (13.5%), podzols, and other soil types [Kowalska et al., 2017]. Characteristic zonation of mountainous vegetation can be distinguished in the area of the Babia Góra National Park, starting from the highest portions: the alpine zone (1650–1725 m a.s.l.), mountain pine zone (1350–1650 m a.s.l.), upper subalpine zone (1100–1350 m a.s.l.), and lower subalpine zone (700–1100 m a.s.l.). The BNP is primarily overgrown with beech, spruce, and fir forests. The *Dentario glandulosae-Fagetum, Luzulo nemorosae-Fagetum, Abieti-Piceetum* association has a considerable share of the park. The *Alnetum incanae* association can be found in the vicinity of creeks; within troughs and submersion areas, the *Caltho laetae-Alnetum* association is also present [Łajczak and Spyt, 2018].

3.3.2 Soil Sampling

The study was conducted using 59 research plots located in the Babia Góra National Park. Soil samples for laboratory analyses were collected from each research plot. Soil samples were taken from the soil horizons that were distinguished from the profiles during fieldwork. Samples for laboratory testing were collected from the organic horizon (Oh, Ofh, Ot, OM), the first mineral horizon (AE, A, AB, or AG), and the subsequent mineral horizon (horizons B, G, and C). Soil samples were taken from horizons according to the depth at which they occurred. On average, soil samples were taken to a depth of 100 cm. Organic horizons had an average depth of 20 cm, a minimum depth of 2 cm, and a maximum depth of 92 cm. The humus forms were determined according to Classification of Forest Soils in Poland (2000). Four subsamples were collected from each plot and thoroughly mixed to obtain a composite soil sample, and the samples from each horizon were put into plastic containers. Research plots were grouped on the basis of species composition, soil type, and altitude. The studied plots were divided into four groups according to the species composition of the stand (Alder: the share of alder (*Alnus incana*) in the stand is more than 80%; Beech: the share of beech (*Fagus sylvatica*) in the stand is more than 80%; Spruce: the share of spruce (*Picea abies*) in the stand is more than 80%; Mixed: stands that contain beech and spruce). Seven soil groups were defined: dystric cambisols (CD), eutric cambisols (CE), fluvisols (FL), gleysols (GL), histosols (HI), podzols (PO), and stagnosols (ST). Additionally, the research plots were grouped according to parent material (I: terrace sediment accumulation; II: fluvial sediments; III: hieroglyphic sandstone; IV: Magura sandstone; V: osielecki sandstone; VI: peat sediments). Three groups of altitude were defined: $<$900 m a.s.l, 900–1100 m a.s.l., and $>$1100 m a.s.l.

3.3.3 Laboratory Analysis

Soil samples obtained in the field were dried and sieved through a 2.0 mm mesh. The particle-size distribution was determined using laser diffraction (Analysette 22, Fritsch, Idar-Oberstein, Germany). The pH of the samples was analyzed in H_2O and KCl using a potentiometric method. The carbon (C) and nitrogen (N) contents were measured with an elemental analyzer (LECO CNS TrueMac Analyzer (Leco, St. Joseph, MI, USA)) and the Ca, Mg, K, and Na contents were determined by inductively coupled plasma optical emission spectrometry (ICP-OES) (iCAP 6500 DUO, Thermo Fisher Scientific, Cambridge, U.K.). The sum of base cations (BC) was calculated. Samples with intact structures were collected in metal cylinders and used to determine the bulk density by the drying-weighing method Grossman and Reinsch [2002]. The stone content in the soil horizon was visually estimated in the field. The share of stones in particular genetic horizons was determined as a percentage. The bulk density and the share of stones were used to calculate the soil organic carbon stock.

Calculation of Soil Organic C Stocks Soil organic C depth interval (SOCstock) and for the whole soil profile (to 100 cm) were calculated according to [IPCC, 2003]. Equivalent approaches at different scales were used by Muñoz-Rojas et al. [2012] Equation (3.1):

$$SOCstock = SOC \cdot BD \cdot d \cdot (1 - \sigma), \tag{3.1}$$

$$SOCstock = \sum_{i=1}^{i...n} SOC_{stocki}, \tag{3.2}$$

where SOC is soil organic C density (Mg ha^{-1}), SOC is soil organic C percentage (g 100^{-1} g^{-1}), BD is bulk density (g cm^{-3}), D is the thickness of the studied layer (cm) and σ is the soil coarse fraction so that (1 – σ) is the fine earth fraction (unitless). The fraction of gravel larger than <2 mm in the soil is the proportion in volume of coarse fragments. Therefore, Equation (3.2), n is the number of soil layers, calculates the total soil organic carbon, i.e., the total SOCstock (Mg ha^{-1}), in the whole soil profile.

3.3.4 Geography Information System and Index

The following inputs were used to obtain the index necessary for the elaboration of the maps. Environmental covariates were derived from the Digital Elevation Model (DEM) of SRTM (NASA SRTM, spatial resolution of 90 m) and calculated using ArcGIS programs. These covariates were analytical hillshade (shadow model according to the relief and light source), slope, aspect, and curvature (model of surface curvature for concave and convex surfaces).

The Topographic Position Index (TPI) is a metric that compares the elevation of each cell in the DEM with the mean elevation of the neighborhood around the specified cell. The local mean elevation is subtracted from the elevation value at the center of the local window. The algorithm is provided as an ESRI-script by Jenness Enterprises (Arizona, USA, 1987), and its local window options are rectangular, circular, and annulus [Weiss, 2017]. Positive TPI values represent locations that are higher than the average of the local window, e.g., ridges, and high positive values represent peaks and ridges. Negative TPI values represent locations that are lower, e.g., valleys. TPI values near zero are either flat areas (where the slope is near zero) or areas with a constant slope (where the slope of the point is significantly greater than zero). TPI is calculated by Equation (3.3):

$$TPIi \quad = \quad Z_0 - \frac{\Sigma_{1-n} \quad Z_n}{n}, \tag{3.3}$$

where Z_0 is the elevation of the model point under evaluation, Zn is the elevation of the grid within the local window, and n is the total number of the surrounding points employed in the evaluation.

3.3.5 Statistical Analysis

The obtained data did not show normality, so a non-parametric test was used for the analysis of the variables. The Kruskal–Wallis test was used. A general linear model (GLM) was used to investigate the effect of soil type, altitude, and type of forest stand on the total soil organic carbon stock and it averages the horizons for comparison. Principal component analysis (PCA) was used to evaluate the relationships between T-SOCstock and the position characteristics, soil, and forest stand type. In accordance with Ward's method, the study plots were agglomerated into groups with different altitudes, slopes, T-SOCstock values, and types of forest stands. The average and standard deviation (SD) are presented in tables and figures. Differences with $p < 0.05$ were considered statistically significant. All analyses were performed using Statistica 12 software (StatSoft, Inc. (2012). STATISTICA (data analysis software system), version 10. www.statsoft.com).

3.4 Results

3.4.1 Basic Properties of the Studied Soils

The studied soils were characterized by diverse properties. The texture of the investigated soils was dominated by sand (average content of 44%) and silt (average content of 41%). The clay content ranged from 1% to 77%, and the pHH2O of soils ranged from 3.1 to 8.1 (Table **??**). The variability in soil organic carbon and nitrogen content was high, with ranges of 4.0–422.0 g kg^{-1} and 1.0–29.0 g kg^{-1}, respectively (Table 3.1). The studied soils were characterized by a high variation in the base cation content (Table 3.1), and the results obtained for the bulk density show diversity in all the profiles (0.1–1.4 g cm^{-3}). Because the percentage of stones is a very influential factor in the estimation of carbon accumulation, it was necessary to determine differences in the stone content in different soil horizons. The results obtained show that the first horizon had an average stone content of 12%, which is significantly lower than the stone content in the other horizons. The percentage of stones increased to over 80% in the lowest horizons (Figure 3.2). The horizons (H) had the following averages; H1 = 11.39 ± 7.48 cm; H2 = 21.80 ± 17.90 cm; H3 = 38.69 ± 17.4 cm; H4 = 26.37 ± 11.63; H5 = 20.50 ± 12.67 cm; and finally H6 = 20.00 ± 0 cm.

Tab. 3.1: The basic properties of the studied soils ($n = 59$).

Variable	Mean	SD	Minimum	Maximum
sand	44	16.7	11	82
silt	41	11.5	12	65
clay	15	8.9	1	77
pH H2O	5	0.9	3.1	8.1
pH KCl	3.98	0.81	2.5	7
SOC	99.0	118.0	4.0	422.0
N	5.0	6.0	1.0	29.0
C/N	17.1	5	7.6	38.9
Na	1.3	2.1	0.2	18.8
Ca	136.5	261.3	0.5	1771.6
Mg	13.9	20.6	0.2	130.6
K	12	11.2	1.1	58.6
BD	1.1	0.4	0.1	1.4

Bulk density (BD) is expressed in g cm^{-3}; Na, Ca, Mg, and K contents are expressed in mg 100 g; Sand, silt, and clay content are expressed as %; soil organic carbon (SOC) and nitrogen (N) content are expressed in g kg^{-1}. Standard deviation (SD)

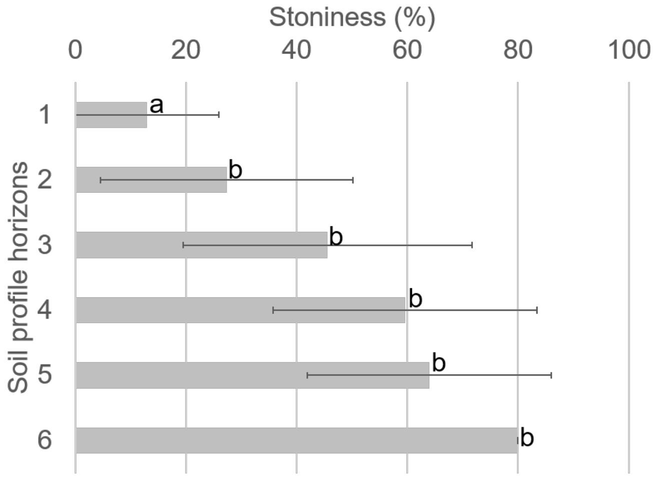


Fig. 3.2: Stratification of stoniness content (%) in the soil profile layers ($n = 59$). Different letters indicate a significant difference ($p<0.05$) between sections of the soil profile according to the Kruskal–Wallis test.

3.4.2 Soil Organic Carbon and Stock in the Studied Soils

As expected, there was clear stratification of organic carbon content (g kg^{-1}) with respect to the horizons (Figure 3.3). Analyses of the first horizon show an average carbon content of 178.2±125.5 g kg^{-1}, which differs from that in the other horizons. There was a decrease in organic carbon content in the second and third horizons, which had values of 59.1±89.1 g kg^{-1} and 26.3±73.1 g kg^{-1}, respectively. A significantly higher SOC content (g kg^{-1}) was noted in the surface horizon (Figure 3.3).

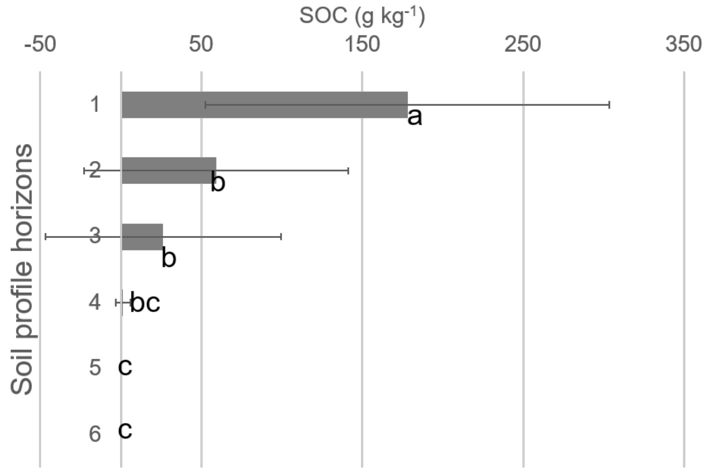


Fig. 3.3: Stratification of soil organic carbon (SOC) content (g kg^{-1}) in soil profiles (n = 59). Different letters indicate a significant difference ($p < 0.05$) between sections of the soil profile according to the Kruskal–Wallis test.

Significant differences in total organic carbon stock in the soils of different forest stands were found (Figure 3.4). The highest total organic carbon stock was in the soils of alder and spruce forests (282.07 ± 237.62 t ha^{-1} and 171 ± 163.74 t ha^{-1}). The transition to lower T-SOCstock was in soils of mixed forest (112.84 ± 45.75 t ha^{-1}), and the lowest T-SOCstock was in soils of beech forest (97.26 ± 20.06 t ha^{-1}) (Figure 3.4).

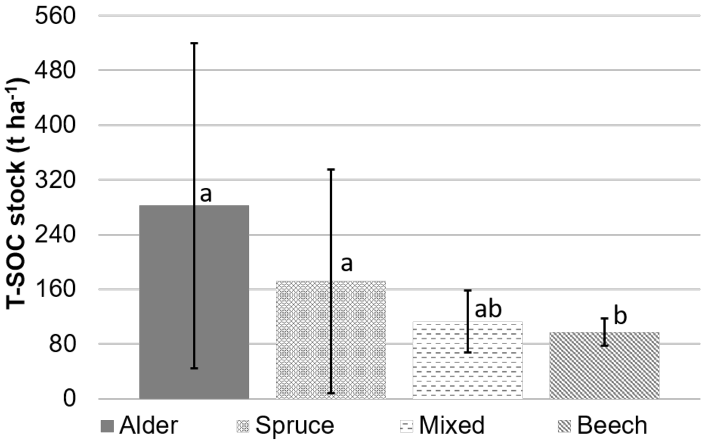


Fig. 3.4: Total organic carbon stock in the soils of different forest stands (t ha^{-1}) (Alder n = 8; Spruce n = 31; Mixed n = 6; Beech n = 14). Different letters indicate a significant difference ($p < 0.05$) between the types of forest stands according to the Kruskal–Wallis test.

The studied soils were divided into seven groups. Significantly higher T-SOCstock was found in histosols (556.23 ± 238.69 t ha^{-1}) compared with the other soil types. Significantly lower T-SOCstock was in fluvisols and eutric cambisols (73.81 ± 20.65 and 99.92 ± 30.08 t ha^{-1}, respectively) (Figure 3.5). Among different types of parent material, peat sediments (VI) had significantly higher T-SOCstock (546.3 ± 255.04 t ha^{-1}). The average T-SOCstock in the soils formed on the remaining parent material was 78% lower (115.65 ± 42.32 t ha^{-1}) (Figure 3.6).

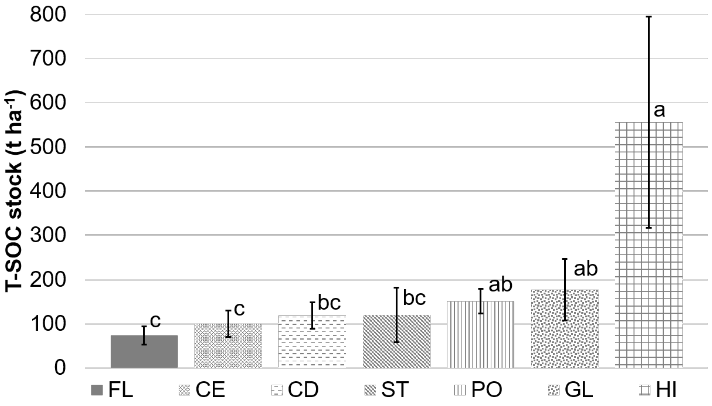


Fig. 3.5: Total organic carbon stock (t ha^{-1}) in different soil types (CD: dystric cambisols, $n = 21$; CE: eutric cambisols, $n = 16$; FL: fluvisols, $n = 3$; GL: gleysols, $n = 4$; HI: histosols, $n = 6$; PO: podzols, $n = 6$; ST: stagnosols, $n = 3$). Different letters indicate a significant difference ($p < 0.05$) between soil types according to the Kruskal–Wallis test.

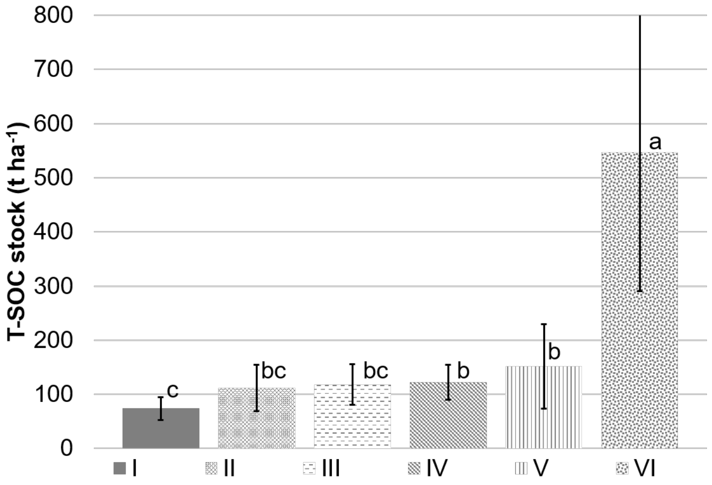


Fig. 3.6: Total organic carbon stock (t ha^{-1}) in soils created on different parent materials (I: terrace sediment accumulation, $n = 3$; II: fluvial sediments, $n = 15$; III: hieroglyphic sandstone, $n = 5$; IV: Magura sandstone, $n = 24$; V: osielecki sandstone, $n = 6$; VI: peat sediments, $n = 6$). Different letters indicate a significant difference ($p < 0.05$) between parent material according to the Kruskal–Wallis test.

The total organic carbon stock in soils was not significantly different between the three altitude groups (>1100, 900–1100, and <900 m) (Figure 3.7). Figure 3.8 maps the values of the total organic carbon stock (t ha^{-1}) in soils with the Topography Position Index (TPI). The highest T-SOCstock (50.10–905.20 t ha^{-1}) was in the valley and on flat mountain areas. Low and intermediate slopes were characterized by a lower T-SOCstock (Figure 3.8).

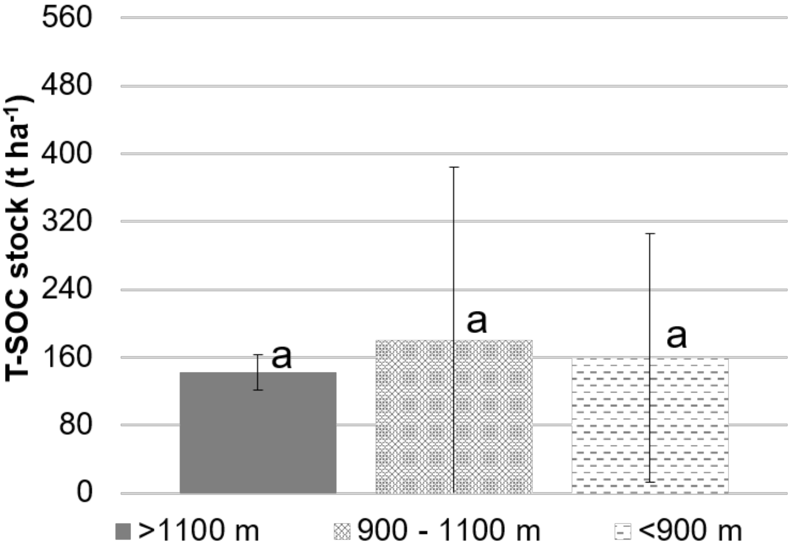


Fig. 3.7: Total organic carbon stock (t ha^{-1}) at different elevations (>1100 m, n = 5; 900–1100 m, n = 15; <900 m, n = 39). Different letters indicate a significant difference (p < 0.05) between soil types according to the Kruskal–Wallis test.

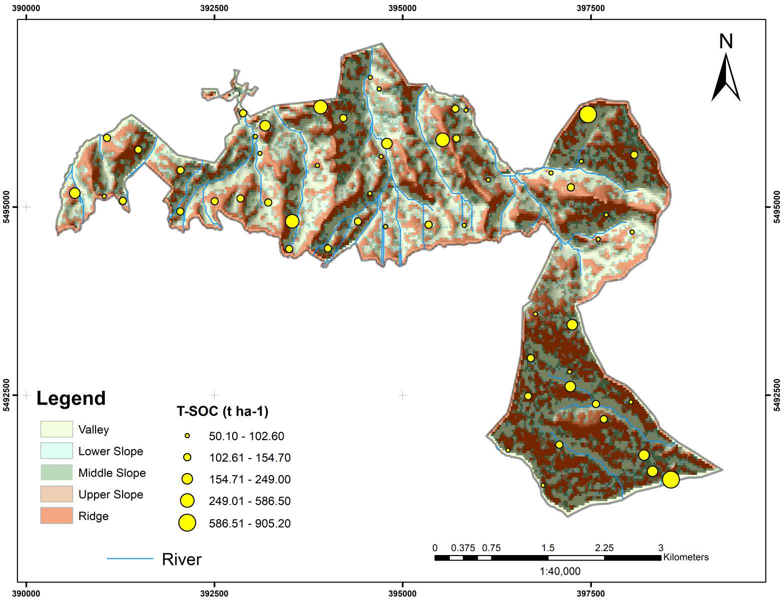


Fig. 3.8: Map of total organic carbon stock (t ha^{-1}) in soil and the Topography Position Index (TPI).

The interaction effects of soil type, altitude, and type of forest stand on the total soil organic carbon stock were confirmed by the general linear model (GLM) analysis (Table 3.2), which indicates that soil type was a more important factor than the other characteristics (Table 3.2). A pronounced increase in the T-SOC stock was found in histosols at a lower altitude under deciduous forest stands. A projection of the variables on the factor plane clearly demonstrates a correlation between total soil organic carbon stock and soil type, altitude, and type of forest stand (Figure 3.9). Two main factors had a significant total impact (41.63%) on the variance of the variables. Factor 1 explains 22.77% of the variance of the examined properties, whereas Factor 2 accounts for 18.86% of the variance. PCA analysis confirms the relationship between T-SOCstock and histosols and the dominance of the alder forest. Cluster analysis was used to classify cases (study plots) into groups called clusters. The results are illustrated with a dendrogram, which enables the identification of two main groups that differ in altitude, slope, T-SOCstock, and type of forest stand. The strongly moistened soils (histosols, fluvisols, gleysols, stagnosols) clearly differ from other soils. The latter group is clearly divided into study plots with podzols and poorer subtypes of cambisols and study plots with richer subtypes of cambisols (Figure 3.10).

Tab. 3.2: Summary of GLM analysis with "sequential" sum of squares (Type I) for the total soil organic carbon stock.

Characteristics	**T-SOCstock**	
	F	*p* value
Altitude	1.55	0.222
Soil type	9.96	<0.001
Type of forest stand	0.26	0.614
Altitude*Soil type	6.88	<0.001
Altitude*Type of forest stand	0.78	0.513
Soil type*Type of forest stand	0.29	0.593
Soil type*Type of forest stand*Altitude	6.67	<0.001

General Linear Model (GLM), Significant effects ($p < 0.05$) are in bold.

3.5 Discussion

Considering that the study area is 1754.5 ha, the carbon contribution to the environment is very important. It was estimated that this area provides 50.10–905.20 t ha^{-1} of soil organic carbon to a 1 m depth. In a study on British forest soils [Vanguelova et al., 2013], the authors noted high values of carbon stock (589 t ha^{-1}) in the top 80 cm, and 664 t C ha^{-1} in the top 1 m of soil, but their results were very dependent on climatic factors and soil type.

In Baritz et al. [2010], the carbon stock in the forest soils of Europe was estimated to range between 1.3 and 70.8 t C ha^{-1} for the O-layer and between 11.3 and 126.3 t C ha^{-1} for the mineral soil to 0–20 cm. In [Jones et al., 2005], forest soil carbon stock of 79 Gt C was reported for all European soils, including peat. In [Vos et al., 2015], total stocks were estimated to be 3.50–3.94 Gt C on the forest floors and

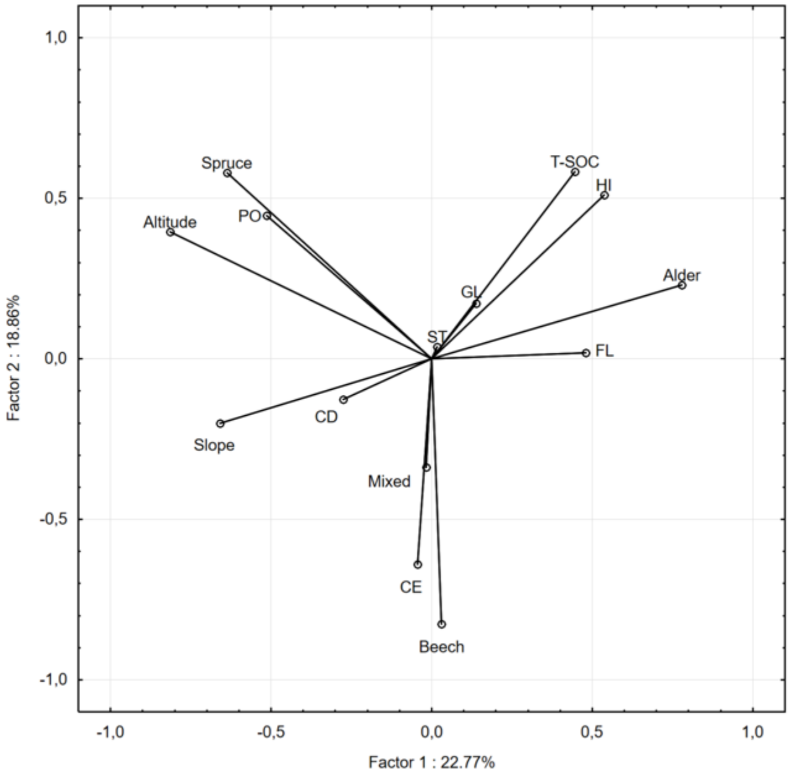


Fig. 3.9: Diagram of PCA with the projection of variables on a plane of the first and second factor for total soil organic carbon stock (T-SOCstock). CD: dystric cambisols; CE: eutric cambisols; FL: fuvisols; GL: gleysols; HI: histosols; PO: podzols; ST: stagnosols.

21.4–22.7 Gt C in the mineral and peat soils to a depth of 1 m in European forests. The topsoil SOC values for agricultural soils across Europe were determined to range between 40 and 250 t C ha^{-1} [Lugato et al., 2013]. The highest SOC values were found in Ireland, the UK, the Netherlands, and Finland, all of which had values of $>$250 t C ha^{-1} and correspond to peatland areas. The carbon stock in the mountain soils of BNP was comparable to the carbon stock for forest soils in other parts of Europe, and it is much higher than the average carbon stock in agricultural soils. Several papers confirm that the greatest organic carbon content is above a depth of 30 cm in different conditions [Don et al., 2007, Börjesson et al., 2018, Marinho et al., 2017].

The obtained results confirm the influence of topography on the accumulation of carbon in the soils of the BNP. The highest carbon stock was recorded in histosols, which are located in the lower positions of the BNP in valleys and on flat mountain areas. A high T-SOCstock was found in spruce stands created on podzols and located at higher altitudes. The temperature decreases with the altitude, which results in a slower rate of organic matter decomposition [Parras-Alcántara et al., 2015]. At the same time, low temperatures reduce the productivity of ecosystems [Zhu et al., 2018]. In [Chen and Tang, 2016], the authors found that SOC stock showed a

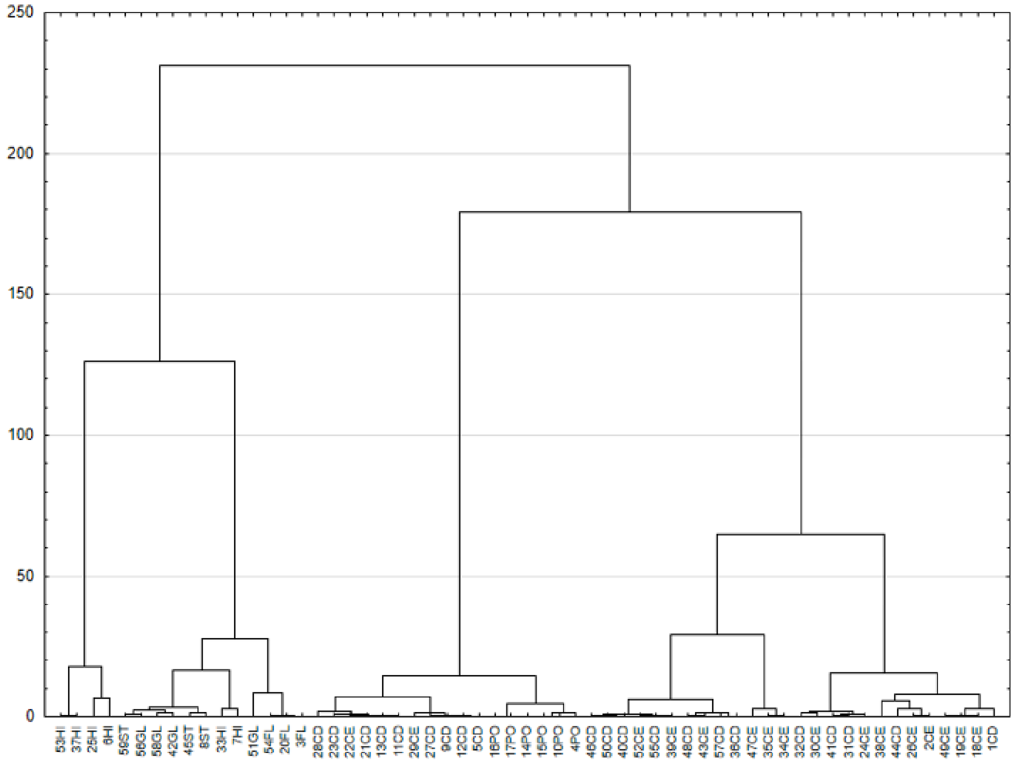


Fig. 3.10: Dendrogram with group identified in the cluster analysis. The altitude, slope, Total SOCstock and type of forest stands were used for diagram preparation (plots number plus symbol of different soil types, CD—dystric cambisols, CE—eutric cambisols, FL—fuvisols, GL—gleysols, HI—histosols, PO—podzols, ST—stagnosols).

decreasing trend at higher elevations, which is where the forest vegetation types transform into alpine shrublands. Altitude importantly but indirectly influences the SOC stock by affecting properties such as lower soil depths, different distributions of forest species, different soil types, and increased percentages of stone content [Kacprzak et al., 2013, Egli, 2016, Bardelli et al., 2017, Soucémarianadin et al., 2018]. In our study, the results of the PCA analysis confirm the importance of the slope in shaping the SOC stock. A lower slope position promotes vegetation growth, which results in higher SOC accumulation. In [Zhu et al., 2017], it was suggested that, at the hill scale, the variability of soil organic carbon stock might be explained by the slope rather than the elevation. Fissore et al. [2017] observed a strong relationship between the slope and the quantity and quality of SOC accumulation in hillslope systems. Specifically, moderate slopes (15%) combined with a concave profile and plan curvature led to greater SOC accumulation.

An important edaphic factor that determines the accumulation of SOC in soils is the parent material [Vos et al., 2015, Zhu et al., 2017]. In our research, high SOC stock was associated with sediments accumulated on terraces, which occur in low-lying valleys and flat mountain areas and are characterized by strong waterlogging.

The results of the cluster analysis identify high SOC stock in highly waterlogged soils. In [Nie et al., 2019, Zhu et al., 2018], the authors confirmed the relationship between SOC and soil water content, and soil moisture explained $>50\%$ of the total variation in SOC stock. The favorable humidity conditions have a positive impact on the productivity of forest ecosystems. Meanwhile, anaerobic conditions that reduce the activity of microorganisms prevail in heavily moistened soils, resulting in a slower rate of organic matter decomposition. In [Błońska et al., 2016, Slepetiene et al., 2018], the importance of soil moisture and acidity for soil organic matter accumulation was confirmed: 89% of the variance in the C content was explained by the hydrolytic acidity and moisture of the soils. In this study, the results show that histosols have the capacity to accumulate more carbon than other soil types. Histosols form when organic matter decomposes more slowly than it accumulates because of a decrease in microbial decay rates. This occurs most frequently in extremely wet areas or underwater. In [Slepetiene et al., 2018], the authors stated that wetland and peatland soils are among the largest organic carbon stocks, and they contribute to carbon emission or accumulation. Most histosols form in environments such as wetlands in which restricted drainage inhibits the decomposition process. The conclusion in [Jonczak, 2015a] asserted that histosols in northeastern Poland are rich in organic carbon content (162.2–459.5 g kg^{-1}). Similar values were found by [Glina et al., 2016], who examined the histosols of forest ecosystems. The organic carbon content in histosols in northeastern Poland was determined to contribute 40% in the first 10 cm [Jonczak, 2015b]. In our study, the soil with the highest supply of carbon was accompanied by alder. Alder species have a greater capacity to incorporate nitrogen (N) into the soil, facilitating the development of nearby vegetation and providing more organic carbon to the soil through the roots [Selmants et al., 2005]. Considerable carbon stock was recorded in soils under spruce stands. High SOC stock under these stands is the result of altitude, as well as the characteristics of this species. Spruce forests have more acidifying effects on the soil than deciduous or mixed forests [Jandl et al., 2007, Błońska et al., 2016], and acidifying species such as spruce decrease the rate of organic matter decomposition. In our research, the lowest SOC accumulation was recorded in the soils of mixed and beech stands. This is the result of these stands being located at a lower altitude, where thermal conditions favor the decomposition process.

3.6 Conclusions

The present study reveals that the SOC stocks in the mountain soils of the BNP are characterized by high variability (from 50.10 to 905.20 t ha^{-1}). The SOC stock in the mountain areas of BNP is affected by interactions between the soil type, altitude, and type of forest stands. The highest carbon stock was found in histosols with alder, which are located at lower altitudes in the valleys and flat mountain areas of the BNP. Higher positions were characterized by a slower rate of organic matter decomposition. A high carbon stock was noted in soil with spruce located at higher altitudes. We ranked the investigated soil types according to their capacity to carbon accumulation: histosols > gleysols > podzols > stagnosols > cambisols > fluvisols. In order to maximize the potential of carbon accumulation in the ecosystem, we emphasize the need to protect histosols, especially from dehydration, and to ensure the proper selection of tree species for breeding programs.

Chapter 4

The influence of tree and soil management on soil organic carbon stock and pools in dehesa systems

This chapter was published in the journal Catena.

Title: "The influence of tree and soil management on soil organic carbon stock and pools in dehesa systems"
Journal: CATENA
Editorial: Elsevier
(Click in the Link): `https://www.sciencedirect.com/science/article/abs/pii/S0341816220300606?dgcid=coauthor`

Authors: **Lizardo Reyna-Bowen**[1,2], Pilar Fernández-Rebollo[2], Jesús Fernández-Habas[2], José A.Gómez[1]

[1]Institute for Sustainable Agriculture-CSIC, 14080, Córdoba, Spain.

[2]Forestry Department, University of Córdoba, Rabanales University Campus, Ctra. Madrid-Cádiz Km. 396, 14014 Córdoba.

4.1 Abstract

This study evaluated the effect on SOC concentration, stock and fractions in a dehesa on the same soil type divided into two areas with different soil management. The first area was a pastured dehesa (P) with young Holm oaks, planted in 1995

(70 trees ha^{-1}, 12 m x 12 m) and, since 2000, grazed by sheep (3 sheep ha^{-1}) with the average period of grazing being six months a year. Prior to this it was managed in the same way as the second adjacent area. The second area was a cropped dehesa (C) with widely spaced mature Holm oak (14 trees in a 12-ha paddock), on which a mixture of vetch and oats was cultivated every three years and tilled with a chisel plough. After 22 years both dehesas showed similar SOC stock distribution amongst areas with different soil management, with approximately 40 t ha^{-1} in the top 100 cm of the soil. The P dehesa only showed higher SOC stock than the C dehesa on the surface 0-2 cm (5.86 ± 0.56 t ha^{-1} vs 3.24 ± 0.37 t ha^{-1}). The influence of the trees, increasing SOC concentration and SOC stock when compared to the area outside the canopy projection, was only detected in the mature trees in the C dehesa. In the area outside the tree canopy, both systems showed a similar distribution of soil organic carbon among their different fractions, with the unprotected fraction being the dominant one, followed by the physically and chemically protected fractions. In the C dehesa, the mature trees' presence significantly modified the distribution of soil organic carbon in their surroundings, increasing the relevance of the unprotected fraction. The distribution of soil organic carbon in the unprotected, and physically and chemically protected, fractions were strongly correlated to the overall organic carbon concentration in the soil indicating the rapid response of these three fractions to the overall carbon budget in the soil, with the biochemically protected fraction showing no correlation, suggesting a high resilience to the changes in carbon budget.

keyword: organic carbon fractions, agroforestry, shift from cultivation to grazing, crop rotation, tree plantation.

4.2 Introduction

Agro-silvo-pastoral systems are a form of land use where trees, crops, and livestock share the same plot of land [Cubbage et al., 2012]. The interaction of these four elements of agro-silvo-pastoral systems provides a variety of benefits and ecosystem services, including CO2 fixation in the woody tissues of trees and in the soil as organic carbon [Nair, 20008, Nair and Nair, 2014]. However, it is well known that soil organic carbon storage and dynamics depend on region, parent material, time, cover vegetation, and soil management [Jenny, 1980, Muñoz-Rojas et al., 2012]. Therefore, local studies are necessary to appraise the potential of soil to store carbon properly. Dehesas are important agro-silvo-pastoral systems in Mediterranean areas, particularly south-western Spain, as well as Portugal and Sardinia in Italy [Eichhorn et al., 2006, Caballero, 2009, Cappai et al., 2017]. In Spain, dehesas cover a total of around 4 million ha of land in the south-western provinces [Andalucia, 2017]. In Spain, the main purpose of dehesas is livestock production. Beef cattle, sheep and Iberian pigs roam freely in the dehesa and feed on pasture and acorns, all of which contribute to soil fertility [Cappai et al., 2017]. The Holm oak (*Quercus ilex*) is the most typical tree in dehesas [Costa, 2006]. Trees may also provide firewood and cork [Carbonero and Fernández-Rebollo, 2014, Cappai et al., 2017]. In some flat areas, dehesas are rotationally cultivated with cereal crops and legumes for hay, silage, grain and straw.

Several papers have addressed the effect of soil management on carbon storage in the dehesa, however it remains a topic which requires further research in this land use system. For instance, Parras-Alcántara et al. [2015], evaluating dehesas in southern Spain found no significant difference in soil organic carbon stock between the conventional and organic management systems, with 74.9 t ha^{-1} and 76.4 t ha^{-1}, respectively. Corral-Fernández et al. [2013], also in Spain, found that soil organic carbon concentration was only slightly different after 20 years of two types of tillage management in dehesas, which was lower in the less intensively farmed, but also noted a strong influence of soil depth on soil organic carbon storage and concentration. In our review, no studies were found evaluating the impact of grazing on soil organic carbon content in dehesa, but comparison to similar systems suggests that this might be dependent on climate type and grazing intensity. So, under arid or semi-arid climates (cool or warm), only low and medium grazing intensities were associated with increased soil organic carbon stocks when compared to ungrazed land, while in a warm, moist climate, all grazing intensity increased soil organic carbon stocks [Abdalla et al., 2018]. Differences in land use and its understory may have a greater impact on soil organic carbon in dehesas than grazing intensity, within the range of stocking density usually described. Pulido-Fernández et al. [2013], in Southern Spain, found the highest soil organic carbon stock in soils from dehesas (42.3 t ha^{1}), being almost twice that of tree-less grasslands (23.9 t ha^{1}) and degraded units (23.7 t ha^{1}). Another case study, by Upson et al. [2016], showed how the conversion of a grassland site in lowland England to either woodland or a silvopastoral-system modified the concentration in the top 150 cm of the soil. Fourteen years after tree planting, the organic carbon content in the 10 cm surface soil layer remained higher in grassland (6.0%), lower in the woodland (4.6%), and intermediate in the silvopastoral system (5.3%), with no differences among treatments below the 20 cm soil depth. Other examples of land use conversion show significant increases in soil organic carbon after the conversion of pasture to forest (8%), cultivation to pasture (19%), cultivation to plantation (18%) and cultivation to secondary forest (53%)[Post and Kwon, 2000, Paul et al., 2002, Guo and Gifford, 2002].

Lozano-García et al. [2016] indicates that in studies on soil organic carbon in the Mediterranean area it is necessary to address the spatial variability induced by slope, aspect, and the position of the trees. The influence of the tree below the canopy area is an important factor in the spatial determination of soil organic carbon. The horizontal and vertical extent of its roots affects the soil organic carbon content below and beyond the tree crown differently. Howlett et al. [2011] found that, in a silvopastoral oak dehesa system (mix of *Quercus ilex* and *Q. suber*) with native pasture and livestock production, there was a higher soil carbon below mature cork oak trees when compared to the area outside the tree canopy projection. Simón et al. [2012] highlighted the positive correlation between tree presence and soil organic carbon stocks up to distances of 8 m away from the tree trunk. The presence of the Holm oak has been noted by previous studies as an important factor for organic carbon sequestration throughout the soil profile [Gallardo, 2003, López-Carrasco et al., 2015]. In other forest systems, it has been shown that the presence of trees increased the soil microbial biomass carbon pool in the first layer [Kara et al., 2008], and improved the soil water holding capacity [Joffre and Rambal, 1993].However, it is unclear how long it takes to develop these differences since tree establishment and the interaction with specific land uses in the dehesa.

Soil organic carbon is a key attribute for soil quality. The impact of soil organic carbon on soil quality can be evaluated more precisely by its distribution amongst different organic carbon fractions with different degrees of protection against physical, chemical, and biochemical degradation. Six et al. [2002]proposed a fractionation method into four classes to evaluate soil quality in arable, afforested, and forest ecosystems. Among these fractions, the physically protected carbon associated with the 53-250 micro aggregates and the chemically protected carbon associated with aggregates <53 microns, are mostly responsible for the changes in long-term accumulation and stability of the carbon pool associated with changes in land use and management. In the same study, Six et al. [2002] showed how the unprotected carbon in macro-aggregates >250 microns are more sensitive to changes in agricultural soil management, while physically or chemically protected carbon greatly contributed to increased SOC stock in afforestation of cultivated land. Vicente-Vicente et al. [2017]analyzed the impact of management factors on soil organic carbon fractions in olive groves managed with a temporary cover crop, which can be considered a similar forest system. Their results suggested that an increase in soil cover by the temporary cover crop increases the capacity to store carbon in three different compartments (unprotected, physically and chemically protected), while the biochemically protected carbon is the most stable throughout the entire soil profile, regardless of management or vegetation cover. We planned the study presented in this manuscript with the aim of developing new knowledge that could contribute to a better quantification of the potential of dehesas to store organic carbon in the soil using different management options. Therefore, the study described in this manuscript encompassed three specific objectives: 1) To quantify the soil organic carbon (SOC) stock in a dehesa with a similar soil type and history, but two different soil management after 22 years. 2) To evaluate the effect on SOC stock of a strategy for tree regeneration in the dehesa based on plantations with a higher tree density, which were introduced in southern Spain as part of the reforestation program promoted by the European Commission in 1992. 3) To explore the implications of two different types of soil management and the influence of the trees on the distribution of SOC in different fractions that differ on the stability.

4.3 Material and methods

4.3.1 Area description

This study was conducted in an experimental farm located at Hinojosa del Duque, Cordoba, Spain ($38°29'46''$ N and $05°06'55''$ W , see Fig. **??**), at 543 meters above sea level. The area has a mean annual rainfall of 437 mm and an average annual temperature of 15.1 $°C$ (average for 2010 to 2017; meteorological experimental station, Hinojosa del Duque). The soil type in the study area is classified as Eutric Cambisol with a shallow depth and rocky outcrops (CSIC-IARA, 1989). This farm was selected because it covers two adjacent areas (Fig. 4.1) with different features, and maintains detailed records about soil management for the last few decades. The first area is a pastured dehesa with young Holm oaks (hereinafter, P). Holm oaks were planted in 1995 at a density of 70 trees ha^{-1} (in a regular frame of 12 m x 12 m). This planting included soil preparation by subsoiling and, during the first five years, the control of herbaceous vegetation with a disc plough. At the time of our sampling, March 2017, the mean diameter at breast height (DBH) was 17.2±2.6 cm and canopy cover was around 10%. The area had been grazed by Merino sheep since 2000, at a stocking rate of 3 sheep per hectare. Grazing is conducted rotationally with at least four grazing periods per year (with the average period of grazing being six months a year). In 2016, natural pastures were fertilized with 40 kg of P_2O_5 ha^{-1}. Prior to 1995 the management of this area was similar to the second area described below.

The second area is a cropped dehesa with widely spaced (1.2 trees ha^{-1}) mature Holm oaks (hereinafter, C). A historical aerial photograph shows that in 1956 these trees, already matured, were already present with the same spatial structure, so it is at least 90-100 years old. At the time of sampling, the mean DBH of Holm oaks was 78.1±13.6 cm and the tree canopy cover was less than 1%. Every three years, a

Tab. 4.1: Soil properties of the two areas - pastured dehesa with young trees (P) and cropped dehesa with mature trees (C). For BC horizon, data shown in this column indicate the depth at which this horizon begins; CEC: Cation exchange capacity; K: Available Potassium ppm; N: Organic Nitrogen; P: Available Phosphorus (Olsen) ppm, S.T.C: Soil Textural Class.

Dehesa	**Hz**	**Depth (m)**	**pH 1/2'5**	**OM %**	**N %**	**P**	**K**	**CEC**	**Ca**	**Mg**	**Na**	**Clay**	**Sand**	**Silt**	**S.T.C.**
								(meq/100g)				**(%)**			
P	A	0.37	6.55	0.78	0.04	117.50	43.40	7.89	4.35	2.80	0.45	8	78	14	Loamy sand
	B	0.63	7.19	0.44	0.03	136.50	32.00	17.02	12.33	3.84	0.57	21	70	8	Sandy clay loam
	BC	>0.63	7.98	0.10	0.01	76.50	10.40	8.75	5.13	2.89	0.57	6	89	6	Sand
C	A	0.36	6.54	1.40	0.07	205.00	28.10	6.27	2.86	2.45	0.47	7	83	10	Sand
	B	0.77	7.75	0.49	0.02	155.50	4.95	19.55	13.07	5.36	0.80	23	68	9	Sandy clay loam
	BC	>0.77	8.65	0.16	0.01	103.00	3.40	14.62	10.36	3.11	0.96	13	81	6	Loamy sand

mixture of vetch and oats is cultivated for hay. Prior to sowing, the area is fertilized with 20 t ha^{-1} of dairy manure. A chisel plough is used for soil tillage, with tillage covering all the plots except the immediate the vicinity (0.3-0.4 m approximately) of the tree trunk. After harvest, and for the following two years of the three-year period between consecutive sowings, the area is grazed, keeping to a similar scheme to that of the pastured area. The main soil properties for both areas, taken from four soil pits made at the time of sampling (two for each area, see section below), are shown in Table 4.1.

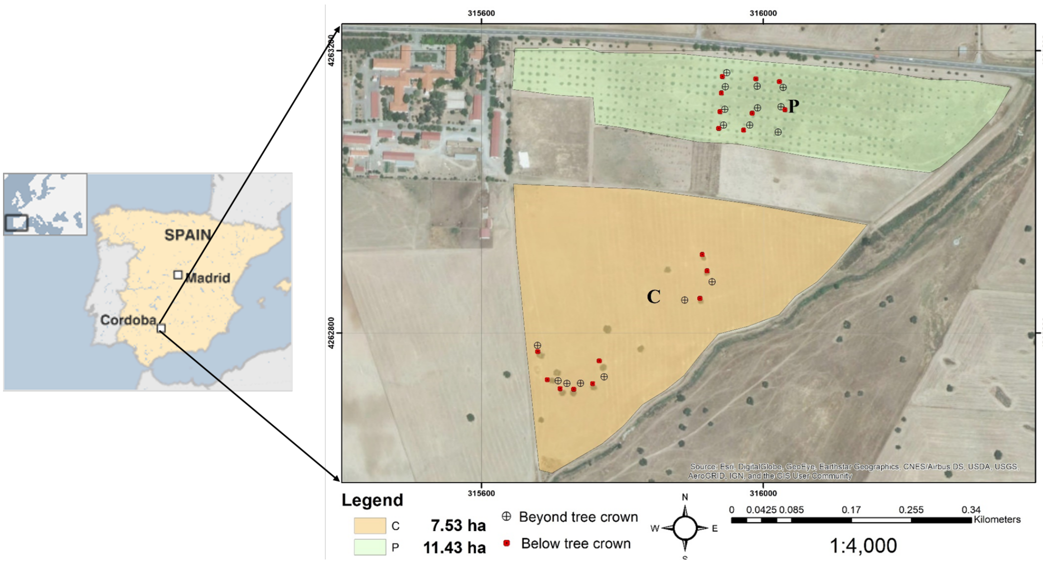


Fig. 4.1: Location of the experimental farm at Hinojosa del Duque, Córdoba, Spain. Study areas are highlighted with different colours: Pastured dehesa with young trees (P) in green, and cropped dehesa with mature trees (C) in brown. Red circles represent soil sampling points below the tree crowns, and dark circles with a cross inside mark sampling points beyond the tree crown projection.

4.3.2 Soil characterization and sampling

Four soil profiles were described by digging four pits, two in each area and with all the pits starting near the tree trunk and crossing below and outside the tree crown projection area. The description of the profile, including visual assessment of root density by root size, was made according the NRCS guidelines, Schoeneberger and Staff [2012], distinguishing between the areas below and beyond the tree crown projection in all the pits. Undisturbed soil samples were taken with a hand soil sampler to determine bulk density (BD). The samples were taken at the 4 pits, distinguishing between pit zone (below and outside the tree canopy) and at four depths (0-5 cm, 20-40 cm, 40-60 cm and 60-100 cm), with two replications totalling 64 samples. The samples were oven-dried at 105 $^{\circ}C$ for 72 hours to a constant mass. The bulk density of the soil was calculated by dividing the dry mass of soil by the volume of the bulk density sampler (98.2 cm^3), according to Hao [2008].

In each area (P and C), 10 random sampling points were selected outside the tree canopy projection. Additionally, the nearest tree to each point was identified and a new sampling point under the canopy was selected, but at a distance from the trunk that located this point within the ploughed area in the C dehesa. Therefore, a total of 20 sampling points per area were sampled. Samples were taken at 8 different depth intervals (0-2 cm, 2-5 cm, 5-10 cm, 10-20 cm, 20-40 cm, 40-60 cm, 60-80 cm and 80-100 cm, where possible) at each sampling point, having previously removed the grass and mulch surface. Soil samples were taken combining a manual soil sampler (for the three top-soil samples) and a hydraulic soil sampler (Giddings®) with a 38.1 mm diameter soil core. Overall a total of 266 soil samples were taken (area x sampling points x depth) because the soil depth at the hard C horizon was less than 100 cm at some sampling points.

4.3.3 Soil analysis

The soil samples were ground, passed through a 2 mm sieve, and homogenised. Stoniness, defined as coarse material whose diameter is $>$2 mm, was determined as a % of mass. Soil organic carbon (SOC), a concentration of fine earth ($<$2 mm diameter) was determined according to Walkley [1947]. Soil organic carbon stocks for each soil depth interval (SOCstock i), and for the whole soil profile, were calculated according to IPCC [2003]: Equation 4.1:

$$SOC_{stocki} = 10000SOCi \cdot BDi \cdot d \cdot (1 - \rho) \tag{4.1}$$

$$SOC_{stock} = \sum_{i=1}^{i=n} SOC_{stock \quad i} \tag{4.2}$$

Where SOCstock i is the total soil organic carbon in a given layer (t ha^{-1}). SOCi is the organic carbon concentration (g g^{-1}), BDi is the bulk density of the soil (t m^{-3}) as defined above, d is the thickness of the depth interval (m), δ is the fraction (0 - 1) of gravel larger than 2 mm in the soil sample, and n is the number of soil layers. Therefore, equation 2 gives the total soil organic carbon, SOCstock (t ha^{-1}) in the whole soil profile discounting the effect of stoniness. BD values for soil depths not sampled were interpolated using mass-conserving splines [Malone, 2017]. Mean values of BD in each area, below and outside of the tree canopy, were used in Equation 4.1.

4.3.4 Soil organic carbon fractionation

Soil organic carbon fractionation was used on a subset of selected samples. For our exploratory analysis, we chose samples from the 0-2 cm, 2-5 cm, 20-40 cm and 40-60 cm depth intervals, and from amongst them, the samples with the maximum and minimum organic carbon concentration values were chosen in both study areas, in the zone below the tree crown and beyond it. Fractionation was carried out following the method proposed by Six et al. [2002]. This method, which combines physical, chemical and biochemical fractionation, allows the determination of four different pools of soil organic carbon: (i) the unprotected fraction, which is the particulate organic carbon in aggregates measuring 2000-250 μm, separated by sieving, plus the light fraction (LF) of the 250-53 μm aggregates, separated by flotation and centrifugation of the $>$53 μm aggregates; (ii) the physically protected fraction, which is the organic carbon in the 250-53 μm aggregates that remains stable after centrifugation, once we have discarded the light fraction by flotation; (iii) the chemically protected fraction, which is the hydrolysable portion, after acid hydrolysis, of organic carbon in aggregates measuring $<$ 53 μm, the slime-sized fractions and clay isolated during the initial sieving and dispersion; and (iv) the biochemically protected fraction, which is the non-hydrolysable organic carbon remaining in the slime and clay fractions after the acid hydrolysis. The concentration of organic carbon in each pool was determined by wet oxidation using sulphuric acid on samples between 0.3–0.5 g using potassium dichromate with an absorbance spectroscope in the range of 600 μ [Vicente-Vicente et al., 2017, Jindo et al., 2016].

4.3.5 Statistical data analysis

In each soil depth sampled, two-factors ANOVAs were performed to evaluate the effects of (i) the soil management, (ii) the tree presence (below and outside the canopy projection) and (iii) their interactions on BD, stoniness, SOC concentration, SOC stock and soil organic carbon fractions. SOC and SOC stock data were transformed to fulfil the ANOVA requirements (inverse and logarithmic functions for SOC and SOC stock respectively). Data of soil organic carbon fractions were grouping in two depths: top (0-2cm, 2-5cm) and deep (20-40 cm, 40-60 cm) layers. The relationship between soil organic carbon fractions was explored by Pearson coefficient correlation. The overall effect of depth on BD, stoniness and SOC was evaluated using a Kruskal-Wallis test and Mann Whitney U test, in the case of soil organic carbon fractions.

BD and stoniness are involved in the calculation of SOC stock and small differences in both variables could affect the final results of carbon stock and mask the differences induced by soil management. Additionally, is known that BD and stoniness can experience in a same area large spatial fluctuation, even at close distances. We used the non-parametric bootstrap approach to assess the uncertainty in SOC stock estimation in C and P related to stoniness and BD (Efron and Tibshirani, 1986). We pooled all stoniness data and, per depth, a new random sample of a size similar as number of sampling points we had at that depth (10 or lesser) was extracted by resampling with replacement. This bootstrap sample of stoniness was then used to calculate SOC stock at each sampling points (10) following equation 1 and 2, where SOC concentration and BD were the original values resulted from samplings. Finally, we averaged the SOC stock from the 10 sampling points and the resulted mean was denoted as SOC stock*. We repeated this process 500 times and compiled the bootstrap distribution of SOC stock* in each area P and C. The uncertainty was quantified by the confidence interval at the 95% level, using the 2.5 and 97.5 percentiles of the bootstrap distributions of SOC stock*. If the confidence interval included the original mean of SOC stock resulting from the sampling, then the effect of stoniness in SOC stock calculation was said to be negligible. A similar procedure was followed with BD, except for the resampling stage. In this case, only BD data from the same area were pooled, due to the significant influence of soil management on BD. The bootstrap sample of BD was then used to calculate SOC stock, keeping in this case the original values of SOC concentration and stoniness from samplings. Calculations and analysis were performed using the R language for statistical computing (R Core Team, 2013) and Statistica SE 14.

4.4 Results

4.4.1 SOC concentration

The SOC concentration was highly stratified, with a clear trend to an exponential decrease in line with depth, particularly in the top 20 cm of the soil (Fig. 4.2). Overall, mean values decreased from 1.81% near the surface to 0.14% between 80 and 100 cm. Soil management affected SOC concentration only in the uppermost 2 cm, reaching average values of 1.30±0.41% in C dehesa and 1.99 ± 0.57% in P dehesa. Holm oak increased SOC concentration in C dehesa by up to 20 cm depth. This increase ranged between 53%, for the top 2 cm, to 142%, for the 10 cm-20 cm soil layer. However, in P dehesa, the presence of the Holm oaks had an insignificant effect on SOC concentration. Roots were concentrated in the A horizon, very fine and fine roots were common under the Holm oak canopy of both dehesas C and P, being more abundant beyond the tree canopy zone in P dehesa (Supplementary material 1, 4.7). Also, in the A horizon, medium and coarse-sized roots were more abundant. In the B horizon, the presence of very fine and fine roots was common under the Holm oak canopy in both areas, but scarce beyond the tree canopy projection area. Very few coarse roots were found in this horizon. In the BC horizon (more than 60 cm depth), no roots were found in either the C or P dehesa.

4.4.2 SOC stock

SOC stock presented a similar depth-distribution amongst areas with different soil managements (P and C) and between zones (below and beyond tree canopy) as those already discussed for SOC concentration (Supplementary material 2, 4.8). Therefore, P dehesa only showed a higher SOC stock than C dehesa at the surface 0-2 cm (5.86±0.56 t ha^{-1} vs 3.24±0.37 t ha^{-1}). Mature trees in the C dehesa contributed to a significant increase in the carbon stock in the topsoil layer (0-20 cm) by an average of 87%, however, young trees had no significant effects in P dehesa.

Figure 4.3 depicts the SOC stock accumulated up to a depth of 20 cm, 40 cm and the total sampled profile. The mean soil depth ranged between 89 cm in P dehesa outside the tree influence, to 81 cm in C dehesa, under the tree canopy. Soil depth reached 100 cm only at 36% of the sampled points. P dehesa showed significantly higher SOC stock than C dehesa in the 0-20 cm profile. However, the differences were not significant when, for carbon stock calculation, 40 cm depth or the total sampling depth were considered. Up to a depth of 20 cm, P dehesa stocked, on

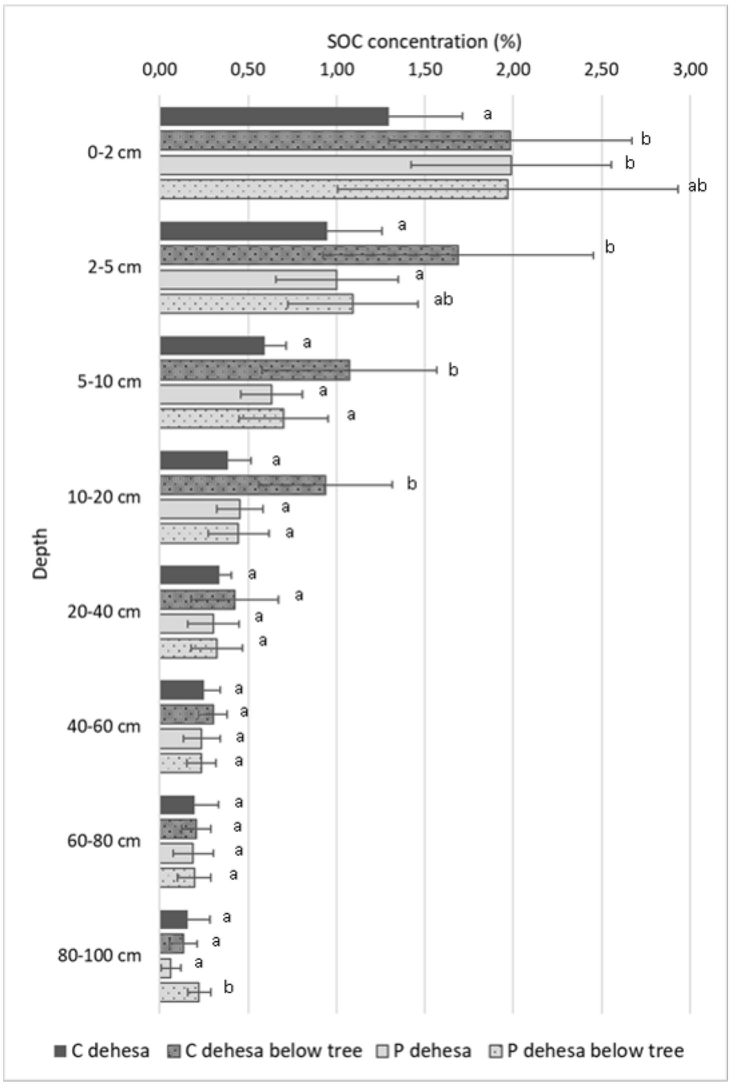


Fig. 4.2: Distribution of SOC concentration with depth at C and P dehesas outside and under the tree canopy (mean and standard deviation). At each depth, different letters indicate a significant difference between soil management and tree influence (beyond-below tree canopy).

average, 6.5 t ha^{-1} more than C dehesa, which represents an increase of 42%. Mature trees significantly increased carbon stocked in C dehesa (up to 20 cm, 40 cm and at total soil depth). Total carbon stocks were 39.9 t ha^{-1} and 38.8 t ha^{-1} in P and C dehesa respectively, and 45.6 and 52.5 t ha^{-1} under the tree canopy (Fig. 4.3). The top 40 cm held, on average, more than 70% of the carbon stocked in the soil profile.

Stoniness increased with depth and was significantly higher in C dehesa (8% and 12% on average in P and C dehesas) (Table 4.2). This slight difference in stoniness could positively affect the computation of total carbon stock in P dehesa, or vice versa in case or C dehesa. However, resampling stoniness led to similar mean of total carbon stock in C and P dehesa (39.7 and 40 t ha^{-1} respectively), given that

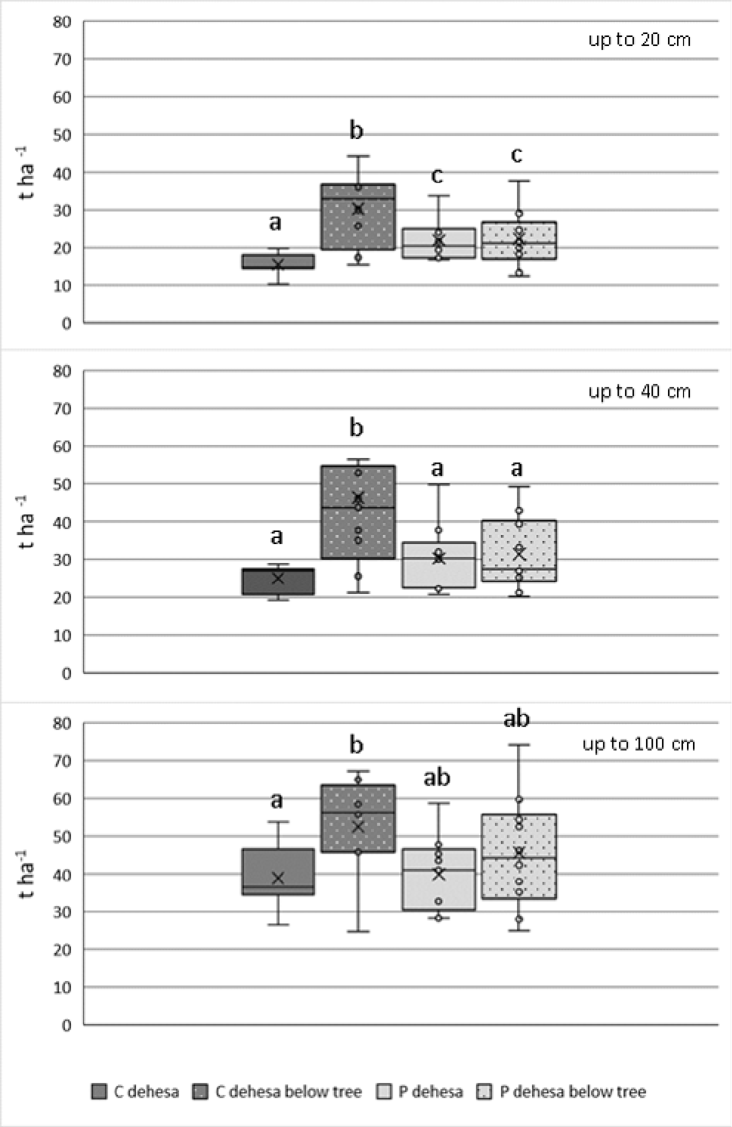


Fig. 4.3: SOC stock at different depths at C and P dehesas outside and under the tree canopy. Different letters indicate a significant difference between soil management and tree influence (beyond-below tree canopy at P and C dehesa).

mean values were within the confidence interval of the bootstrap distribution of SOC stock* (Fig 4.4, A and B). Like stoniness, soil bulk density increased significantly with depth (Table 4.2). On average, BD ranged from 1.49 t m^{-3} in the top layer (0-2 cm) to 1.64 t m^{-3} at the deepest depth interval measured (80-100 cm). The effect of different types of soil management on bulk density was restricted to the topsoil layer (0-5 cm), with higher values in the P dehesa. In both areas (P and C), trees significantly decreased the soil bulk density of the top layer under their canopy. Figure 4.4 (C and D) showed bootstrap distribution of SOC stock* after BD resampling. According to the position of the mean value in the range of confidence interval, the use of raw data of BD from each area instead of mean value, resulted in slight higher value of stock in C dehesa but similar in P dehesa (39.7 t ha^{-1} in P dehesa and 39.4 t ha^{-1} in C dehesa).

Tab. 4.2: Mean values and standard errors of soil bulk density and stoniness according to depth in P and C dehesa, below and outside the tree canopy projection. At each depth, different letters indicate a significant difference according to two-factors ANOVA ($p<0.05$).

Depth (cm)	Bulk density (t m^{-3})				Stoniness (%)			
	P-dehesa	P-dehesa below canopy	C-dehesa	C-dehesa below canopy	P-dehesa	P-dehesa below canopy	C-dehesa	C-dehesa below canopy
0-2	1.62 (0.05) a	1.53 (0.13) b	1.53 (0.02) b	1.28 (0.05) c	6.8 (1.22) a	7.5 (0.77) a	10.7 (1.45) b	9.2 (1.29) b
2-5	1.61 (0.05) a	1.53 (0.12) b	1.53 (0.01) b	1.23 (0.04) c	8.4 (0.97) a	7.1 (1.15) a	11.3 (1.85) b	10.6 (1.79) b
5-10	1.57 (0.06) a	1.54 (0.01) a	1.53 (0.01) a	1.35 (0.05) b	2.1 (0.94) a	4.6 (2.06) a	9.8 (2.96) b	7.7 (1.55) b
10-20	1.49 (0.09) a	1.54 (0.07) a	1.52 (0.03) a	1.44 (0.04) a	5.0 (0.97) a	5.3 (1.52) a	12.4 (2.75) b	10.7 (2.97) b
20-40	1.46 (0.09) a	1.56 (0.06) a	1.55 (0.05) a	1.59 (0.05) a	8.0 (0.78) a	7.4 (1.36) a	8.5 (1.70) a	11.4 (2.23) a
40-60	1.70 (0.03) a	1.61 (0.11) a	1.65 (0.02) a	1.67 (0.04) a	9.9 (1.52) a	8.3 (1.28) a	12.4 (1.82) a	11.3 (2.28) a
60-80	1.69 (0.08) a	1.55 (0.12) a	1.69 (0.05) a	1.69 (0.02) a	14.2 (2.97) a	9.6 (2.33) a	10.0 (2.08) a	11.4 (1.51) a
80-100	1.63 (0.09) a	1.50 (0.11) a	1.71 (0.08) a	1.71 (0.05) a	9.5 (1.95) a	7.0 (2.50) a	13.9 (2.85) b	17.0 (2.00) b

Tab. 4.3: Mann-Whitney U test of the soil organic carbon (SOC) concentration and stock at each fraction with soil depth as independent factor. Significance is noted as: ns: not significant; *: $p< 0.05$; **: $p<0.01$; ***: $p<0.001$.

Effect	Variable	Z	p-value
Depth	SOC unprotected	2.669	**
	SOC physically protected	2.947	**
	SOC Chemically protected	3.829	**
	SOC Biochemically protected	-1.137	ns
	Stock unprotected	1.880	ns
	Stock physically protected	0.859	ns
	Stock Chemically protected	-0.580	ns
	Stock Biochemically protected	-3.133	**

4.4.3 Soil organic carbon pools

Figure 4.5 depicts the SOC concentration in the different pools by soil management (C and P) and location to the tree canopy at top and lower soil layers. The absence of the tree effect on SOC concentration in the different pools, as has been already shown for bulk SOC concentration, is apparent from the analysis in P dehesa. In the topsoil of C dehesa, there is higher concentration of SOC in the unprotected and the chemically protected pools in samples located below the tree canopy. No differences were detected between types of dehesa in the SOC concentration of the different pools with the exception of the biochemically protected. Additionally, Figure 4.5 compares the SOC concentration of the different pools between the under-tree canopy of both types of dehesa. It is apparent how the differences in SOC concentration are concentrated in the unprotected pool, which tends to be higher in C dehesa. Overall, there was a clear effect of depth on SOC concentration of all the fractions except the biochemically protected ones (Table 4.3).

There was a clear correlation between SOC concentration of the unprotected, and physically and chemically protected, fractions with SOC concentration in the bulk soil (Table 4.4). SOC concentration for the physically and chemically protected pools was higher than that of the bulk soil, particularly the chemically protected pool, while the unprotected fraction presented a lower concentration than that of the

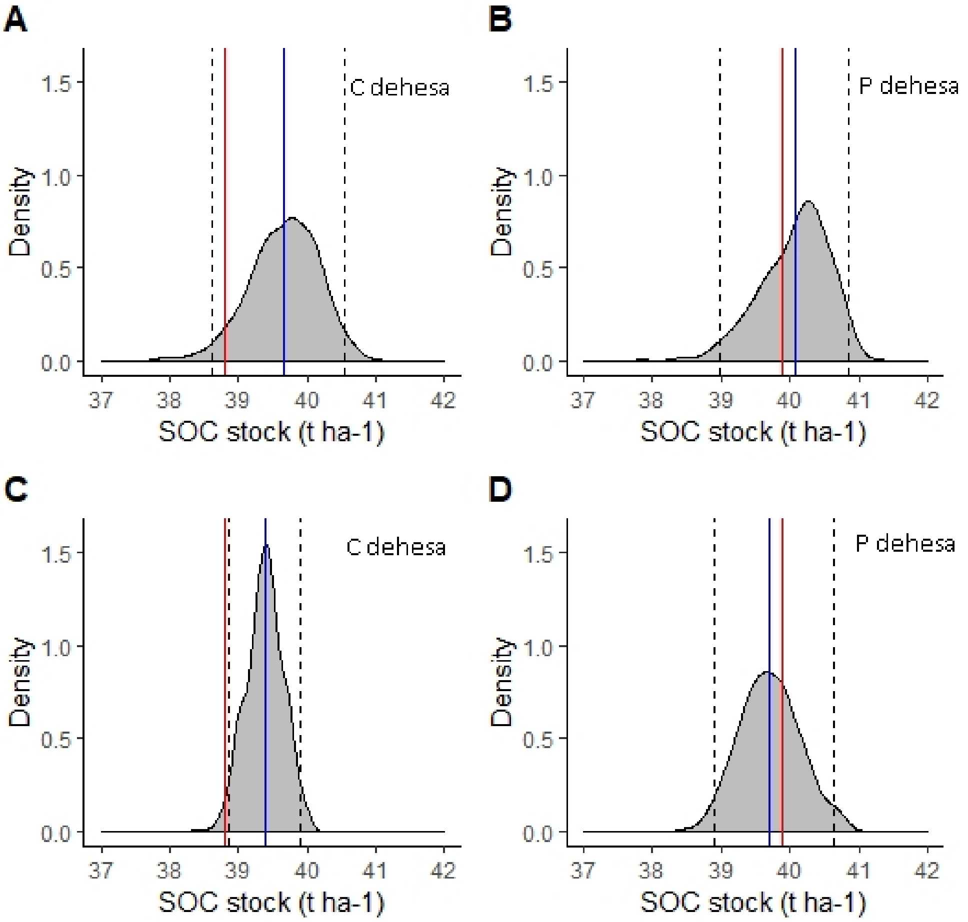


Fig. 4.4: Bootstrap distribution of SOC stock after stoniness (A and B) and bulk density resampling (C and D) showing the mean (blue lines) and the confidence interval (2.5 and 97.5 percentiles; dotted lines). The red line shows the mean SOC stock resulting from sampling points.

bulk soil. There was no correlation between the SOC concentration of biochemically protected pool and that of the bulk soil.

The distribution of the total SOC among the different pools is depicted in Figure 4.6. In P dehesa the largest pool of organic carbon was in the unprotected fraction, at around 30%-45%, although the other three pools had a slightly lower contribution, ranging from 15% to approximately 30%, with the exception of the biochemically protected pool in the topsoil itself beyond the tree canopy, which represented a small fraction of around 3%. There was a tree effect on the relative contribution of the unprotected pool, which tended to decrease under the tree canopy, and in the biochemically protected pool, which tended to increase in relative contribution under the tree. In C dehesa, the unprotected carbon pool was also the fraction with the largest contribution to the total SOC, although this time with a higher magnitude than in P dehesa, ranging from 30% to 75%. The other two organic carbon fractions with a larger contribution were the physically and chemically protected fractions,

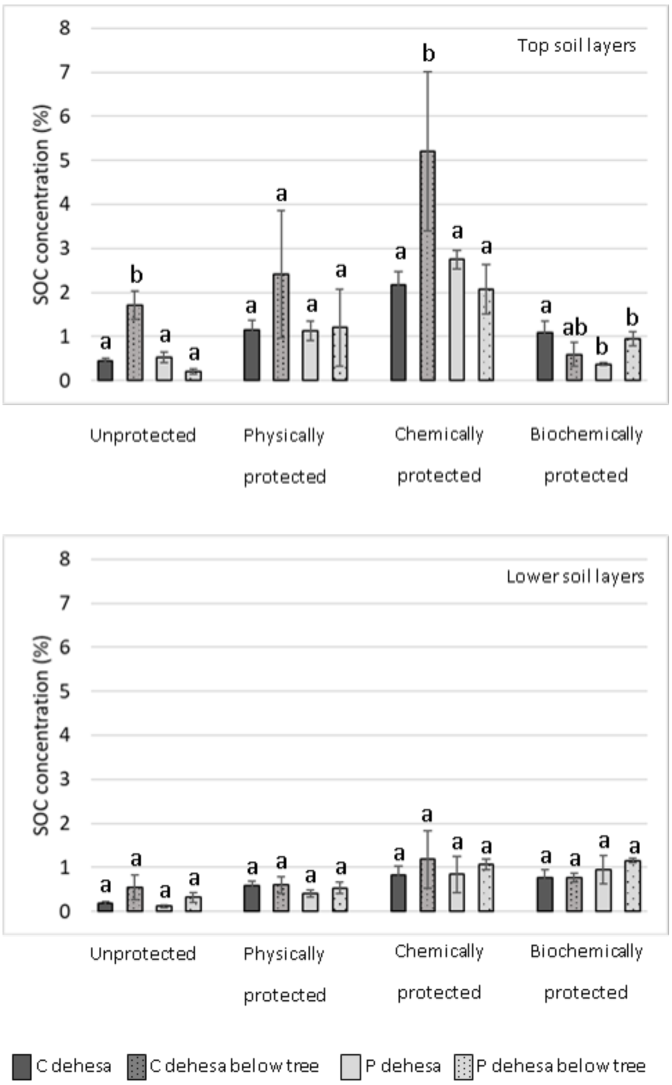


Fig. 4.5: Soil organic carbon (SOC) concentration by fractions at C and P dehesa outside and under the tree canopy by depth. Top layers (0-2 cm, 2-5 cm), lower layers (20-40 cm, 40-60 cm). In each fraction, different letters indicate a significant difference according to soil management and tree influence.

both ranging from 10% to approximately 25%. The biochemically protected pool contribution presented a wider variation, ranging from 2% in the top layer of the under-canopy area up to 20% in the lower soil layer. In C dehesa there was a significant tree-effect on the relative contribution of the unprotected carbon pool, which tended to have a higher contribution under the tree canopy. Table 4.3 showed that depth does not affect to the relative contribution of each fraction to the overall SOC stock, which only varies in the biochemically protected fraction due to the increase of their relative contribution as the soil depth increases.

Tab. 4.4: Pearson correlation coefficient between the soil organic carbon concentration in the bulk soil and in the different soil fractions. Significance is noted as: ns: not significant; *: $p< 0.05$; **: $p<0.01$; ***: $p<0.001$.

	SOC fractions			
	Unprotected	Physically protected	Chemically protected	Biochemically protected
Bulk soil	0.98***	0.72***	0.87***	-0.29 ns
Unprotected		0.61**	0.82***	-0.30 ns
Physically protected			0.85***	-0.25 ns
Chemically protected				-0.45*

4.5 Discussion

4.5.1 SOC concentration in dehesa

Our results clearly show that SOC concentration decreases with depth in the dehesa system. These results are consistent with the literature [González et al., 2012, Pulido-Fernández et al., 2013, Francaviglia et al., 2017]. In fact, the highest SOC concentration was found in the top 0-5 cm, although with a high variability, ranging from 1.2% to 2.0%. These results are similar to those reported by Pulido-Fernández et al. [2013] for dehesas in Leptosols, 2.3% and Luvisol 1.1%. The authors attributed this variation in the first 5 cm to the type of land use. Higher values may be found in other dehesas, an example being a study consisting of 36 different dehesas in Mainland Spain featuring Holm oaks with scattered tree cover [González et al., 2012]. The authors reported the same mean SOC concentration as in our study (1.6%). However, this was in the first 20 cm of soil, suggesting even higher values in more superficial layers. Such a discrepancy may be explained by differences in the climatic conditions, as our study site was more arid, and also had lower thickness and clay content in the surface layer.

The decreases in SOC concentration were clearly stratified at depths of up to the 20 cm, after which the trend continued without significant differences. This 20 cm depth threshold, in which the depth-dependent relationship of SOC concentrations was strongest, coincides with the A horizon in our soils. It has previously been shown that both SOC and depth of the A horizon are highly correlated with the soil type [Premrov et al., 2017], as well as the rhizosphere depth, the historical use of the land, and the orography [Marinho et al., 2017]. The type of vegetation has a further influence on the rhizosphere width due to significant differences in the root systems, such as woody plants having deeper root systems than herbaceous plants [Pulido-Fernández et al., 2013, Zhou et al., 2016]. In our experiment, most of the roots of the trees and herbaceous vegetation were found in the A horizon.

4.5.2 Effect of soil management on SOC concentration

With respect to soil management, in our study cereal-legume cultivation in rotational 3-year cycles, plus sheep grazing, resulted in a similar SOC concentration to no-tillage farming and exclusively sheep grazing, albeit with the exception of the topsoil layer (0-2 cm). It is also generally accepted that a change of land use from agriculture into permanent grassland can increase soil organic carbon concentration, at least in the first years after the transformation [Mohanty, 2017, Abdalla et al., 2018]. The relevance of our study is to show that, for the conditions studied, this transformation from three-year rotation into permanent grassland has not increased the SOC concentration after 20 years. This might be explained by the relatively low tillage intensity of the three-year rotation employed together with the organic fertilization applied to the crop, by the moderate productivity of the permanent grassland under rain-fed Mediterranean conditions in the study area which did not receive additional fertilization, and by the fact that the plantation of trees in the P dehesa required periodical tillage during the first five years to control competition for soil water by the weeds growing naturally on the farm. In the latter explanation, such intense work on the soil could result in a decrease in SOC concentration during the first few years, which the soil may still be recovering from. Nevertheless, there are other examples in the literature that did not find the expected increase to changing soil management under Mediterranean conditions, particularly in more arid areas. For instance, Romanyà et al. [2010] already stated that state that after abandoning agricultural lands, the capacity of C sequestration would be greater in wet areas and lowest in semiarid. Low levels of organic C in semiarid and Mediterranean soils suggest that the recovery of C after the abandonment of arable may not take place mainly as a result of ecological and soil constraints existing in dry and semiarid areas it can take a very long time. Romanyà et al. [2000] estimated that the original SOM content can be reached after 60-100 years after reforestation with P. radiata of cereal fields.

4.5.3 Effect of the trees on SOC concentration

SOC concentration not only depends on depth, but also on other factors relating to the presence of trees. However, in this study, higher SOC concentrations were only found in the presence of mature trees, up to a depth of 40cm, but not in the presence of younger trees. This finding echoes several research works carried out in dehesas [Gallardo, 2003, Howlett et al., 2011, Simón et al., 2012, González et al., 2012, Pulido-Fernández et al., 2013] and in other agroforestry systems [Fernández-Ondoño et al., 2010, Monroe et al., 2016, Upson et al., 2016]. Since the trees' influence on SOC is strongly related to their roots, their presence was evaluated in the A horizon of both dehesas in our study. Thus, lower root density was found in the open area compared to the area below the tree canopy of both young and mature trees (samples from P and C dehesa, respectively), but the highest root density was found under the mature trees. We also documented a sharp decrease in root density from the A to B horizon. Although several studies have recorded that oak roots can extend to deep soil horizons [Moreno et al., 2005], many studies have found that most of the root biomass is concentrated in the top 30cm of the soil horizons [Canadell, 1996, Moreno and Obrador, 2007, Ojeda et al., 2017, Meier et al., 2017]. This might explain why Pulido-Fernández et al. [2013] found differences between SOC concentration from samples taken beneath tree canopies when compared to those solely from the 0cm-5cm layer in open areas. Moreover, another factor affecting the topsoil underneath the canopies is the accumulation of fallen leaves, as was studied in a sessile oak site [Kara et al., 2008]. Nevertheless, this effect is more evident in soils under deciduous trees rather than evergreen trees.

Our results reinforced the hypothesis that trees can significantly increase SOC concentration after a relatively long period of time (more than 22 years, but less than 90 years in our case), with it being necessary to incorporate a temporal dimension when appraising the impact of trees on SOC concentration in the dehesa system. This is in line with results obtained in other agroforestry systems, such as those studied by Upson et al. [2016] in England, where the introduction of ash trees (Fraxinus excelsior) into pasture grazed by beef cattle had not increased SOC 14 years after planting. Accordingly, introducing trees onto grasslands does not necessarily increase SOC content in the one or two decades after planting. In fact, after tree plantation, SOC could even decrease because of the priming effect of new root exudates and dead roots [Cardinael et al., 2018]. In this connection, Haile et al. [2010], indicate that agroforestry systems can help soil carbon sequestration in the long-term, while, in the short-term, carbon conservation may be unstable, mainly due to certain soil management practices. This seems to be the case with the P dehesa in our study, where the dominant source of organic carbon in the soil remains herbaceous vegetation and animal manure. Several studies indicate that the fine roots of herbaceous crops and pastures are more abundant than those of trees, while also having a higher renovation rate [Persson, 1983, Canadell, 1996, Moreno et al., 2005]. It is easier for these fine roots to integrate into the soil in a more protected form of carbon [Rasse et al., 2005].

4.5.4 SOC fractions

Our study is one of the few studies evaluating the effect of management and tree influence on SOC fractions at several depths in dehesa. In P dehesa, after more than 22 years' afforestation, the oak trees did not have a significant influence on the SOC concentration of the different carbon pools, or on the relative contribution of each pool to the total SOC, when compared to the area outside the tree canopy. Poeplau [2013] indicate how most of the changes in soil organic carbon tend to happen a few decades after afforestation. This lack of differentiation might be due to the homogenising effect of permanent grazing across the whole plot, which is a dominant factor overcoming the moderately higher presence of tree roots in the below canopy areas, particularly of sparse coarse roots (S1). Moreover, the overall moderate SOC concentrations mean that none of the depths and areas in the P dehesa are carbon saturated, with carbon saturation deficits from 0.20 to 0.81, as defined by Stewart et al. [2009]. A different situation was observed in the C dehesa which holds much older trees. In this dehesa, the under-canopy area presented a higher SOC concentration in the unprotected and chemically protected pools, resulting in a higher fraction of the carbon being contained in the unprotected pool, when compared to the area beyond the canopy projection. The unprotected pool is the most sensitive to changes in SOC [Poeplau, 2013], which suggests that these differences might be due to a higher addition of fresh plant and root material in the vicinity of the trees, as is apparent due to the higher tree root density in this dehesa ((4.7)). Given the correlation between the bulk SOC content and that of all the pools except the biochemically active pool (shown in Table 4.4), the absence of a difference in organic carbon concentration translates into the lack of differences in the different carbon pools. The biochemically protected carbon pool has a very slow response to changes in SOC content [Stewart et al., 2009] and this seems to be the case here.

The dominant organic carbon pool in both dehesas tended to be the unprotected pool, represented from 29% to 77% of the total carbon pool depending on the dehesa, depth and sampling point. There were only in two situations, both in the pastured dehesa, where another pool displayed a similar share of the total carbon pool. This was in the lower soil layer outside the canopy projection, where the biochemically protected form represented more than 25% of the total organic carbon, and in the topsoil layer under the canopy in which the chemically protected pool was 30% of the total carbon pool (slightly higher than the 29% share of the unprotected fraction). This proportion of unprotected carbon (which might average around 35-40% for the two dehesas and depths sampled) in the higher ranges of the share of the unprotected fraction in the total soil organic carbon pool is one reflected by Poeplau [2013] for some grassland and forest areas in Northern Europe.

Although there were some slight differences in the method to determine the different carbon pools, our results suggest that in the edaphic-climactic conditions of our study, and despite a relatively high carbon saturation deficit, the two strategies for dehesa management store a significant fraction of soil organic carbon in the unprotected pool. The fraction of SOC stored in the unprotected pool is higher than that reported for other agricultural systems (e.g. [Poeplau, 2013]) even for another typical Mediterranean cropping system, that of olives, for which Vicente-Vicente et al. [2017] reported an average share of the unprotected fraction ranging from 35%, in the top 5 cm of the soil, to 22%, in the 5 cm-15 cm soil depth. The other two dominant pools are the physically and chemically protected fractions which ranged from a 10% to a 35% share, similar to that shown by Vicente-Vicente et al. [2017] for olives. The dehesas studied displayed biochemically protected fractions which were slightly lower (particularly in the topsoil) than those reported for other agricultural systems [Poeplau, 2013, Vicente-Vicente et al., 2017]. As a result, these dehesa systems are more sensitive to changes in conditions (e.g. more intensive management, warmer climate, etc) which can cause rapid depletion of the other less protected pools [Six et al., 2002].

4.5.5 SOC stock

The total SOC stock in our work reached values from 38.8 t ha-1 to 52.5 t ha^{-1}. These values are within the observed range in other studies carried out on dehesa systems. For instance, Howlett et al. [2011], in a dehesa with a similar soil depth, up to 100cm, found a total SOC stock of 28 t ha^{-1}. In a dehesa in a relatively more humid area (582 mm of annual rainfall) used as permanent grassland, Román-Sánchez et al. [2018] found a mean value of 43.8 t ha^{-1}, sampling a soil profile at a depth of 30 cm which held, on average, 70% of the total SOC stock. Nonetheless, as these authors highlighted, SOC stock varied considerably across the landscape, in which Cambisol, Regosol and Leptosol soil types were alternated (17.0 to 94.1 t ha^{-1}). Corral-Fernández et al. [2013], found a mean total SOC stock of 77 t ha^{-1} in different dehesa farms with Cambisol, with this value decreasing to 58 t ha^{-1} in cases where Leptosols were present.

The different management carried out on P and C dehesas had resulted in a different SOC stock, when a soil depth of 20 cm was taken into account for calculation, with the area devoted to permanent grassland stocking more carbon - 6.5 more t ha^{-1}. As we discussed above, there were no significant differences in SOC concentration between different types of management in most of the soil layers considered in this study, with the exception of the upper topsoil layer (0-2 cm). Therefore, this difference in carbon stock could be due to the contribution of other variables involved in SOC stock calculation, such as stoniness or soil bulk density, the latter depending on soil management. In fact, as a result of grazing, P dehesa showed higher soil bulk density at the surface than C dehesa, in which the occasional tilling reduced BD in the layer worked by farming implements. The simulation of the calculation of SOC stock through the permutation of the raw data of stoniness and BD, albeit referring to the total soil profile, led to slight variations on SOC stock, somewhat more accentuated in C dehesa where the measured stoniness was higher and the BD lower. Although these differences, within the range of 0.5-1 t ha^{-1}, were small in the context of total stock, they may have importance when comparing different land uses and soil management. Along these lines, Mohanty [2017], found that SOC stock increases when arable land was devoted to permanent grassland (from 28 t ha-1 to 32 t ha^{-1}). Moreover, these variables can also be important to the dynamics of the organic matter, by regulating processes such as the flow of water, circulation of air in the soil, or even the formation of micro-aggregates [Uribe et al., 2014, Ferreiro-Domínguez et al., 2016, Seddaiu et al., 2018]. However, the differences disappeared when increasing the depth for stock calculation. The carbon stocked in the first 20 cm of the soil represented around 55% of the total SOC stock in P dehesa, and 40% in C dehesa, which coincides with the results of Corral-Fernández et al. [2013], who found more than 41% of the total carbon stored in a Cambisol soil type (100cm deep), or 48% in a leptosol (65cm deep), in this layer. Authors such as Parras-Alcántara et al. [2015] and Román-Sánchez et al. [2018], reported an average of 70-80% of the total carbon accumulated as being in the first 25-30 cm of the soil.

Several studies demonstrate that the presence of trees has a big impact on SOC stock in dehesa [Howlett et al., 2011, Simón et al., 2012]. In our study, we found contrasting results depending on the maturity of the trees. In P dehesa, with relatively young trees (22-year-old) there was no impact on SOC stock, while in C dehesa with mature trees, SOC stock increased by 35% under the tree canopy (from 38.8 to 52.5 t ha^{-1}). These results are in line with the findings of Upson et al. [2016] in a temperate agroforestry system where trees were 14 years old. Howlett et al. [2011] found an increase of 22 t ha^{-1} on SOC stock under the canopy of mature cork oaks in comparison with areas beyond the tree canopy (from 28 to 50 t ha^{-1}). Furthermore, **?** highlighted that trees adjacent to grazing had a positive influence on the ability of soils to store soil C and N. Young trees with low growth, as occurs with the Holm oak, have still a constricted root system and canopy and this fact may limit the accumulation of SOC in the area underneath tree. However, this represents a clear future opportunity for P dehesa to fix organic carbon in soil and woody tissues. To do so, some kind of tree density control would be necessary in the future to overcome possible competition amongst the trees for soil nutrients and water that would collapse growth.

The carbon soil stock in C dehesa, with current tree canopy cover, reached a value of 38.9 t ha^{-1}, while in P dehesa was 40.5 t ha^{-1}. Although the difference was not significant, the change in land use from a crop-pasture rotation to a permanent grassland with holm oaks, have resulted in an increment of SOC stock in the order of 2 per mil per year, which mean a 50% of the target proposed by "4 per 1000" initiative.

4.6 Conclusion

Our results provide insight into the impact of specific types of management in dehesa on the modification of SOC and SOC stock, as well as its effect on its distribution among the different organic carbon fractions. It is apparent that 22 years after transformation of crop-pasture rotation and low tree density into permanent grassland used exclusively for low intensity grazing, and with a high tree density (70 trees ha^{-1}), both dehesas presented a similar SOC stock of approximately 40 t ha^{-1} in the top 100 cm of the soil. The dehesa with permanent grassland only showed higher SOC stock than the crop-pasture rotation dehesa on the surface (5.86±0.56 t ha^{-1} vs 3.24±0.37 t ha^{-1}). The influence of the trees, increasing SOC and SOC stock when compared to the area outside the canopy, was only detected in mature trees in the cropped dehesa. 22 year-old Holm oaks were still not able to induce an increase in SOC and SOC stock. The lack of differences in SOC stock between the two dehesas can be explained by the lack of differences in SOC stock between the two managements, crop rotation vs grazing at low density, especially away from the trees, and that the significant increase of SOC stock, found only beneath old tree canopies, did not translated in significant differences at plot scale because the low tree density.

Permanent grassland and crop-pasture rotation presented a similar distribution of soil organic carbon amongst different functional fractions, with the unprotected fraction being the dominant one (30%-45%), followed by the physically (15%-25%) and chemically (15%-25%) protected fractions. The presence of mature trees significantly modified the distribution of soil organic carbon in their surroundings, increasing the importance of the unprotected fraction (40%-70%), and decreasing the relative importance of the physically (10%-25%) and chemically protected (9%-29%) fractions, probably for the higher contribution of fresh organic matter from roots and leaves to the soil. The distribution of soil organic carbon in the unprotected, and physically and chemically protected fractions was strongly correlated to the overall organic carbon concentration in the soil, with the biochemically protected fraction showing no correlation.

Tab. 4.5: One-way ANOVA of the soil organic carbon (SOC) concentration and stock at each fraction with, (i) soil management as independent factor at tow depth (top and deep soil) and (ii) tree presence as independent factors in C and P dehesa at two depths (top and deep soil). Significance is noted as: ns: not significant; *: $p < 0.05$; **: $p<0.01$; ***: $p<0.001$.

Effect	Variable	Topsoil layer F	Topsoil layer p-value	Lower soil layer F	Lower soil layer p-value
Soil management	SOC unprotected	0.244	ns	2.191	ns
	SOC physically protected	0.006	ns	1.611	ns
	SOC Chemically protected	2.530	ns	0.001	ns
	SOC Biochemically protected	8.177	*	0.215	ns
	Stock unprotected	0.012	ns	0.193	ns
	Stock physically protected	0.219	ns	1.523	ns
	Stock Chemically protected	1.214	ns	0.113	ns
	Stock Biochemically protected	1.968	ns	0.256	ns
Tree in C-dehesa	SOC unprotected	14.023	*	1.453	ns
	SOC physically protected	0.738	ns	0.006	ns
	SOC chemically protected	2.728	ns	0.263	ns
	SOC biochemically protected	1.818	ns	0.002	ns
	Stock unprotected	11.633	*	2.432	ns
	Stock physically protected	8.705	*	0.780	ns
	Stock Chemically protected	4.226	ns	0.054	ns
	Stock Biochemically protected	2.295	ns	0.000	ns
Tree in P-dehesa	SOC unprotected	5.412	ns	3.234	ns
	SOC physically protected	0.006	ns	0.726	ns
	SOC chemically protected	1.256	ns	0.285	ns
	SOC biochemically protected	12.43	*	0.441	ns
	Stock unprotected	11.640	*	0.092	ns
	Stock physically protected	0.051	ns	2.488	ns
	Stock Chemically protected	0.168	ns	0.003	ns
	Stock Biochemically protected	5.011	ns	0.634	ns

4.7 Supplementary materials

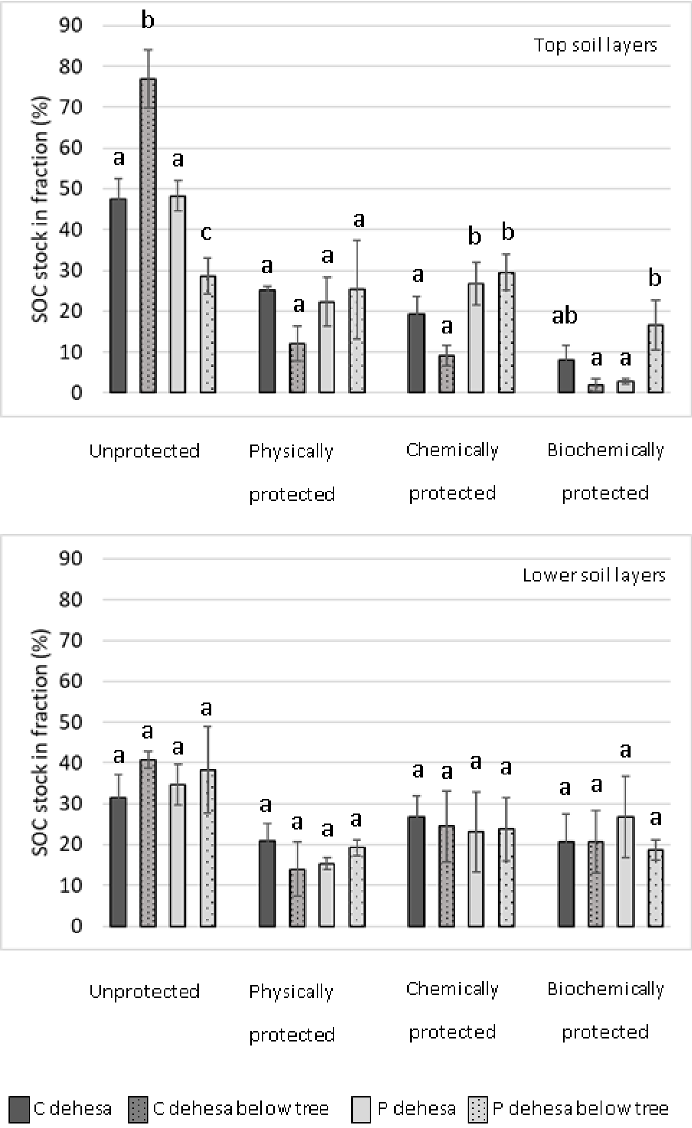


Fig. 4.6: Distribution of soil organic carbon (SOC) stock by fractions according to soil management (C and P) and tree influence (beyond-below tree canopy) in tow depths, top layer (0-2 cm, 2-5 cm) and lower layer (20-40 cm, 40-60 cm). In each fraction, different letters indicate a significant difference according to soil management and tree influence

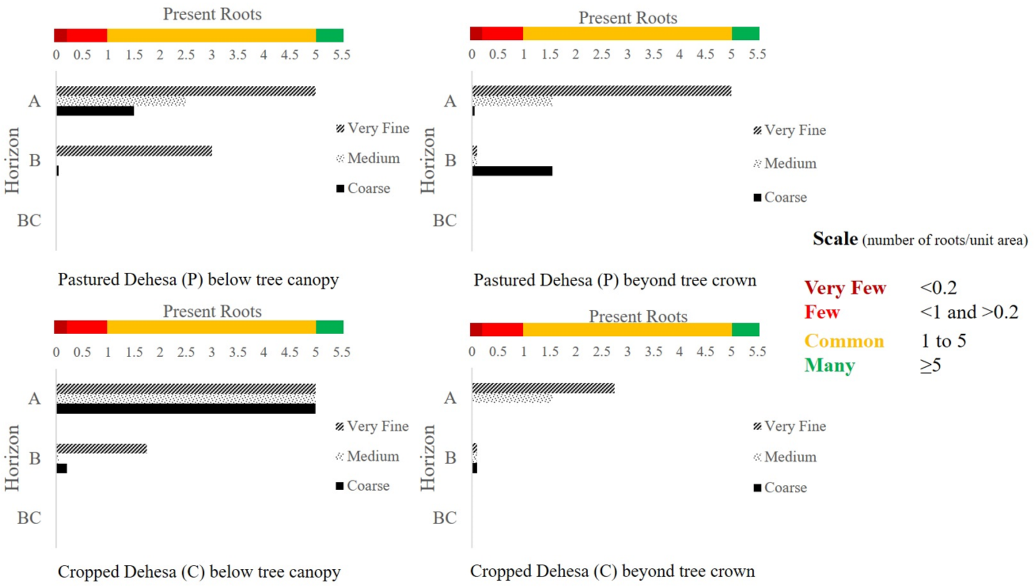


Fig. 4.7: Visual abundance of roots by root class in different soil horizons in pastured (P) and cropped dehesa (C). Size classification of the roots is Very Fine (<1mm), Medium (2 to <5mm) and Coarse (5 to <10mm).

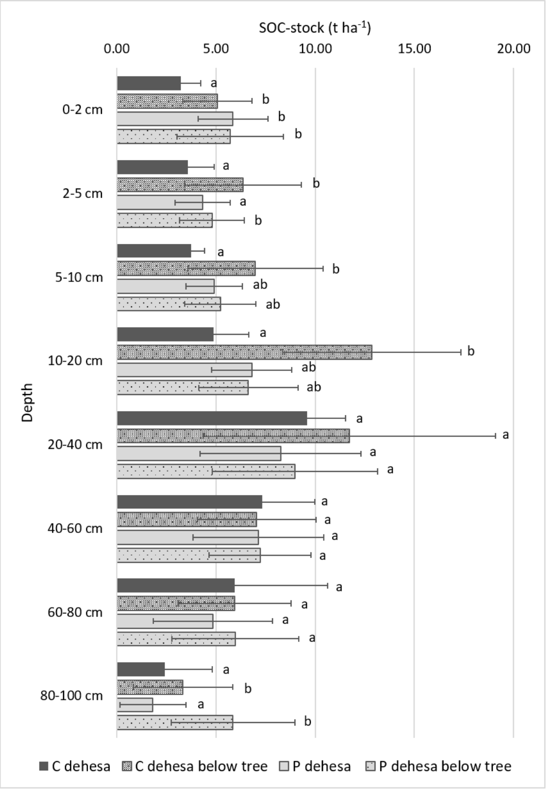


Fig. 4.8: Distribution of SOC stock with depth at C and P dehesas outside and under the tree canopy (mean and standard deviation). At each depth, different letters indicate a significant difference between soil management and tree influence (beyond-below tree canopy).

Chapter 5

Stock of soil organic carbon and functional fractions in response to grazing intensities in permanent pasture of dehesa system

Preliminary results of this chapter were presented at an international congress in Spain and Poland, see below, and the current version is a working manuscript for submission to Geoderma.

Title: Efecto de la intensidad de pastoreo sobre la concentración y acumulación de carbono orgánico en el suelo en Dehesa.
Event: 58^{a} Reunión Científica de la Sociedad Española para el Estudio de los Pastos
Country: España, Sevilla
Presentation: Poster

Title: Effect of grazing intensity on organic carbon concentration, accumulation and its fraction in pasture soils
Event: 8th. International conference for younger researchers in Agriculture, Forestry and Technology Country: Poland
Presentation: Poster

Authors: **Lizardo Reyna-Bowen**[1,2], María T. Hidalgo[1], Ramón Leal[1],José A.Gómez[2], Pilar Fernández-Rebollo[1]

[1]Forestry Department, University of Córdoba, Rabanales University Campus, Ctra. Madrid-Cádiz Km. 396, 14014 Córdoba.
[2]Institute for Sustainable Agriculture-CSIC, 14080, Córdoba, Spain.

5.1 Abstract

The present work aims to analyze the effect of three grazing intensities, high (H), moderate (M) and without grazing (W), maintained for a long time, on the concentration and stock of soil organic carbon (SOC) in an agrosilvopastoral system (dehesa). Soil samples were taken outside the tree canopy projection area at five different depths: 0-2, 2-5, 5-10, 10-20 and 20-30 (cm). The SOC concentration of each sample and the SOC stock was calculated in the whole profile as well as the distribution in the four different functional carbon fractions (unprotected and physically, chemically and biochemically protected). The highest SOC concentration was obtained at the surface layer (0-2 cm), with 1.59±0.43%, decreasing to 0.47±0.11% in the deepest layer (20-30 cm). The SOC concentration was significantly affected by the intensity of grazing, where the area grazed at moderate intensity showed the highest values. However, SOC stock was similar in the three areas: total SOC stock in the whole analyzed profile (0-30 cm) was 27t ha^{1} for grazed areas, regardless of grazing intensity, and 26t ha^{1} for the no grazed area. The unprotected fraction had the lowest organic carbon concentration in all areas compared to other fractions, whilst chemically protected fractions showed the highest. In general, the organic carbon concentration of the unprotected and physically protected fractions decreased with depth, with grazing increasing the organic carbon concentration of the biochemically protected fraction. In the upper soil layer, the unprotected SOC was the dominant fraction in all areas, whereas in the 10-20 cm soil layer the unprotected fraction shared importance with physically and chemically protected fractions. Grazing significantly modified the distribution of soil organic carbon stock between fractions in the 10-20 cm soil layer, increasing the relative importance of the biochemically protected fraction (from 6% in ungrazed areas to 10% in grazed areas) and decreasing the contribution of the unprotected fraction (from 35% in ungrazed areas to 21% in grazed areas). These results show that extensive grazing can be a valuable tool for increasing the pool of stable carbon and, therefore, sequestering carbon in Mediterranean dehesa soils.

keyword: organic carbon fractions; agrosilvopastoral system; permanent grassland; grazing exclusion; livestock density

5.2 Introduction

Grassland ecosystems cover approximately 40% of terrestrial land surface and provide important ecosystem services: provisioning services such as food production and clean water supply; regulating services like erosion prevention or carbon sequestration and storage; supporting services, for instance habitats for species; and cultural services, being, for example, a place for recreation or aesthetic appreciation [Papanastasis et al., 2015]. Provisioning services are capital in grasslands, which are the main food source for herbivores, both wild and domestic. Wild animals and livestock, as users of grasslands, influence them and, ultimately, regulate their structure and functioning. Humans, through management, can modify grasslands themselves (e.g. fertilizing or reseeding) and modulate the grazing effects on grasslands (e.g. regulating the stocking density or grazing season).

Grassland soils represent a large potential reservoir for carbon storage. As in any other ecosystem, the major factors that influence soil organic carbon (SOC) storage are thought to be related to environmental variables and soil type, amongst which it is worth noting the role of the rainfall and the amount of fine particles (clay and silt) in the soil [Wang et al., 2019]. Grazing intensity is also likely to influence SOC storage, and previous studies have found contrasting effects: increase, decrease and no effect [Piñeiro et al., 2010, Ferreiro-Domínguez et al., 2016, Zhou et al., 2017]. Abdalla et al. [2018] stated that grazing below the carrying capacity of the grassland results in a decrease in SOC storage, although the response is climate-dependent: whilst in humid and warm climates grazing increases carbon storage, in humid and cold climates it decreases, regardless of the intensity of grazing; carbon storage increases in dry climates (warm or cold) when grazing is conducted at low intensity. Moreover, McSherry and Ritchie [2013] found an interaction between rainfall and soil texture in grazing effects on SOC storage, where an increase in rainfall resulted in a decrease in grazing influence on carbon storage on finer texture soils and an increase on sandy soils. Species composition of grassland also play an important role in the sequestration of organic carbon, because of their different ability for biomass production, its allocation below and aboveground and the chemical composition of their tissues [Gatti et al., 2016]. The impact of grazing on carbon storage in soils is also mediated by species composition (e.g. C4 vs C3) [Abdalla et al., 2018, McSherry and Ritchie, 2013]. Furthermore, shifts in species composition and abundance may be driven by grazing [Díaz et al., 2007, Piñeiro et al., 2010]. Hence, grazing influences the factors that control SOC storage, interacting with plants and soils in a complex way at each climatic scenario. The effect of grazing on the quantity of SOC sequestered is of interest, but also on the quality of the organic carbon. Soil organic matter is considered to be composed of several functional fractions that differ in their intrinsic degradability [Six et al., 2002]. The unprotected organic matter is an important nutrient source for soil microorganisms, having a rapid turnover. On the contrary, organic matter could be protected from soil microorganisms through the formation of microaggregates, occlude within silt and clay particles or within more recalcitrant compounds, extending the permanence of organic matter on soil. Although some studies have reported that manure fertilization increased the SOC content of more stable fractions [Tian et al., 2017, Li et al., 2018], the effects of grazing on different functional SOC fractions remain unexplored.

Dehesa is the most spread agro-silvo-pastoral systems in the Mediterranean regions [Carbonero and Fernández-Rebollo, 2014], covering around 4 million ha in south-western Spain. This system is the result of human intervention over natural oak woodlands, reducing trees and shrubs coverage to enhance pasture and oak production and to enable occasional cereal and legume cropping. Hence, the vegetation is characterized by a two-layered structure in the same unit of land: a tree layer at low density and an understory of natural pasture or crop. Although Dehesa is a multipurpose system, livestock is the main source of income: flocks of sheep and herds of beef cattle, combined with Iberian pigs, graze extensively, feeding themselves mainly on pasture and acorns. Several studies have assessed the SOC stock in dehesa system, focusing on the effects of land use [Fernández-Romero et al., 2014, Lozano-García et al., 2016], tree canopy [Howlett et al., 2011, Simón et al., 2012], and soil management [Parras-Alcántara et al., 2014, Reyna-Bowen, 2020]. Even though livestock is the main economic activity of dehesa farms and, hence, grazing is widely spread, studies aiming to investigate the impacts of grazing on SOC content and functional fractions are still scarce in the dehesa system. Understanding the effects of grazing intensity on SOC storage would be interesting in order to provide a framework for enhancing SOC management in dehesa farms.

When analyzing the effects of grazing intensity on SOC stock, time is a relevant variable, and long-term grazing or ungrazing condition should be considered [McSherry and Ritchie, 2013]. In areas with short grazing life, negligible effect might be shown. Moreover, a gradient of grazing intensity can be obtained by sampling grassland soil at different distances from a water trough, since water troughs act as a livestock concentration point and the frequency of grassland use is viewed as distributed concentrically around these points [Holechek, 1988]. We followed a sampling approach analogous to this one to examine the effects of grazing intensity on total SOC stock and their functional fractions in grassland soils of dehesa that have been grazed for a long-term. Additionally, as a contrast area, we choose an adjacent grassland without grazing for the last 20 years. We hypothesized that both, grazing at high intensity and the absence of grazing would have a negative effect on carbon sequestration since, in both situations, grassland production may be limited although by different mechanisms (C and N constraint respectively). Testing this hypothesis, quantifying the effect of different grazing intensities on SOC stock and functional fractions is the objective of this study.

5.3 Materials and Methods

5.3.1 Area description

This study was conducted in a commercial farm of Southern Spain (Figure 1), located at the municipality of Pozoblanco in the north of Córdoba province Lat. $(38^\circ 22' 62''$ N and Long. $(4^\circ 45' 59''$ W). The farm altitude is 543 m.a.s.l. The area has a mean annual precipitation of 612 mm and the average annual temperature is 15.1 °C (climate-data.org). The predominant type of soil on the farm is Eutric Cambisol, which is considered to have low chemical fertility. Table 5.1 describes the main characteristic of the soil profile in the sampling area.

Farm is covered by natural pasture and holm oak-woodland savannah (dehesa system), and is devoted to extensive grazing with beef cattle, sheep and swine since more than 50 years (since 1958 according to owners). Farm is divided into several paddocks and grazing is conducted in a rotational way, alternating grazing and resting periods. Under normal conditions, it is common to graze the same paddock two or three times during the growing season of the pasture and once more during the summer, when the pasture is dry. However, the annual grazing calendar depends largely on weather conditions, mainly on rainfall amount and distribution. Swine only graze freely on the farm during the autumn (from November to January), when the acorn reaches the maturity and falls. The livestock density of the farm has decreased in recent years and is now in the range of 0,50-0,60 LU ha^{-1}. There are occasional crops on the farm, alternating natural pasture with cereals (in rotation of more than ten years), although in recent years they are less frequent.

In a farm paddock of 24 ha, three adjacent zones with distinct levels of grazing intensity (high, medium, and without grazing) were selected (Figure 5.1). The first zone selected, representative of a high intensity of grazing (H), was the vicinity of the watering trough. It is widely accepted that the distribution of water points is an important environmental factor that affect the patterns of livestock use across the landscape [Holechek, 1988]. After fencing, water is the most frequently used tool for affecting livestock distribution in large paddocks. Livestock are attracted to water and center their activity around these points, resulting in uneven patterns of pasture use: a more frequent use (grazing and trampling) in the vicinity of water points and a less frequent use in remote areas. For sampling purposes, H zone was established as a circle of 30 m of radius from watering trough. The second zone, denoted as area with moderate intensity of grazing (M), was set as the area included in a circular crown with radius 60 and 90 m from the watering trough. Finally, an area of 0.70 ha excluded from livestock use 20 years ago was selected as zone without grazing (W).

The distance of W from the watering trough was 60 m. The main soil properties for the areas, taken from two soil pits made at the time of sampling (one for grazing area and another for without grazing appear in Table 5.1.

Pastures of H and M were dominated by annual herbs. They showed a medium to high plant diversity. Among the specie present are grasses as *Aegilops geniculata* Roth, *Avena barbata* Pott ex Link, *Bromus rubens* L, B. *diandrus* Roth , B. *hordeaceus* L, *Dactylis glomerata* L., *Lolium multiflorum* Lam or *Vulpia* spp; forbs as *Anthemis arvensis* L., *Diplotaxis* spp., *Erodium* spp., *Eryngium campestre* L., Sp. Pl., *Plantago coronopus* L., P. *lanceolata* Forssk., *Senecio lividus* L.; and abundant legumes as *Astragalus pelecinus* L. Barneby, A. *hamosus* L., *Ornithopus compressus* L., *Trifolium subterraneum* L., *T. cherleri* L., *T. tomentosum* L., or *T. glomeratum* L.. Bare soil and animal paths are more abundant in H. Pasture of W was less specie-rich and exhibited a different plant species composition, with the perennial grass *Dactylis glomerata* L. being dominant and scarce the legume.

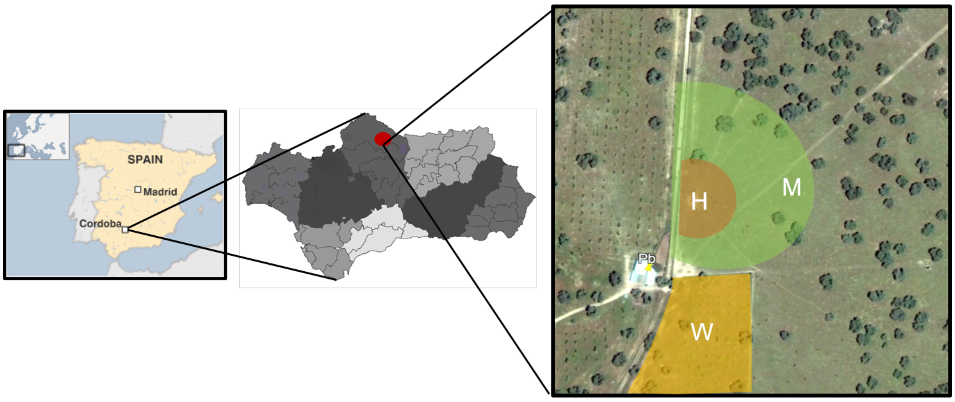


Fig. 5.1: Location of the experimental farm at Pozoblanco in Córdoba province, Spain. Study areas are highlighted with different colors. High grazing (H) in brown, moderate grazing (M) in green, and without grazing (W) in yellow.

Tab. 5.1: Soil properties in H, M (grazing area) and without grazing in dehesa. *BC horizon, data shown in this column indicate the depth at which this horizon begins; N: Organic Nitrogen; K: Available Potassium assailable p.p.m.; P: Available Phosphorus (Olsen) p.p.m.; CEC: Cation exchange capacity; S.T.C: Soil Textural Class.

Area	Hz	Depth (m)	pH 1/2'5	N	K	P	CEC	Ca	Mg	Na	Clay	Sand	Silt	S.T.C.
				%				(meq/100g)				(%)		
Grazing	A	0.23	6.41	0.11	215	9.30	6.38	3.73	1.85	0.50	4	82	14	Loamy sand
	B	0.41	5.73	0.05	129	9.40	11.63	7.98	2.95	0.37	13	72	15	Loamy sand
	BC*	>0.41	7.71	0.01	58	3.50	13.42	7.01	5.65	0.15	9	89	2	Sand
Without Grazing	A	0.16	7.86	0.14	309	20.60	13.42	8.92	3.40	0.32	6	82	12	Loamy sand
	B	0.36	8.30	0.06	151	9.30	9.89	6.00	3.20	0.31	8,7	74	17	Loamy sand
	C*	>36	8.45	0.02	205	10.40	17.98	13.47	3.75	0.37	16	73	11	Loamy sand

5.3.2 Field soil sampling

In December of 2017, two pits 1 m depth were dug, one in M and other in W zone, and soil profiles were described according the NRCS guidelines, [Schoeneberger and Staff, 2012]. In H zone, a shallower pit of 40 cm was dug for measuring soil bulk density. From these three pits, undisturbed soil samples were taken with a hand soil sampler of a volume of 98.2 cm^3 to determine bulk density (BD). The samples were taken at three depths (0-10 cm, 10-20 cm, 20-30 cm), with six replications totalling 54 samples. The samples were dried in an oven at 105 °C for 72 hours to a constant mass. The bulk density of each soil layer was calculated by dividing the dry mass of soil by the volume of the bulk density sampler, according to Hao [2008].

Twelve random sampling points were selected this outside the area below the tree canopy projection in each zone (H, M and W), avoiding the influence area of the holm oaks. Soil samples were taken at each sampling point to a depth of 30 cm, dividing them into 5 depth intervals: 0-2 cm, 2-5 cm, 5-10 cm, 10-20 cm and 20-30 cm. Pasture and surface mulch were previously removed. These depths were chosen because previous studies, had found that farm management practices could affect soil characteristics down to a depth of 20-30 cm [Reyna-Bowen, 2020]. Soil samples were taken combining a manual soil sampler for the top-soil samples and a hydraulic soil sampler (Giddings®) with a 38.1 mm diameter soil core for the rest. In total, there were 180 soil samples (3 zones x 12 sampling points x 5 depth intervals).

In February of 2017, after an accumulated rainfall of 47.8 mm, soil penetration resistance (cone index, hereafter CI) was evaluated using a cone penetrometer with a fine tip (cone diameter 12.6 mm, tip angle 30° and rod diameter 9.5 mm) following the recommendations established by standard ASAE S313.2 for soils with high mechanical impedance. Four random points, far away of the canopy projection of the trees were chosen in each zone (H, M, and W) and, around these selected points ten CI measurements were taken with the penetrometer up to a depth of 30 cm, recording data of resistance to penetration at intervals of 0.5 cm. Then, we averaged the ten CI readings to produce a single profile of penetration resistance for each point. CI of the following depths 0-2 cm, 2-5 cm, 5-10 cm, 10-20 cm and 20-30 cm were also average. In addition, soil samples were taken in selected four points of each zone with a manual soil sampler at 0-5 cm, 5-10 cm, 10-20 cm and 20-30 cm of depth to determine soil water content. Soil samples were stored in plastic containers sealed and placed in a portable cooler. Once in the laboratory, samples were weighted before and after oven-dried (105°C for 72 hours) and gravimetric soil water content was determined.

5.3.3 Soil analysis

To obtain soil organic carbon (SOC) concentration, the 180 samples were ground, passed through a 2 mm sieve and homogenized. The remainder coarse material whose diameter is $>$2 mm, was weighted to determine stoniness as % in mass. SOC concentration of fine earth was determined according to Walkley [1947]. The stock of soil organic carbon for each soil depth interval (SOC stock) and for the whole soil profile, were calculated according to IPCC [2003]. Equation 5.1:

$$SOC_{stocki} = 10000 SOCi \cdot BDi \cdot d \cdot (1 - \rho) \tag{5.1}$$

$$SOC_{stock} = \sum_{i=1}^{i=n} SOC_{stock \quad i} \tag{5.2}$$

Where SOCstocki is total soil organic carbon in a given layer (t ha^{-1}). SOCi is organic carbon concentration (g g^{-1}), BDi is bulk density (T m^{-3}), d is the thickness of the depth interval (m), ρ is the fraction (0 - 1) of gravel larger than 2 mm in the soil, and n is the number of soil layers. Thus, equation 5.2 gives the total soil organic carbon, SOCstock (t ha^{-1}) in the whole soil profile. BD values for upper soil depths not sampled were interpolated using mass-conserving splines [Malone, 2017].

The concentration of organic carbon in the different functional soil fractions was measured, following the method proposed by Six et al. [2002], in a subset of selected samples: six per zone (H, M and W) and depth interval (2-5 cm and 10-20 cm). Overall, 36 samples were analyzed. Fractionation was performed on soil samples to separate unprotected, physically, chemically and biochemically protected fractions. Unprotected fraction was integrated by the particulate organic carbon in aggregates of 2000-250 μm plus the light fraction (LF) of the 250-53 μm aggregates. Particulate organic carbon was separated by sieving and the LF by flotation and centrifugation. After the LF was discarded by flotation, the aggregates of 250-53 μm that remained stable after centrifugation were collected to obtain the physically protected fraction. The aggregates measuring $<$53 μm, such as slime-sized fractions and clay that had been isolated during the initial sieving and dispersion, were acid-hydrolyzed and the hydrolysable portion was collected to obtain the chemically protected fraction. Finally, the remaining non-hydrolysable organic carbon after the acid hydrolysis was collected to obtain the biochemically protected fraction. All residues were oven-dried at 60 °C and weighed. The concentration of organic carbon in each pool was determined by wet oxidation using sulphuric acid on samples between 0.3–0.5

g using potassium dichromate with an absorbance spectroscope in the range of 600 μm [Vicente-Vicente et al., 2017].

5.3.4 Statistical data analysis

One-way analysis of variance (ANOVA) were performed to analyze the effect of grazing intensity on SOC concentration and stock, BD, stoniness, Cone Index (CI) and gravimetric soil humidity at each soil section sampled and, for SOC stock, also in the whole soil profile. Differences were considered significant al $P < 0.05$. A post-hoc LSD test were carried out to compare differences among treatments (W, M and H), when ANOVA resulted significant. The effect of depth on these variables was analyzed in each zone using the Kruskal-Wallis test, due to a lack of homoscedasticity of variances.

The concentration of SOC in the different functional fractions (unprotected, physically, chemically and biochemically protected fractions) and their contribution to total SOC were analyzed using a factorial ANOVA with the treatments (W, M, and H) and the depth (2-5 cm and 10-20 cm) as fixed factors and their interaction. Additionally, at each zone and depth, the weight of the different SOC fractions to total SOC was compared through a one-way ANOVA. Linear models were used to evaluate the relationship between SOC concentration in the different functional fractions and the SOC concentration in the bulk soil. All statistical analysis was performed using the Statistica v 6.0 software.

5.4 Results

5.4.1 Variation of SOC concentration, bulk density, stoniness and penetration resistance with grazing intensity and depth

As expected, a clear downward stratification of the carbon concentration in the soil was found (Table 5.2). The average of the highest carbon concentration was around 1.59±0.43% at the surface (2-5 cm), decreasing the concentration up to 0.47±0.11% at the last depth (20-30 cm). We found differences in SOC concentration between grazing intensities in the 5-10 and 10-20 cm soil layers, being higher in moderate grazing zone. Soil bulk density in 0-5 cm section was significantly higher in the zone heavily grazed compared with the other two zones. Bulk density decreased with depth in H, whilst it showed similar values in all the sampled profiles in W and M. In general, the stoniness increased with depth in all zones. The surface stoniness (0-5 cm) was higher in the zone grazed at moderate intensity. As well as the stoniness, the CI increased with depth in all three zones, with the CI of H being higher at the surface (0-2 cm) than that of W. Differences in the CI were also found in the soil layer of 10-30 cm, with the moderate grazing zone showing lower CI values compared to the others. At the time of CI sampling, gravimetric soil water content was similar between zones.

Tab. 5.2: Distribution of soil organic carbon concentration (SOC), bulk density (BD), Stoniness in (%), and Resistance of soil penetration in mega Pascal (MPa) by depth. Different lowercase letters indicating difference among depths in each grazing intensities. High (H), Moderate (M) and Without grazing (W), and different capital letters indicating difference between grazing intensities in soil depth according to Kruskal-Wallis test in SOC, and (BD, Stoniness and Resistance of soil penetration) with ANOVA test. $P<0.05$

SOC concentration (%)			
Depth (cm)	**H**	**M**	**W**
0-2	1.61±0.51a	1.48±0.3a	1.69±0.5a
2-5	1.11±0.25a	1.22±0.23ab	1.16±0.31a
5-10	0.74±0.27b B	1.1±0.27bc A	0.83±0.31ab B
10-20	0.59±0.19bc B	0.87±0.34cd A	0.57±0.18bc B
20-30	0.41±0.12c	0.45±0.12d	0.56±0.11c
Bulk Density (g cm^{-3})			
0-2	1.88±0.18aA	1.41±0.2aB	1.48±0.23aB
2-5	1.84±0.15aA	1.41±0.17aB	1.49±0.22aB
5-10	1.68±0.11abA	1.43±0.1aB	1.51±0.17aAB
10-20	1.44±0.15ab	1.48±0.08a	1.54±0.11a
20-30	1.66±0.31b	1.55±0.16a	1.55±0.2a
Stoniness (%)			
0-2	19.4±4.22ab B	24.64±4.27a A	16.16±2.55a B
2-5	19.38±4.7ab B	24.69±4.5a A	18.78±4.2ab B
5-10	14.44±4.81a B	25.72±6.95a A	21.89±5.46bc A
10-20	23.46±4.51bc	25.58±7.11a	26.54±6.24c
20-30	34.29±13.75c	38.54±11.91b	27.5±6.93c
Resistance of soil penetration (MPa)			
0-2	0.79±0.12a A	0.62±0.27a AB	0.39±0.15a B
2-5	2.07±0.15ab	1.72±0.76ab	1.35±0.31a
5-10	2.67±0.09bc	2.04±0.59b	2.15±0.24ab
10-20	3.03±0.2bc A	2.35±0.24b B	2.75±0.16b A
20-30	3.42±0.1c A	2.56±0.43b B	3.03±0.46b AB
Humidity (%)*			
0-5	9.63±1.58a	9.81±1.32	10.55±1.71
5-10	7.61±0.79b	7.03±0.58	7.52±1.39
10-20	7.38±0.17b	7.42±0.43	7.09±1.26
20-30	10.66±3.31a	9.69±4.45	9.48±3.71

5.4.2 Effect of grazing intensity on SOC stock

Figure 5.2 shows the SOC stock in each soil section and the accumulation of the SOC stock with depth. Only at surface (0-2 cm), H and W stocked more soil carbon than M. With depth, the differences in accumulated organic carbon were diluted, disappearing from 5 cm depth onwards. The total SOC stocked at 30 cm depth averaged 27 t ha^{-1} in both grazing areas, and 26 t ha^{-1} in the area without grazing.

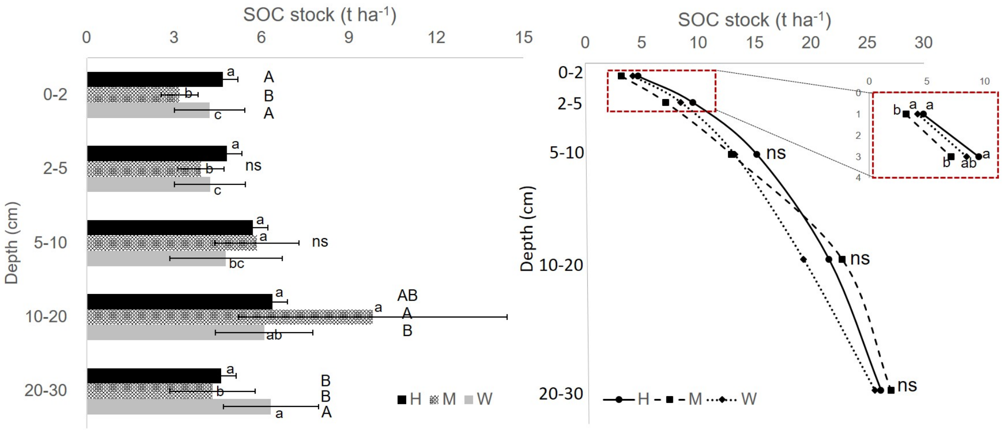


Fig. 5.2: Soil organic carbon stock (SOC stock) according to grazing intensity (high H, moderate M and without grazing W) at each soil section, in left side (whiskers show the standard deviation). Different lowercase letters indicate difference among depths at each grazing intensity, and different capital letters indicate difference between grazing intensities at each soil section. In the right side, SOC stock accumulation with depth in the zones grazed at different intensities. Different letters indicate a difference between grazing intensities

5.4.3 Effect of grazing intensity on SOC concentration in different functional fractions

The SOC concentration of the chemically protected fraction showed the highest values at both sampled depths, ranging from 3.4 to 3.8 (g C / 100 g soil fraction) in the upper soil section and from 2.2 to 2.3 (g C / 100 g soil fraction) in the lower section (Figure 5.3). In turn, the lowest SOC concentrations corresponded to the unprotected fraction, which fluctuated between 0.4 and 0.7 (g C / 100 g soil fraction) in the upper soil section and from 0.1 and 0.3 (g C / 100 g soil fraction) in the lower one. The SOC concentration of the unprotected fraction decreased significantly with depth in the three zones. There were no significant differences in the SOC concentration of the unprotected fraction amongst grazing treatments in the upper soil section; however, in the lower section, it was lower in H compared to W. At each depth, the SOC concentration of the physically protected fraction was similar independently of the grazing intensity. Moreover, a significant decrease in SOC concentration of this fraction with depth was observed in H and W, but not in M. The same pattern was observed in the chemically protected fraction, except that, in this case, the decrease with depth was only significant in H. The SOC concentration of the biochemically protected fraction decreased with depth only in W. In the upper soil layer, we did not find differences between grazing treatments in this fraction but, in the lower soil layer, grazing, irrespectively of its intensity, increased the SOC concentration.

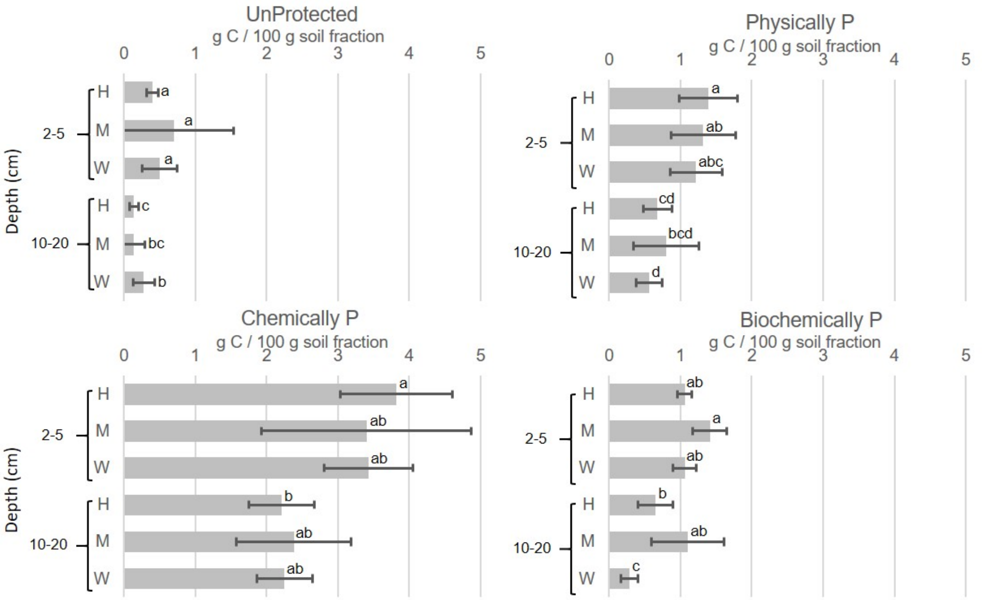


Fig. 5.3: SOC concentration of the unprotected, physically, chemically, and biochemically protected fractions according to grazing intensity (high H, moderate M and without grazing W) and soil depth (whiskers show the standard deviation). In each functional fraction, different letters indicate differences by grazing intensity, depth and their interaction.

Figure 5.4 shows the contribution of the functional fractions to the bulk SOC. In the upper soil layer, the unprotected SOC was the dominant fraction, ranging from 36% in H up to 47% in W. Next in importance was the physically protected fraction, which weighed between 24% and 30% and chemically protected, which ranged from 18% to 23%. Lastly, the biochemically protected fraction contributed with around 10%. Grazing treatment did not induce differences in the contribution of any of the functional fractions in this soil layer. In the subsurface layer of the soil, the unprotected fraction was not the dominant one; it shared importance with physically and chemically protected fractions mainly. Grazing intensity modified the contribution of unprotected and biochemically protected fractions: the weight of the unprotected fraction was lower in H (24%) and M (18%) compared to W (35%)

and, conversely, the contribution of the biochemically protected fraction was higher in H (16%) and M (21%) in relation to that of W, which barely reached 6%.

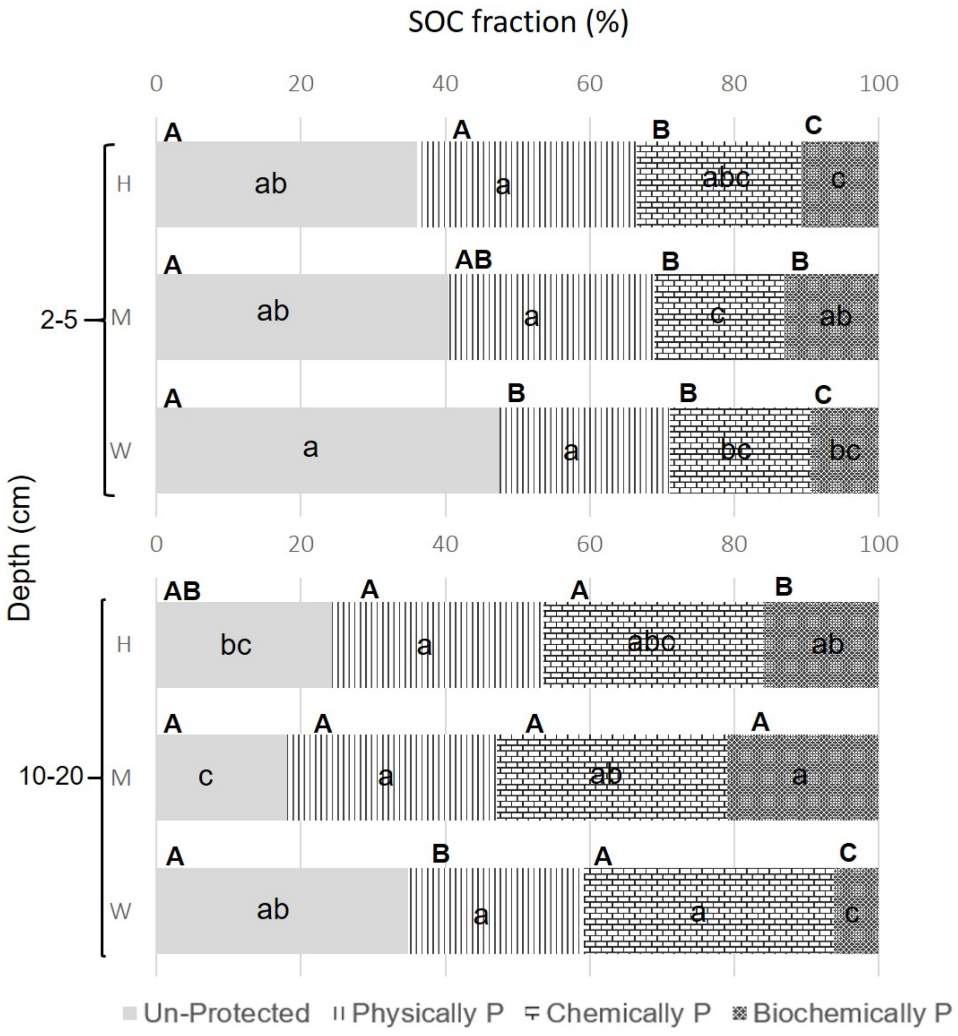


Fig. 5.4: The contribution of the SOC functional fractions to the bulk SOC per grazing intensity (high H, moderate M and without grazing W) and depth. The lowercase letters indicate differences in each fraction according to grazing intensities and depth. The uppercase letters indicate differences between fractions in each grazing intensity and depth.

5.4.4 Relationship between SOC concentration in the different functional fractions and overall SOC concentration in bulk soil

Figure 5.5 depicts the relationship of the SOC concentration in the different functional fractions with the SOC concentration of the bulk soil. It can be seen that the SOC concentration of the unprotected fraction was positively correlated whit the SOC concentration of the bulk soil in most of the situations (the upper and lower soil layers of W; the lower layer of M; and the upper layer of H). Concerning the physically protected fraction, this relationship was only significant in the lower soil layer of W and M and in the upper soil layer of H. No relationship was found between the SOC concentration in the chemically protected fraction and the SOC concentration of the bulk soil in any case. Finally, the concentration of the SOC in the biochemically protected fraction was positively related to the SOC concentration of the bulk soil in the lower soil layer of M.

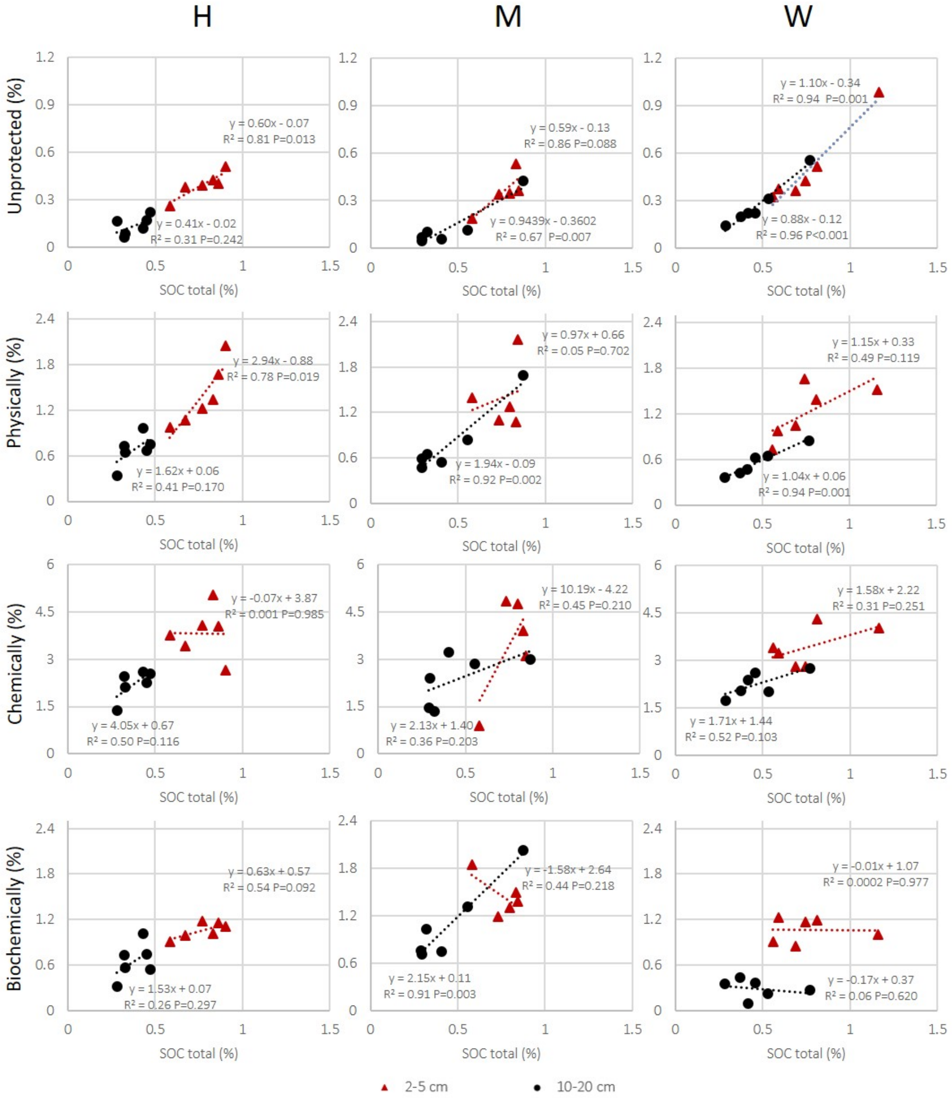


Fig. 5.5: The relationship of SOC concentration in the different functional fractions with the SOC concentration of the bulk soil

5.5 Discussion

5.5.1 Effects of grazing intensity on SOC concentration

Moderate grazing intensity has resulted in higher SOC concentration in the 5-10 and 10-20 cm soil depths as compared to the ungrazed sites for 20 years or grazed at high intensity. There were no significant differences in the shallower soil sections sampled (0-5 cm), nor in the deepest one (20-30 cm). Changes in the SOC concentration induced by grazing are more likely to occur in the topsoil layers, where livestock can modify some soil and plant variables and where the majority of pasture root biomass, the main SOC input, occurs [Soussana and Lemaire, 2014]. Several research works have found a positive effect of grazing on SOC concentration at light to moderate grazing intensities that turn to a negative effect as grazing intensity increases from moderate to heavy [Wei et al., 2011, He et al., 2011, Medina-Roldán et al., 2012, Li et al., 2013, Ferreiro-Domínguez et al., 2016, Zhou et al., 2016].

Grazing exerts an influence on several factors controlling SOC concentration that may explain our results. Firstly, grazing affects pasture composition and structure (life form) that, in turn, affects the amount, cycling and storage of nutrients [Moir et al., 2012, Soussana and Lemaire, 2014]. In our study area, pasture composition differed between grazed and non-grazed sites. Thus, the non-grazed site was dominated by the perennial tall grass *Dactylis glomerata*, being this specie scarce at grazed sites. Annual species were widespread at the grazed site, among which there were abundant prostrate legume species as *Astragalus* pelecinus, *Ornithopus* compressus, *Trifolium* subterraneum, T. tomentosum or T. glomeratum. Legumes were scanty at the ungrazed site. Díaz et al. [2007], analyzing plant traits induced by grazing from 197 research works carried out all over the world, concluded that grazing benefits plants of small size, prostrate growth habit and with rosette and stolonifera architectures. In addition, this study found that grazing favors annual over perennial species. In Mediterranean pasture, grazing may also encourage the presence of annual legumes, mainly those with a prostrate growth habit [Rochon et al., 2004, Lloveras, 2006, Tárrega et al., 2009]. Hence, the exposed effects of grazing on plant traits are in accordance with our observations of pasture composition in the current study.

Secondly, grazing animals may accelerate N-recycling through the excreta, increasing the size of the inorganic N pool available in the soil at times [Augustine and McNaughton, 2006, García-Moreno et al., 2014], which stimulate pasture production and N plant content [Mikola et al., 2009]. For instance, [Moir et al., 2012] found, in a glasshouse experiment with sixteen pasture grasses, amongst which *Dactylis* glomerata was present, that although aerial and root mass varied significantly between species, in all cases it increased with the application of dairy cow urine. Additionally, N availability in soil is expected to increase in pastures rich in legume species, which act as nitrogen fixers [Soussana and Lemaire, 2014]. As legumes were scarce in our non-grazed site, N availability may be limiting pasture production in this site compared to grazed sites, where legumes were abundant and N-recycling mediated by grazing may occur.

Different pasture composition and N availability in soil, may result in differences in pasture production and, specially, in the proportion allocated to roots and other organs, that ultimately control SOC concentration and storage [Soussana and Lemaire, 2014], and could explain the differences found in SOC concentration amongst the grazed and ungrazed sites. However, it fails to explain the differences found in the SOC concentration between sites with different grazing intensities, since both sites showed similar pasture composition. At this point, it is worth considering that defoliation is a third livestock effect that affects pasture production and shoot:root biomass allocation. Frequent and repeated defoliation of pasture and treading, as will occur at the intensive grazing site, reduces photosynthetic tissue, which may mean a decrease in biomass above and below ground [Gatti et al., 2016, juan Gao et al., 2018] and pasture ground cover [Jones and Tracy, 2017], decreasing soil C input. In fact, pasture ground cover was lower at our high intensity grazing area. Additionally, frequent defoliation may reduce the depth that roots reach [Ingram et al., 2008]. On the other hand, it has been found that a light defoliation may stimulate pasture production [Gatti et al., 2016] and root biomass [Gong et al., 2014]. The results of soil penetration resistance also suggest that soil strength could be impeding the root growth at different soil depths in each site. The length of time that a soil is suitable for root elongation is controlled, at high soil water content, by a lack of oxygen and, at low soil water content, by wilting point combined with soil strength. Some authors point out that 3 MPa of soil strength could be the threshold beyond which pasture root growth becomes limited [Materechera et al., 1991, Bengough et al., 2011]. In our case, this level of soil strength is reached at a depth of 10 cm at the high-intensity grazing site, 20 cm in the ungrazed area and was not reached in the soil layers sampled of the moderately grazed area. Precisely, the stratification ratio of the SOC concentration (calculated as the SOC ratio of the upper to the lower layer) decreases at these depths in each site and, according to Franzluebbers [2002], an increase in this ratio may be related to the rate and amount of SOC sequestration.

It is worth remembering that the response of SOC concentration to grazing intensity is strongly dependent on climate and soil type, as pointed by Piñeiro et al. [2010], Abdalla et al. [2018] and [McSherry and Ritchie, 2013]. These authors concluded that in semiarid climate like the Mediterranean, grazing at moderate intensity may increase SOC concentration, as occurs in our study, whilst in wetter climates it may be reduced. Nevertheless, soil texture interacts with climate, modulating the effect of grazing on SOC concentration in different ways. McSherry and Ritchie [2013] found in a meta-analysis about grazing effects on SOC, that grazing had more positive effects on sandy soils than on clay soils at higher precipitation. Soils of our study site are sandy (around 85% of sand) and mean annual precipitation is relatively high in the Mediterranean context (612 mm), hence, according to McSherry and Ritchie [2013] finding, a relative high and positive effect of grazing on SOC would be expected, which is in line with the results obtained, at least in terms of increasing SOC concentration across the subsoil profile.

5.5.2 Effects of grazing intensity on SOC stock

SOC concentration gives us valuable information about the capacity of soils to act as carbon sinks (i.e., SOC concentration in relation to soil saturation level), however, the role of livestock in the control of carbon storage must be analyzed using SOC stock, due to grazing effects on the soil bulk density. Despite finding significant differences in SOC concentration, we did not find differences in SOC stock amongst the three treatments with very different grazing intensities. Differences in BD induced by grazing could explain this result. The site grazed at high intensity showed higher BD in the 0-10 cm soil section than the other two sites, which contributes positively to the SOC stock calculation in this site. This result agrees with the general idea that the effect of livestock grazing and trampling on BD is restricted to upper soil layer, being changes of BD at a depth below 10 cm infrequent [Donkor et al., 2002, Fernandez Rebollo, 2004, Houlbrooke et al., 2010]

Contrary to the initial hypothesis, we did not find differences in BD between sites grazed at moderate intensity and ungrazed. Even the mean values of BD in the ungrazed site were higher than those reached in the grazed site within the whole soil profile. Livestock trampling may compact soil, decreasing total porosity, particularly the volume of large pores [Kulli, 2003, Drewry et al., 2008] and therefore BD increase. However, some authors have reported negligible change in BD between grazed and non-grazed areas in sandy soils [Donkor et al., 2002, Greenwood and McKenzie, 2001] or silt loam soils [Daniel et al., 2002]. In this context, Houlbrooke et al. [2010] suggest a compensatory micropore response to trampling, through which, a decrease in macro porosity due to trampling could be compensated by increasing micropore space, and hence little difference in total porosity or BD could be found. This compensatory micropore response to trampling is probably more common on sandy soils, as those of our study site, where organic matter has a relevant role in the construction of micropore, as suggested by Stock and Stock and

Downes [2008]. In the vicinity of watering trough, the intense livestock trampling could decrease not only the macro porosity of soil, but also the amount of new micropore space. It is worth noting that we did not find differences in CI between sites in those soil layers where the BD differed, except at the soil surface. This result disagrees with other findings, which provide a positive relationship between BD and CI [Daniel et al., 2002, Kurz et al., 2006]. However, for a same soil water content, CI depends on soil organic matter, decreasing when the organic matter increases [Stock and Downes, 2008]. The higher SOC concentration found in deep layers sampled in the moderate grazing site might explain the lower CI.

On the other hand, differences in stoniness also contributed to compensate differences in SOC stock amongst sites, since the one that was grazed at normal intensity (with a higher concentration of SOC in the 5-20 cm soil section) showed a higher surface stoniness. However, the comparation of SOC stock was not significant when the calculations were made apart from the stoniness (results not shown). The total SOC stock reached a value of 27 t ha^{-1} in grazing sites and 26 t ha^{-1} in the site excluded to grazing. These values are in line with other research works carried out in dehesas with similar precipitation. For instance, in a similar dehesa, Reyna-Bowen [2020] found a total SOC stock of 26 t ha^{-1} in the first 30 cm of soil layer and Román-Sánchez et al. [2018] 43.8 t ha^{-1} in a dehesa in a more humid area.

5.5.3 SOC stock distribution in different functional pools. Effect of grazing intensity

Our study is one of the few studies that evaluates the effect of grazing intensity on SOC fractions at several depths in dehesa. It is apparent that grazing had no effect on the SOC concentration of different soil functional fractions or on the relative contribution of each pool to the total SOC stock at the surface layer (0 to 10 cm depth). However, at 10-20 cm, grazing significantly increased the carbon concentration of the biochemically protected fraction and its relative contribution to the total SOC stock and decreased the concentration of the unprotected fraction as well as the contribution of this fraction to the total SOC stock. The unprotected fraction is composed by plant residues and microbial debris partially decomposed [Six et al., 2002], consequently the decreased input of plant roots at the high-intensity grazing site could lead to a low carbon concentration in this fraction. This is in line with the limitation on root elongation suggested by the result of soil penetration resistance at this site. The biochemically protected fraction represents a more stable form of carbon in the soil and depends on the chemical composition of the organic input residues and the reactions that transform the organic matter in the soil into recalcitrant compounds [Six et al., 2002]. Clearly, the type of soil input residues varies amongst grazed and non-grazed sites. Firstly, pasture composition differs amongst sites and, therefore, the chemical composition of the shoot and root tissues may also differ, affecting the chemical composition of soil organic matter. For instance, juan Gao et al. [2018] reported that the incorporation of milk vetch and rape to the soil as a green manure increased the degree of aromaticity, humification and average molecular weight of dissolved organic matter and made it more stable in red paddy soil. Secondly, livestock dung is abundant in the grazing site, especially in the vicinity of the watering trough, whilst fresh plant and root material are the main source of residues in the ungrazed site. In extensive grazing systems, around 50% of above-ground biomass could be consumed by livestock. Most of the digestible carbon of the diet is respired after intake and, after two microbial fermentation plus a gastric digestion at the ruminant digestive tract, undigested plant material, cell wall polysaccharides mainly, are excreted and return to the soil as dung [Peco et al., 2017, Dungait et al., 2005]. Dung has a low degradability and C:N ratio, and a considerable fraction of it can be incorporated directly into the soil organic matter [Dungait et al., 2005]. In this line, Tian et al. [2017] and Li et al. [2018] found that fertilization strategies that include manure can increase the pool of stable carbon in the surface layer of crop systems. Grazing may also modify the reactions that transform the soil organic matter into recalcitrant compounds by altering soil properties, as gas exchange or soil moisture and soil microbial activity [Drewry et al., 2008, Li et al., 2013].

The dominant organic carbon pool in the upper soil layer tended to be the unprotected pool, which represented from 36% to 47% of the total carbon pool, where the lower contributions correspond to the grazing site and the higher to the ungrazing site. Nonetheless in the grazing area, the physically protected pool had a similar contribution to the total carbon than the unprotected pool. The fraction of SOC stored in the unprotected pool in the upper soil layer is within the range of those found in other dehesa farms [Reyna-Bowen, 2020], but slightly higher than that reported for other agricultural systems. For instance, Yang et al. [2018b] sampling the first 20 cm of soil depth in maize and rice fields of a subtropical climate region in China, reported an average share of the unprotected pool ranging from 17 to 31%. In Mediterranean climate, Vicente-Vicente et al. [2017] reported an average share of the unprotected fraction of 22% in the top 5 cm of the soil of olive groves. At a deeper soil layer (10-20 cm), the unprotected pool still remains dominant in the ungrazed area, although sharing importance with the chemically protected pool, whilst in the area moderately grazed all four pools contribute with a similar weight to the total carbon pool. The contribution of the biochemically protected fraction to total carbon stock of grazing areas was higher to those found in other dehesa farms of the same region in the topsoil, but similar in the lower soil layer [Reyna-Bowen, 2020]. In contrast, the contribution of the biochemically protected pool to total carbon stock in ungrazed area was lower (particularly in the lower soil layer) than those reported for dehesa [Reyna-Bowen, 2020] and other agricultural systems [Poeplau, 2013, Vicente-Vicente et al., 2017, Yang et al., 2018a]. As a result, the suppression of grazing can shift the dehesa to a more sensitive system regarding changes in conditions (e.g. warmer climate), which can cause rapid depletion of the other less protected carbon pools [Six et al., 2002]. In other words, grazing can be a valuable tool to increase the pool of stable carbon and, therefore, to sequester carbon in dehesa soils.

5.5.4 Response of SOC concentration within functional pools to total SOC concentration

The SOC concentration of the unprotected fraction increased with the increasing total SOC in both depths sampled, suggesting that none of the three areas are carbon saturated and they might still have a potential to store more carbon in this pool under the prevailing management conditions. This result was in agreement with that reported in previous studies showing that the unprotected fraction has a linear relationship with SOC concentration [Stewart et al., 2009, Reyna-Bowen, 2020, Yang et al., 2018a]. The SOC concentration of the physically protected fraction had a positive relationship with total SOC in deeper soil layer sampled of the ungrazed and moderately grazed areas, whilst in the heavily grazed area this positive relationship was found only at soil surface. Although previous studies revealed that soil microaggregates have a limited capacity to occluded organic carbon and a curvilineal relationship has been proposed, recent studies in different soil types have found a linear relationship similar to that found in our site [Yang et al., 2018a, Reyna-Bowen, 2020]. Carbon concentration of the chemically protected fraction had no obvious further increase with total SOC concentration, suggesting that the current concentration in this fraction is approaching to C saturation level. Reyna-Bowen [2020] found in dehesas of the same region and with similar texture a positive relationship, although the reported SOC concentrations for this fraction were lower than those at our site. As occurs with the chemically protected fraction, SOC concentration of the biochemically protected fraction did not increase with increasing total SOC concentration, except in the 10-20 cm soil layer of the site grazed at moderate intensity, where a positive relationship was found. This result seems to indicate that, in the current conditions, soil might have capacity to stabilize more C through biochemical mechanisms.

5.6 Conclusion

Our results provide insight on how grazing intensity may affect the concentration of organic carbon and its storage in pasture soils of dehesa system, and, especially, on how the distribution of carbon stored into different functional fractions may be affected. Moderate grazing intensity, sustained over time, has resulted in higher SOC concentration compared to sites without grazing for a long time (20 years) or intensively grazed on a recurring basis. Grazing influences the factors that control SOC storage, interacting with plants and soils in a complex way at each climatic and edaphic condition. The acceleration of N-recycling through the excreta, increasing the size of the inorganic N pool available in the soil; the shifts in species composition and abundance, encouraging annual legumes of prostrate growth habit; or the stimulation of growth through a light defoliation could lead to an increase in pasture production with extensive grazing which, in turn, would result in an increase in SOC concentration. Despite finding an effect on SOC concentration, SOC stock was similar in all pasture soils, regardless of the intensity of grazing, suggesting that the impact on SOC stock might be negligible or very small, at most. The total SOC stocked in a 30 cm soil depth reached a value of 27 t ha^{-1} in grazing sites and 26 t ha^{-1} in the site excluded to grazing.

Grazing had an inconspicuous effect on the distribution of soil organic carbon amongst different functional fractions at soil surface. The unprotected fraction was the dominant one (36%-47%, increasing the contribution from heavily grazed areas to ungrazed areas), followed by the physically (24%-30%), chemically (18%-23%) and biochemically (9%-13%) protected fractions. Deeper into the ground (10-20 cm), grazing significantly modified the distribution of soil organic carbon, increasing the relative importance of the biochemically protected fraction (from 6% in ungrazed areas to 19% in grazed areas) and decreasing the contribution of the unprotected fraction (from 35% in ungrazed areas to 21% in grazed areas).

The SOC concentration of the unprotected fraction was positively correlated to the overall organic carbon concentration in the soil, suggesting that none of the three areas are carbon saturated and they might still have a potential to store more carbon in this pool under the prevailing management conditions. The SOC concentration of the physically protected fraction was positively correlated with total SOC concentration in deeper soil layer, whilst, in general, no relationship was found amongst the SOC concentration in the chemically or biochemically protected fractions except in deeper soil layers of the site grazed at moderate intensity, where a positive relationship was found in the biochemically protected fraction. Apart from the effects that grazing could have on the provision of the ecosystem services in the dehesas, such as increasing plant biodiversity or reducing the frequency and intensity of forest fires, extensive grazing can be a valuable tool for increasing the pool of stable carbon and, therefore, sequestering carbon in Mediterranean dehesa soils.

Chapter 6

Prediction of SOC using Vis-NIR spectroscopy. A comparison of devices for spectra recording

Preliminary results of this chapter were presented at an international congress in Austria.

Title: "Prediction of SOC and SOC fractions using Vis-NIR spectroscopy. A comparison of devices for spectra recording"
Event: EGU General Assembly 2018, Vol. 20, EGU2018-12493, 2018
Country: Austria, Vienna
Presentation: Poster

Authors: **Lizardo Reyna-Bowen**[1,2], María T. Hidalgo[1], Ramón Leal[1],José A.Gómez[2], Pilar Fernández-Rebollo[1]

[1]Forestry Department, University of Córdoba, Rabanales University Campus, Ctra. Madrid-Cádiz Km. 396, 14014 Córdoba.
[2]Institute for Sustainable Agriculture-CSIC, 14080, Córdoba, Spain.

6.1 Abstract

Soil analysis is critical for research and precision agriculture, and there is a need to find alternatives to, or complementary methods for, traditional laboratory analysis to overcome limitations derived of high cost or limiting processing times. In the present work, our objectives were a) To compare models calibrated from spectra taken with different devices to estimate the soil organic carbon (SOC) concentration based on visible near infrared (Vis-NIR) spectral signals of soil samples, and b) To evaluate the ability of equations to predict carbon concentration in similar soil types with different land uses. Two hundred and sixty six soil samples taken at different depth from two dehesa farms with different land use were employed. Soil reflectance spectra were measured with LabSpec 5000 spectrophotometer. Spectra were recorded with: (i) Contact probe, (ii) Muglight accessory with a circular tray adapter. SOC concentration was determined by the Walkley & Black method. The spectral data were calibrated against the SOC concentration through Partial Least Squares Regression (PLSR) and Modified Partial Least Squares Regression (PLSR-modified) techniques. The accuracy of the prediction models was verified by validation and cross-validation, using the coefficient of determination (R^2), the root mean squared error (RMSE), the residual predictive deviation (RPD) and the range error ratio (RER). The accuracy of SOC models was very good. R^2 and RPD were higher than 0.95 and 4.54, respectively, and RER higher than 20. External validation provided more conservative accuracy metrics, although RPD indexes were above 3.12, indicating excellent predictions, without differences between the two devices. Both, the Muglight and the contact probe showed good results in RMSE (0.222 vs 0.244) and R^2 (0.90 vs 0.89) respectively. Although both devices accurately predict SOC, the use of the contact probe could reduce the time needed to record the spectra of soil samples compared to the Muglight. The accuracy of the selected model was reduced when was used for predicting organic carbon concentration of similar soil of another farm with different land uses (R^2= 0.66, RPD = 1.09, and RMSE =0.57).

keyword: SOC, organic-matter, Walkley&Black, reflectance, PLSR model

6.2 Introduction

The high variability of the soil organic carbon (SOC) content makes accurate estimates at field or larger scales difficult [Arrouays, 2003].However, SOC must be spatially quantified for several purposes, one of them to determine actual potential, or impact, for carbon sequestration. The high cost of traditional laboratory techniques to measure the concentration of SOC is a major drawback [Bernoux et al., 2002]. another been their time consuming nature. However, there is another alternative to traditional laboratory techniques whose use has been growing rapidly in the last decades.

Visible Infrared Spectroscopy (Vis-NIR) has been used for the prediction of SOC and many soil properties [Rossel et al., 2006, Stenberg et al., 2010, Chen and

Tang, 2016]. VIS-NIR spectrum contains information from two main regions of the electromagnetic spectrum: the visible region (Vis, 350 - 700 nm) and the near-infrared region (NIR, 700 - 2500 nm), which can be used for the acquisition of information on different chemical compounds of soils [Ouerghemmi et al., 2011, Marmette, 2018]. There are specific wavelength ranges that have strong relation with soil carbon and they can be used for predicting the content of soil organic and inorganic carbon [Wenjun et al., 2014, Xu et al., 2018, Vitharana et al., 2019]. For example, Wight et al. [2016] found that the region between 700 nm and 800 nm was correlated with organic carbon prediction and Ostovari et al. [2018] found also relation of soil organic matter (SOM) with spectral reflectance at wavelengths of 490, 671, 785, 1090, 1420, 1860 and 2420 (nm). As a laboratory technique for soil organic carbon determination, spectroscopy is ten times cheaper than traditional methods, such Walkley-Black, due to reduced need for sample handling and used of reagents [LeRoy., 1969, Huang et al., 2010, Rossel et al., 2016, Ji et al., 2016, Marmette, 2018]. According to Rourke and Holden [2011], the estimated time to analyze a sample with the traditional method is similar to that needed to process 5 to 10 consecutive samples with the spectrophotometer, including the repetitions that are necessary for each soil sample. However, the use of Vis-NIR information for SOC prediction requires a previous development of robust models from a wide set of soil samples, which must be analyzed by laboratory standard methods. Different mathematical models have been used to calibrate equations for predicting SOC from the spectral footprint. A partial least squares regression model (PLSR) has been the most widely used for that purposed Wold et al. [2001]. However, in recent years, the performance of others mathematical models have been analyzed, providing most of them satisfactory results: support vector machines (SVM), randomized forest (RF), multivariate adaptive regression splines (MARS), and regression trees (CART), [He et al., 2007, Baldock et al., 2013, Tamburini et al., 2017, Minu and Shetty, 2018]. Most of these models were developed to be applied locally and their usefulness in other areas with similar soil characteristics is still uncertain.

For Vis-NIR spectroscopy, the time spent on processing the soil samples, and hence the cost, is also related to the different type of devices for collecting the spectral information. Some types of devices, such as trays or capsules, should be filled with the soil sample and then slightly compacted, keeping the same compaction for all samples. Between consecutive samples, the device should be cleaned to avoid mixing of soil particles and this task can take quite some time, even more than the recording of the spectrum itself. Other devices, such as contact probes, reduce the time needed for sample preparation, since the spectral signal can be recorded directly from the container containing the soil sample. In addition, cleaning the device after the recording of the spectral signal is easier. However, sources of error could increase with the use of the latter type of device, as contact between the sample and the device depends largely on the operator's ability. For example, the angle of light incidence on the sample may change slightly if the operator does not always hold the probe vertically. These slight variations can affect the spectral signal collected from the sample. Therefore, in this study we pursued these two objectives: a) to compare performance of Vis-NIR models calibrated for SOC concentration estimation from spectra recorded with different devices using Partial Least Squares Regression (PLSR), and b) to validate the ability of the models to predict the SOC concentration of a soil type similar to that used for calibration phase but with different land use.

6.3 Materials and Methods

6.3.1 Site characteristic

This work was carried out from soil samples coming from two dehesa farms located in the province of Córdoba, Spain. The soil type in both study zones is classified as Eutric Cambisol (CSIC-IARA, 1989). For more detail on the characteristics of the sites, see chapters 4 and 5. Although both farms are dehesa and their main use is livestock, there were differences in land use between the farms. On the first farm sheep were the only livestock present, while on the second a flock of sheep, a herd of cattle and a herd of pigs grazed on the farm. In addition, some fields on the first farm were occasionally cultivated with a mixture of vetch and oats and other fields were devoted to permanent pasture, while on the second farm crops were less frequent, with permanent pasture dominating. On the second farm, an abandoned field was included in the sampling scheme.

6.3.2 Soil samples

Two sets of soil samples were collected, each one from one of the farms. The soils of the first farm was sampled on March 2017. The samples were taken below and outside the tree canopy projection at different depth intervals (0-2 cm, 2-5 cm, 5-10 cm, 10-20 cm, 20-40 cm, 40-60 cm, 60-80 cm and 80-100 cm) in a permanent pasture field and in a crop-pasture rotation field. In total, two hundred and sixty-six soil samples were collected because the depth of the soil at the hard C horizon was less than 100 cm at some sampling points. This set was used for calibrating Vis-NIR models. The second farm was sampled on December 2017. Soil samples were taken at five different depth intervals (0-2, 2-5, 5-10, 10-20 and 20-30 cm), out of the tree canopy influence, in a field grazed at high and moderate intensity and in an abandonment field. In total one hundred and eighty soil samples were collected from the second farm. This set was used to analyze the ability of the calibrated models to be extrapolated to other areas with different uses.

6.3.3 Laboratory reference analysis for soil carbon concentration

The soil samples of farms were dried at 40°C, passed through a 2 mm sieve, and homogenized. SOC concentration was determined according to Walkley [1947]. The results of SOC concentration of both farms are shown in table 6.1.

Tab. 6.1: Mean of soil organic carbon (SOC) concentration (%), standard deviation (Std. Dev.), coefficient of variation (CV), median, minimum and maximum values for sampling carried out in farm 1 and farm 2. Results of farm 1 are also shown divided into calibration and validation sets.

Farm	Samples	n	Mean	Std. Dev.	CV	Minimum	Maximum	Median
1	Total set	266	0.72	0.70	96.53	0.00	3.90	0.43
	Calibration	216	0.72	0.71	98.85	0.00	3.90	0.43
	Validation	50	0.73	0.64	87.10	0.02	2.21	0.49
2	Total set	180	0.96	0.49	51.54	0.19	2.56	0.89

6.3.4 Sample preparation and spectral measurement

The samples set were scanned with a portable LabSpec 5,000 spectrometer (350–2,500 nm; ASD Inc., Boulder, Colorado, USA) using IndicoPro 6.0 spectrum acquisition software (ASD Inc., Boulder, CO, USA). The raw channel data sampling rate of 1.4 nm in the visible and near-infrared region (350–1,000 nm) and 2.2 nm in the short wavelength infrared region (1,001–2,500 nm) are interpolated to 1 nm intervals across the full spectrometer range from 350 nm to 2,500 nm. The nominal spectral resolution varies with the spectrometer region. The visible and near-infrared region has a spectral resolution of 3 nm at 700 nm, and the short wavelength infrared region has a spectral resolution of 10 nm at 1,400 nm and 2,100 nm.

The two hundred and sixty six samples were measured using High Intensity Muglight, model-A122100, (ASD Inc.) equipped with a sapphire window using an ASD sampling tray adapter with a quartz window having a 110 mm2 spot diameter (ASD Inc.). The probe features a built-in light source and acts as a workstation, so that samples can be placed on top of the probe. Four replicates of each sample were scanned, two for each tray adapter by rotating it 45°. An average of 50 spectra was collected from each replicate and stored as an average spectrum. White reference scans (with a Spectralon panel) were taken between every sample scan. The final spectrum was obtained by averaging the four composite scans. Additionally, the same samples were measured using Contact Probe, model A122317 (ASD Inc.), equipped with a 20 mm diameter quartz window which leaves a smaller footprint on the samples and enable to access samples in tighter spaces. Samples were placed in a plastic sampler and scanned from overhead. Three replicates scans (each been the average of 50 internal scans) were obtained for each sample, mixing between scans. The final spectrum was obtained by averaging the three composite scans. White reference scans (with a Spectralon panel) were taken between every sample scan. The samples of second farm were only measured with this Contact Probe, with the same methodology explained above, Figure 6.1.

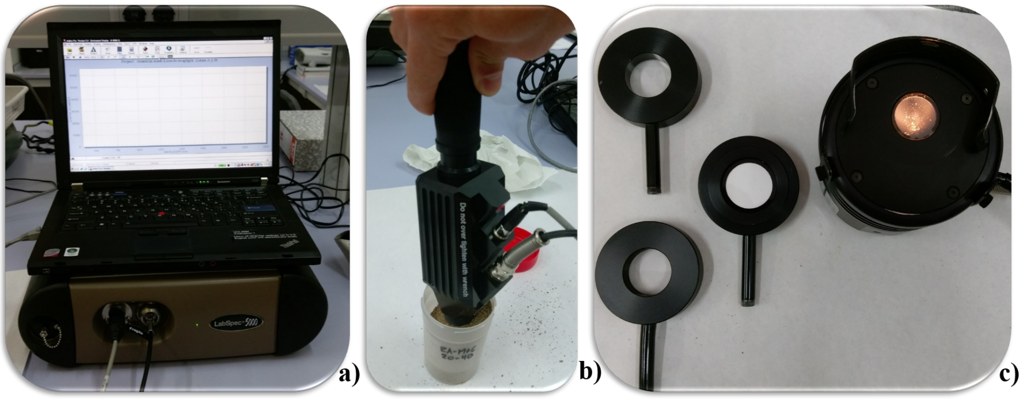


Fig. 6.1: Instruments used: a) LabSpec 5000 (Analytical Spectral Devices, Inc. (ASD), b) Contact-Probe sensor and c) Muglight sensor.

6.3.5 Spectral preprocessing and calibration models

We used the software package WinISI IV (version 4.6.8, Infrasoft International, LLC, State College, PA, USA) for spectral preprocessing. To eliminate noise at the edges of each spectrum, the raw spectra were reduced to between 570 and 2450 nm. To further improve the signal-to-noise ratio, several spectral pre-processing algorithms were tested: standard normal variate (SNV) and standard normal variate and detrending (SNV&D). According to Barnes et al. [1989], this step serves to reduce the dispersion and effect of particle size, and also to eliminate the linear or curvilinear trend of each spectrum. The SNV&D and SNV were used in combination with the first and second derivative of the spectra, using different intervals for derivative calculation (4, 5 and 10 nm), as well as smoothing algorithms.

The spectral data were calibrated against the SOC concentration through Partial Least Squares Regression (PLSR) and Modified Partial Least Squares Regression (PLSR-modified) techniques. Again, we used the software package WinISI IV. The PLSR is a method that relates two data matrices, X of predictors and Y of responses, by a linear multivariate model. PLSR is close to Principal Component Regression (PCR). Unlike PCR, PLSR models the structure of Y and integrates compression and regression steps to select the successive orthogonal factors that maximize the covariance between X and Y [Wold et al., 2001]. Previously, the soil set of the first farm was divided into calibration and validation subset (80% of samples for calibration and 20% for validation) using the algorithm “select” implemented in WinISI software. The spectral outliers that could affect prediction model building were identified by principal component analysis (PCA) considering a Mahalanobis distance less to 3 from the average spectrum [Martens, 1989, Terra et al., 2015].

6.3.6 Model performance evaluation

The best model (PLSR or PLSR-modified with different spectral preprocessing) was selected based on statistic metrics from calibration and cross-validation processes [Huang et al., 2010, Luce et al., 2014]. For this purpose, we used the coefficient of determination (R^2) and the standard error of calibration (SEC). SEC was calculated by equation 6.1:

$$SEC = \sqrt{\sum_{i=1}^{n} \frac{(y_{ii} - y_i)^2}{n - p - 1}} \tag{6.1}$$

where yii and yi are the measured and predicted values of sample i, respectively, n is the number of samples and p is the number of PLSR variables used by the model.

For cross-validation, the calibration dataset was randomly divided into four folds. Four models were built leaving, each time, one of the four folds apart for validation. This way, four measures of R^2 and SEC of the predictions were made. The final result of the cross-validation is the average of R^2 and SEC values thus obtained, denoted by 1-VR and SECV respectively.

Additionally, in order to assess the model performance, we calculated the range error ratio (RER) and the ratio of performance to deviation (RPD), define as:

$$RER = \frac{(Min - Max)}{SEC} \tag{6.2}$$

$$RPD = \frac{SDval}{SECval} \tag{6.3}$$

According to Roberts et al. [2004] values of RER>20 indicate that model is excellent; RER $\geq$15 to $\leq$20, the performance of the model is successful; from RER $\leq$10 to <15 the model is moderately successful and finally a value of RER $\leq$8 to <10 indicates that the model is moderately useful. Pinheiro et al. [2017], evaluate the model prediction capacity through RPD index as follows: RPD <1.0 indicates very poor predictions, unsuitable for analysis; 1.0 RPD <1.4 indicates poor predictions; >1.4 RPD <1.8 indicates fair predictions, suitable for assessment and correlation; >1.8 RPD <2.0 indicates good predictions, suitable for quantitative assessment; >2.0 RPD <2.5 indicates very good quantitative predictions, and RPD >2.5 indicates excellent predictions.

The model outputs were validated via external validation, using the validation subset, the 20% of soil samples of the first farm reserved apart. The SOC values measured against those predicted by the model were compared using the root mean square error (RMSE) (Equation 6.4). RER and RDP indexes were also calculating for validation considering the range, SD and RMSE of validation subset.

$$RMSE = \sqrt{\sum_{i=1}^{n} \frac{(y_{ii} - y_i)^2}{n}} \tag{6.4}$$

where yii and yi are the measured and predicted values of sample i, respectively, and n is the number of samples. Finally, we predicted the SOC of the second farm soil samples using their spectra and the best model selected. RMSE was used to study the fit of the data.

6.4 Results and discussion

6.4.1 Variations of soil spectral reflectance with soil depth

The reflectance spectra were analyzed in the range of 570 to 2450 nm. In a general comparison, the reflectance recorded with the Contact-Probe device was lower that the reflectance gathered with the Muglight instrument (figure 6.2 a). This is in line with the size of the lighting window, of larger diameter in Muglight device. However, the spectra retain the same curve styles, only varying in the degree of reflectance (figure 6.2 b). This same behavior was seen in the work of [Gandariasbeitia et al., 2017], where the same device was compared, obtaining the same results in the reflectance of the devices found in this study.

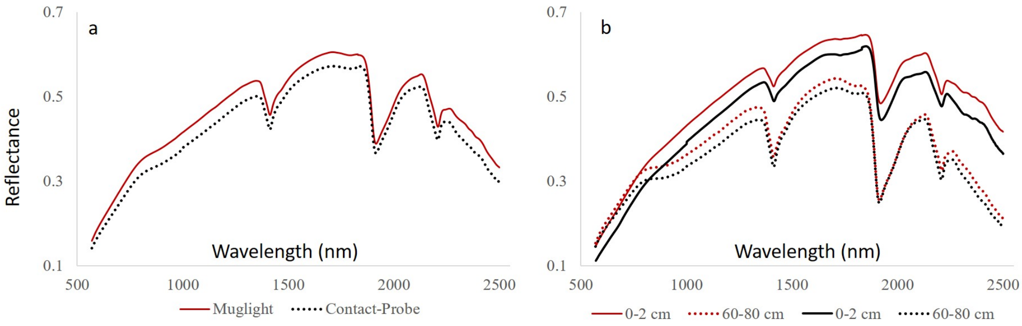


Fig. 6.2: Comparison of the spectral curve of soil samples of the first farm recorded with different sensors: Muglight represented with a continuous line and Contact Probe sensor represented with a segmented line. (Left) the average of the total soil spectral and (right) the average soil spectral for two depths: 0-2 cm and 60-80 cm

The results of the two hundred and sixteen soil samples scanned with both sensors showed two main groups: the soil spectra of the upper layers (0-2, 2-5, 5-10, and 10-20 cm) that exhibited higher values of SOC content, showed also higher reflectance, while the reflectance of the soil samples from deepest layers (40-60, 60-80, and 80-100 cm), with lower SOC content, was also lower (Figure 6.3).

Analyzing the first sample group and sections with similar clay content (i.e. 0-2, 2-5, 5-10 and 10-20 cm). The overall results of the carbon content show a clear stratification in the soil profile. Also, the variations of the reflectance of the spectra are visible according to the carbon content of the soil. Figure 6.3a shows the spectra of farm 1 under the canopy of the young tree in the pastured dehesa, in which the 0-2 cm and 2-5 cm samples with 1.98% and 1.65% soil carbon content showed the lowest reflectance. On the other hand, the 5-10 cm and 10-20 cm depth samples with 0.75% and 0.40% carbon content respectively, showed higher reflectance.

Figure 6.3b shows the samples in the area outside the canopy projection of the young tree canopy. The results showed that the samples with the highest organic carbon content were those taken at 0-2 cm. The mean spectra of those samples, showed lower reflectance than the spectra of samples taken at 2-5, 5-10 and 10-20 cm. Figure 6.3c, the samples taken under the canopy of the adult tree showed similar organic carbon contents in the first depths. This was reflected in the spectra that follow the same pattern without differences. However, soil samples taken outside the influence of the adult tree canopy showed a variation in spectra profile. These samples also showed a stratification of carbon content, but in an lesser degree than the other groups.

As several studies show, the relationship between carbon content and spectral curves is that the more carbon, the lower reflectance [Guillén et al., 2013, Omran, 2017, Xu et al., 2018, Kusumo et al., 2018b]. Another fundamental factor that affects the reflectance curves is the mineralogy of the soil. The greater the amount of clay, the lower the soil reflectance [Stenberg et al., 2010, Siirt, 2016, Omran, 2017, Kusumo et al., 2018a]. In the second group of soil samples, the soil samples from deepest layers (40-60, 60-80, and 80-100 cm), the behavior of the soil mineralogy can be better assessed. As there are no differences in the organic carbon content, the variability observed in the spectral curves is due to the variation in clay, silt and sand content. In figure 6.3c and d, showing the pastured-crop area, it can be seen that mean spectrum curves of soil section 40-60 cm and 60-80 cm are above those of soil section 20-40 cm and 80-100 cm, probably reflecting the higher clay content in these soil layers. Instead, in the pastured area, the clay stratification it seems to follow a different pattern. Figure 6.3c seems to show an increase in clay content with deep in soils under the tree canopy, while outside of the tree canopy the stratification is less marked (figure 6.3b). These agree with clay distribution in soil with deep showed in chapter 4.

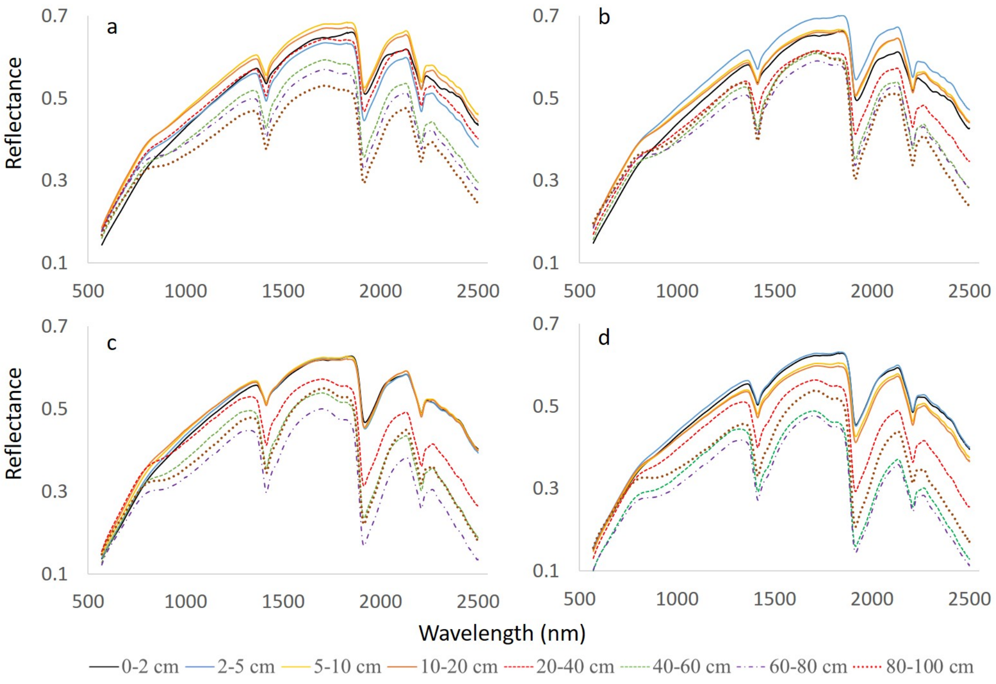


Fig. 6.3: Soil reflectance of soil samples from first farm at different depths using the Muglight sensor for recording the spectra: a) pastured area below the canopy of young Holm oak tree; b) pasture area outside the canopy of young Holm oak tree; c) pasture-crop area below the canopy of mature Holm oak tree; d) pasture-crop area outside the canopy of mature Holm oak tree.

The absorption feature in the range 350–1000 nm might be due to the Fe oxides in the soil, mainly hematite and goethite, and the influential wavelengths located between 1000 and 2500 nm can be attributed clay minerals and organic matter

[Rossel and Behrens, 2010]. Figure 6.4 showed the first derivative reflectance spectra of soil at different depths and recorded with both sensors.The curves of first derivative of raw spectra was similar, independently of the device using for recording spectra. The results of the reflectance with the first derivative in different sections of the soil profile (See Figure 6.4) showed that the range of 1400, 1900, and 2200 nm were the absorption peaks possibly related to C-H, O-H, and C-O combinations. The spectra also showed a small absorption peak at approximately 2200 nm, which is related to the clay lattice Al-OH absorption band [Clark et al., 1990]. These peaks coincide with several works where they works related to the carbon content and the mineralogy of the soil [Omran, 2017, Xu et al., 2018, Douglas et al., 2018, Chen et al., 2018]. In addition, slight differences in spectral shapes were present throughout the spectral range, indicating subtle absorption peaks.

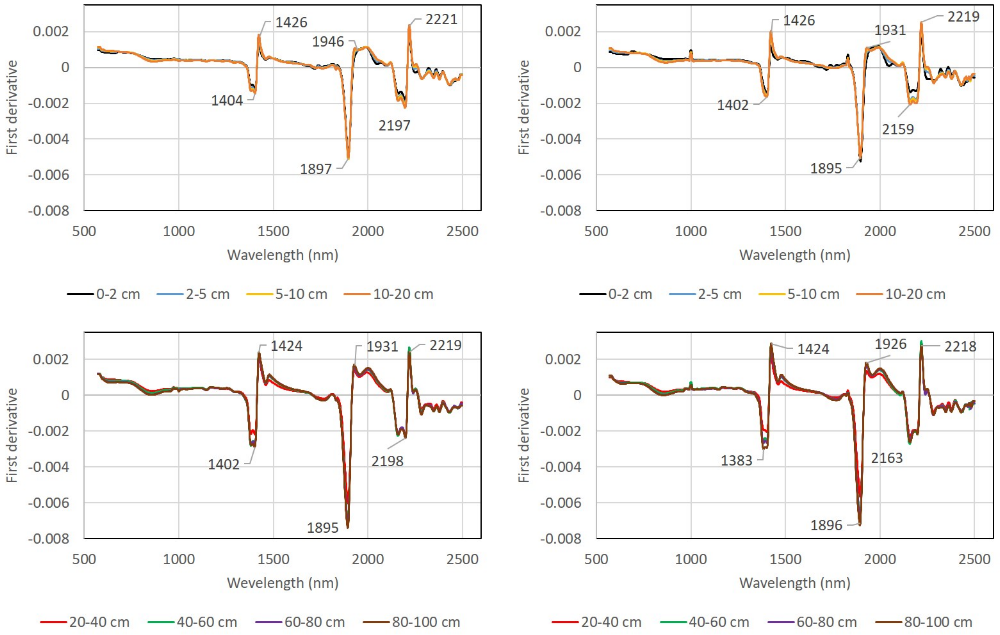


Fig. 6.4: Mean first derivative reflectance spectra (FDR) of soil at different depths with recorded with different sensor: Muglight sensor (Left) and Contact-Probe sensor (Right).

6.4.2 Calibration and Prediction of SOC

The calibration with the two devices showed good results, determining the percentage of carbon obtained in the soil samples. Likewise, all the models obtained were good, regardless of the device used to take the spectra. Among the models used to predict organic carbon values, the best was the PLSR-modified. Compared to other works, the pre-processing of the spectra improves the calibration models in our case. According to quality indicators such as R2, SEC, 1-VR, SEVC, RDP and RER the selected models showed in table 6.2 are excellent (There is a full table in annex

1). Figure 6.5 shows the results of the validation (50 soil samples from farm 1). The validation gave good results with R^2 values of 0.90 for the Muglight device and 0.89 for the Contact-Probe.

Tab. 6.2: Result of the calibration using Contact-Probe and Muglight recording devices. Coefficient of determination (R^2); residual predictive deviation (RPD); Range Error Ratio (RER), and coefficient of determination of cross-validation (1-VR) from PLSR-modified models.

	Regression	**Spectral preprocesing**	**n**	**Mean**	**Range**	**Std.**	**SEC**	**R^2**	**SEVC**	**1-VR**	**RPD**	RER
Contact-Probe	PLSR-modified	SNV 1,4,4,1	200	0.642	0.00-3.90	0.616	0.110	0.968	0.124	0.959	5.0	31.37
Muglight	PLSR-modified	SNV&D 1,10,5,1	198	0.589	0.00-2.46	0.509	0.089	0.969	0.112	0.952	4.5	21.88

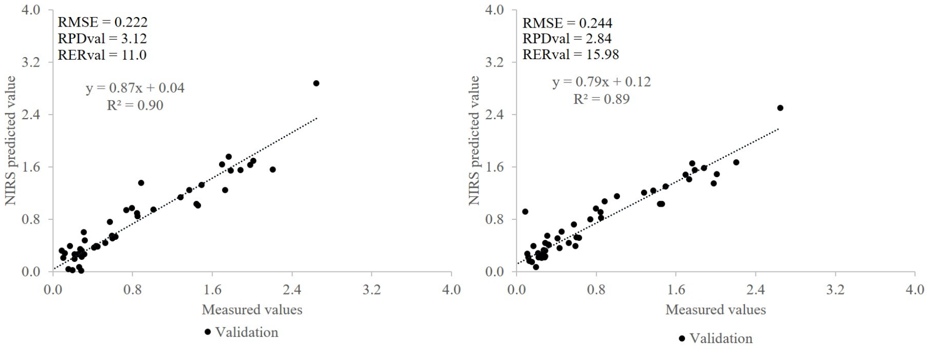


Fig. 6.5: The relationship between SOC laboratory measurement and SOC predicted values. 50 samples of SOC prediction and samples of calibration model; from the C variation values. Muglight sensor (left) and Contact-direct sensor (right). Root Mean Squared Errors of validation (RMSE), Residual Predictive values (RPD) and Range Error Ratio (RER) with calibration and validation.

Literatures shows how models calibrated for other soil types have obtained an adjustment of R^2 ranging from 0.75, to 0.99 [Douglas et al., 2018, Kusumo et al., 2018a,b, Luce et al., 2017, Hosseini et al., 2017, Makovníková et al., 2017]. Minu and Shetty [2018] obtained similar results in calibration with R2 = 0.85 and RPD = 2.58 using two hundred samples for calibration. The values obtained in our work are within the highest predictive capabilities shown in the literature. The results obtained for predictions with the validation soil samples with the two devices (Contact-Probe with a value of R^2 = 0.89, RMSE = 0.244, RPD = 2.484, and Muglight with a value of R^2 = 0.90, RMSE = 0.222, and RPD = 3.12) are successful according to [Pinheiro et al., 2017]. The results obtained in this work suggest that the instrument giving the best results for our conditions was the Contact-Probe, since the time required to record the spectra was less.

Figure 6.6 shows the results obtained in farm 2. The prediction of the carbon content values with an R^2 = 0.66, RPD = 1.09, and RMSE = 0.57. Comparing with the results obtained with farm 1 with the Contact-Probe device with a value of R^2 = 0.89, RMSE = 0.244, RPD = 2.484, these results show that the potential of the model decreases significantly when extrapolating in farm 2 the model calibrated for farm 1. In general, the model underestimates the values of SOC concentrations, specially those corresponding to samples taken in the abandoned area of the second farm (blue dots in figure 6.6). There is a tendency to better predict the SOC concentration of soil samples taken in heavily grazed areas. Low values of SOC concentration of abandoned area were the worst predicted. These results agree with the idea that predictions with site-specific calibrations are better that those made with calibrations of other site or derived from soil spectral libraries at regional scale. However, as stated by Lobsey et al. [2017], the used of "local" spectroscopic techniques that use a small representative set of site-specific spectrum with a selected subset of data from soil spectral libraries, could improve the accuracy of the prediction.

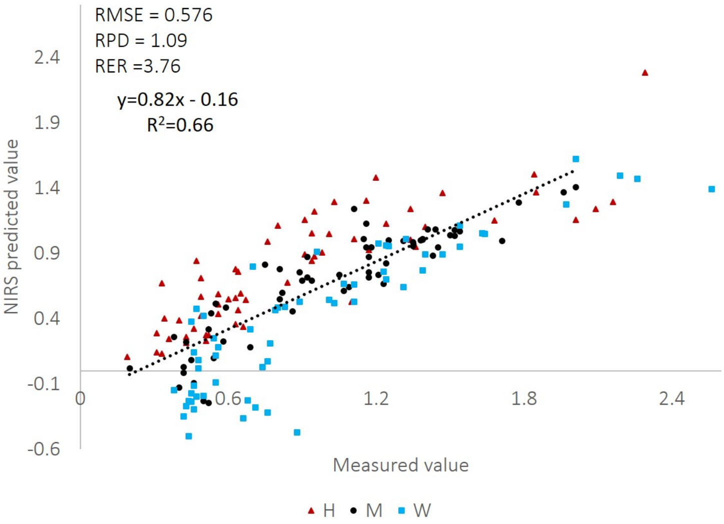


Fig. 6.6: The relationship of SOC concentration measured at farm 2 and Vis-NIRS predicted values (180 samples). Land used with different grazing: high intensity (H), moderate intensity (M), and without grazing (W). Root Mean Squared Errors of validation (RMSE), Residual Predictive values (RPD) and Range Error Ratio (RER).

However, this shows that the PLRS prediction models can be calibrated for different types of soils for their accuracy in predicting organic carbon. However, the prediction in the second farm, where there is the same type of soil and with different management (intensive, moderate and without grazing), was a poor prediction according to Pinheiro et al. [2017] of the carbon content values. However, although the model adjustment was moderate, we consider that it is not convenient to use it on farm 2 despite the fact that the soil has similar characteristics.

6.5 Conclusion

The accuracy of calibrated models to predict SOC concentration by Vis-NIR spectroscopy was high. The coefficient of determination of cross-validation and RPD were higher than 0.95 and 4.54, respectively, and RER was higher than 20. Both devices used for recording the spectra, the muglight and the contact-probe, provided models of similar accuracy. The use of the contact-probe reduces the time required to record the spectra of soil samples compared to the muglight and may therefore reduce the analytical cost and improved the financial feasibility of soil spectroscopy. The accuracy of the best model using the contact-probe device to record the spectra was reduced when it was used for predicting the organic carbon concentration of similar soil from another farm with different land uses ($R^2 = 0.66$, RPD = 1.09, and RMSE =0.57). The accuracy and robustness of the model can be improved by adding site-specific samples to the calibration data set.

annex 1

Full table of the result of the calibration using Contact-Probe and Muglight recording devices. Coefficient of determination (R^2); residual predictive deviation (RPD); Range Error Ratio (RER), and coefficient of determination of cross-validation (1-VR) from PLSR-modified models.

Contact-Probe	**Regression**	**Scatter**	**Derivative**	**Samples**	**Mean**	**Range**	**Std.**	**SEC**	**RSQ**	**SEVC**	**1-VR**	**RPD**	**RER**
	PLS	SNV&D	1,5,5,1	200	0.601	0.00-3.90	0.559	0.121	0.953	0.14	0.938	3.99	27.79
			1,10,5,1	199	0.623	0.00-3.90	0.582	0.126	0.953	0.137	0.945	4.25	28.39
			2,5,5,1	205	0.645	0.00-3.90	0.608	0.143	0.945	0.23	0.857	2.64	16.91
			2,10,5,1	200	0.629	0.00-3.90	0.587	0.127	0.953	0.165	0.921	3.56	23.58
			1,4,4,1	200	0.621	0.00-3.90	0.572	0.12	0.956	0.143	0.938	4.00	27.20
			2,4,4,1	202	0.624	0.00-2.82	0.541	0.166	0.906	0.274	0.744	1.97	10.26
		SNV-ONLY	1,5,5,1	200	0.614	0.00-3.90	0.568	0.119	0.956	0.133	0.945	4.27	29.25
			1,10,5,1	202	0.625	0.00-3.90	0.580	0.117	0.959	0.134	0.947	4.33	29.03
			2,5,5,1	205	0.645	0.00-3.90	0.608	0.143	0.945	0.23	0.857	2.64	16.91
			2,10,5,1	200	0.629	0.00-3.90	0.587	0.127	0.953	0.165	0.921	3.56	23.58
			1,4,4,1	200	0.614	0.00-3.90	0.568	0.117	0.957	0.135	0.943	4.21	28.81
			2,4,4,1	208	0.661	0.00-3.90	0.621	0.117	0.919	0.304	0.762	2.04	12.80
	PLS - modified	SNV&D	1,5,5,1	200	0.635	0.00-3.90	0.609	0.111	0.967	0.123	0.960	4.95	31.63
			1,10,5,1	197	0.618	0.00-3.90	0.594	0.114	0.963	0.120	0.959	4.95	32.42
			2,5,5,1	203	0.635	0.00-3.03	0.564	0.087	0.976	0.173	0.907	3.26	17.46
			2,10,5,1	204	0.638	0.00-3.17	0.589	0.112	0.964	0.146	0.934	4.03	21.64
			1,4,4,1	201	0.647	0.00-3.90	0.622	0.114	0.967	0.125	0.960	4.98	25.28
			2,4,4,1	200	0.610	0.00-3.90	0.562	0.109	0.962	0.190	0.885	2.96	20.47
		SNV-ONLY	1,5,5,1	203	0.649	0.00-3.90	0.626	0.119	0.964	0.131	0.956	4.78	29.69
			1,10,5,1	201	0.635	0.00-3.90	0.608	0.119	0.962	0.127	0.956	4.79	30.63
			2,5,5,1	201	0.633	0.00-3.03	0.567	0.086	0.977	0.166	0.914	3.42	18.19
			2,10,5,1	204	0.638	0.00-3.17	0.589	0.112	0.964	0.146	0.939	4.03	21.64
			1,4,4,1	200	0.642	0.00-3.90	0.616	0.110	0.968	0.124	0.959	4.97	31.37
			2,4,4,1	200	0.610	0.00-3.90	0.562	0.109	0.962	0.190	0.885	2.96	20.47
Mugligth													
	PLS	SNV&D	1,5,5,1	199	0.595	0.00-2.46	0.518	0.104	0.959	0.119	0.948	4.35	20.59
			1,10,5,1	200	0.600	0.00-2.46	0.522	0.109	0.956	0.119	0.049	4.39	20.59
			2,5,5,1	201	0.597	0.00-3.03	0.515	0.093	0.968	0.14	0.927	3.68	21.57
			2,10,5,1	200	0.586	0.00-2.46	0.496	0.107	0.953	0.128	0.933	3.88	19.14
			1,4,4,1	198	0.604	0.00-2.46	0.524	0.098	0.965	0.118	0.949	4.44	20.76
			2,4,4,1	201	0.616	0.00-3.03	0.536	0.09	0.972	0.168	0.902	3.19	17.98
		SNV-ONLY	1,5,5,1	200	0.620	0.00-3.20	0.552	0.116	0.956	0.133	0.943	4.15	23.98
			1,10,5,1	201	0.600	0.00-2.64	0.520	0.111	0.954	0.121	0.946	4.30	21.74
			2,5,5,1	201	0.597	0.00-3.03	0.515	0.093	0.968	0.14	0.927	3.68	21.57
			2,10,5,1	202	0.602	0.00-2.64	0.519	0.111	0.954	0.134	0.933	3.87	19.63
			1,4,4,1	202	0.625	0.00-3.03	0.554	0.11	0.961	0.131	0.945	4.23	23.05
			2,4,4,1	201	0.616	0.00-3.03	0.536	0.09	0.972	0.168	0.902	3.19	17.98
	PLS - modified	SNV&D	1,5,5,1	202	0.607	0.00-2.46	0.524	0.106	0.959	0.13	0.939	4.03	18.85
			1,10,5,1	198	0.589	0.00-2.46	0.509	0.089	0.969	0.112	0.952	4.54	21.88
			2,5,5,1	204	0.619	0.00-3.03	0.548	0.082	0.978	0.135	0.940	4.06	22.37
			2,10,5,1	202	0.602	0.00-2.46	0.520	0.107	0.958	0.126	0.941	4.13	19.44
			1,4,4,1	201	0.604	0.00-2.46	0.526	0.093	0.969	0.123	0.946	4.28	19.92
			2,4,4,1	205	0.613	0.00-3.03	0.535	0.079	0.978	0.151	0.921	3.54	20.00
		SNV-ONLY	1,5,5,1	201	0.604	0.00-2.46	0.525	0.099	0.964	0.120	0.948	4.38	20.42
			1,10,5,1	202	0.605	0.00-2.46	0.524	0.106	0.959	0.125	0.943	4.19	19.60
			2,5,5,1	204	0.619	0.00-3.03	0.548	0.082	0.978	0.135	0.940	4.06	22.37
			2,10,5,1	201	0.595	0.00-2.46	0.511	0.105	0.957	0.124	0.941	4.12	19.76
			1,4,4,1	201	0.613	0.00-2.46	0.534	0.093	0.970	0.124	0.946	4.31	19.76
			2,4,4,1	200	0.583	0.00-3.03	0.491	0.076	0.976	0.138	0.921	3.56	21.88

Chapter 7

General Discussion

In this Thesis, the effect of land use and management on soil organic carbon stock was evaluated under different conditions to provide an overall view of the impact of land use and soil management. Three different land uses were addressed, agriculture in Ecuador, agro-silvo-pastoralis in Spain and forestry in Poland. This study of soil organic carbon stock under diverse conditions was chosen to provide a comprehensive training to the doctorate candidate on the subject, allowing also a broader perspective on different environment and management conditions. In chapter 2 this thesis addressed the effect of different land uses on soil organic concentration and stock under different Land Uses in the Carrizal-Chone Valley in Ecuador. Using sixty-four soil pits for soil mapping purposes in the valley, we took advantage of them to evaluate the impact of land use on soil organic carbon (SOC). Our results showed that in the top soil horizon (A) with an average depth of 40 cm, the higher concentration of SOC (1.80 and 1.60% respectively) corresponded to two tree crops, banana and cocoa, followed by maize and cacao (1.33 and 1.07%, respectively), while the lowest values were found in lemon orchards and fallow land plowed before a rotation (0.35% SOC in both cases). It is worth mentioning that the areas with natural vegetation ranked, in terms of top soil SOC in an intermediate position, around 0.8% SOC. There was a significant influence of soil texture, with higher SOC concentration in soils with of clay loamy or clay texture, which reflects both the higher affinity of the soil to preserver organic carbon as well as their better capability to sustain biomass production of the crops, which might interact with the effect of land use. Nevertheless, our study shows how agricultural uses that provide a significant return of biomass to the soil and a soil management that does not disturb aggressively the soil can achieve high SOC concentrations in the top soil, which might be compatible with provision of Ecosystems Services related to a high SOC content in the top soil with agricultural production. The interpretation of the analysis of SOC concentration into stock is more complicated since it also depends on the total soil depth, bulk density and rock content, making more complicated to link results to land use with such a limited (64) soil pits. Anyway, our SOC stock results were in the higher range of those described for the A horizon in Ecuadorian

Amazon reinforcing the idea that appropriate management of agricultural crops can result in the storage of a significant amount of OC in the top A horizon.

In chapter 3 this thesis studied factors controlling SOC distribution in a forested area of a completely different environment, on continental and humid climate in Poland. This study reflected a high variability among different forest stands and a high impact of the soil type on SOC concentration and stock, but not of different elevation of the forest stands, which might have an effect on forest biomass productivity. Nevertheless, in spite of this, there is a clear trend towards an increase in SOC stock in Alder and Spruce forests as compared to mixed stand and Beech, reflecting the higher productivity and age of the first two stands. Overall these forest areas stored a large amount of SOC in the top 1-m of the soil, with values ranging between 80 t ha^1 and 240 t ha^1. This study contributed to quantify this potential for SOC storage as well as exploring some of their sources of variability for a better appraisal at regional and national scales.

Chapters 4 and 5 changed scale, studying in detail the effect on SOC stock and concentration of different management strategies in an agroforestry system of high natural relevance, dehesa. Chapter 4 studied the effect on SOC concentration and stock of replacing old dehesas at low tree density (1.2 tree ha^1) in a rotation with one seeding every 3 year and low grazing intensity with one at high tree density (70 tree ha^1), non-plowed and also at low grazing intensity. Our results showed that after 22 years the high tree density dehesa showed the same SOC stock in the whole soil profile, due probably to the limited soil carbon accumulation in both dehesas, in a semiarid environment, which can´t compensate possible SOC losses during the soil disturbance required to install the high tree density dehesa. Overall, the new dehesa only showed higher SOC concentration in the very top 0-2 cm soil layer, and the effect of the tree on enhancing SOC concentration was only noted in the vicinity of the mature tree of the low density dehesa. These results indicate a low rate of carbon accumulation into the soil in this system, suggesting that any attempt of increasing SOC in dehesa should be planned at very long-term, preserve mature trees, and expect a moderate increase with current dehesas, providing there are not overgrazed or cultivated intensively. Chapter 4 also presents, to the best of our knowledge, the first attempt of evaluation the distribution of SOC in dehesa in the four fraction standardized by Six et al. [2002]. Our results showed that in this study permanent grassland and crop-pasture rotation presented a similar distribution of soil organic carbon amongst different functional fractions, with the unprotected fraction being the dominant one (30% - 45%), followed by the physically (15% - 25%) and chemically (15% - 25%) protected fractions. The presence of mature trees significantly modified the distribution of soil organic carbon in their surroundings, increasing the importance of the unprotected fraction (40% - 70%), and decreasing the relative importance of the physically (10% - 25%) and chemically protected (9%

- 29%) fraction. Chapter 5 studied another management variable which might affect SOC concentration and fraction distribution in the topsoil of dehesa, grazing intensity. We studied three different grazing intensity high, moderate and none. The average of the livestock density per ha was within 0.5-0.6 (LU ha^1), for the high-intensity grazing area in this case. The vicinity of the watering trough was chosen as a high intensity grazing area, with the understanding that it is more frequented by grazing animals, sometimes more than twice as much, and can exemplify adequately what happens in soil subjected to high intensity grazing and trampling. The moderate grazing intensity represents the common livestock range managed in dehesa farms of this region, 0.5-0.6 LU ha^1, and an area excluded from livestock 20 years ago is representative of the absence of grazing. Despite the finding of significant increase in SOC concentration with moderate grazing, non-differences among the three grazing intensities was found in SOC stock, suggesting compensating mechanisms at the grazing intensities studied. The abundance of legumes induces by grazing and the acceleration of N-recycling through the excreta could increase pasture production of grazed areas compared to non-grazed areas and compensate the carbon loss in the diet selected by livestock. The trampling of heavily grazed areas increases the bulk density of the soil, which could lead, in the calculation process, to a greater reserve of SOC, since the apparent density of the soil is a multiplier variable. Overall, grazing significantly increased the carbon concentration of biochemically protected fraction and its relative contribution to the total SOC stock. In the top soil of all areas the dominant fraction, in terms of total SOC distribution was the unprotected one, as in the dehesa of Chapter 4. However in deeper soil layers of moderately grazed areas, all four fractions contributed with a similar weight to the total carbon stock, while in non-grazed areas the unprotected fraction still remained as dominant. This finding suggest that grazing could be a valuable tool moderately increase the pool of stable carbon and, therefore, to sequester more stable carbon in dehesa soils. To our knowledge, there are sow few studies that evaluate grazing effect on the distribution of SOC content in the different functional fractions proposed by Six et al. [2002] that the findings of Chapter 5 constitutes a relevant contribution to this issue.

Chapter 6 of this thesis explored the improvement of prediction of SOC using Vis-NIR spectroscopy, evaluating methodologies and different devices. All the soil samples managed in Chapters 4 and 5 were recorded through the LabSpec 5000 spectrophotometer in order to have a wide spectral soil library for developing predictive equations on SOC using different devices for recording the spectral signal: Mugligth accessory with a circular tray adapter and a contact probe. The accuracy of the SOC models was excellent and similar for both devices. R^2 of cross validation and RPD (ratio between the standard deviation of the validation dataset and the standard error of cross validation) were always higher than 0.95 and 4.5, respectively. In addition, the RER index (ratio between the range of calibration dataset and the standard error of cross validation) reached values above 20 with RMSE = 0.222.

External validation provided more conservative accuracy metrics. Given that the use of both devices provide predictive equations of similar performance, and the time spent in processing the sample for reading the spectral signal is lower when using the contact probe, the prediction of SOC concentration with the contact probe ensembled to the LabSpec 5000 spectrophotometer is presented as the most suitable option. Comparing with traditional methods for soil organic carbon concentration assessment, the use of Vis-NIR technology would reduce the resources and time spending for this task and could enhance our capabilities of measure a large amount of soil samples in a monitoring program. However, the performance of the developed model was reduced when we use it to predict SOC concentration in an area with a similar soil but with different management (non-grazed for 20 years). These results suggest that, in addition of having extensive soil spectral libraries, specific site calibration models should be developed to encompass the huge variability in soils and management practices.

Overall, the research developed in this Thesis has underlined three major issues in the study of SOC on agricultural and forest soils. Chapters 2 and 3 have underscored the great effect of climate, land use and soil type on SOC concentration and total stock, indicating how significant increases in them can be achieved, regardless of the environment, with a proper land use (like banana and cocoa tree crops in Ecuador) or forest stands (like Alder and Spruce forests in Poland), although the maximum SOC will be regulated by climate conditions and soil type. These chapters underscored the large variability in determining SOC stock due to the different sources of variability, soil depth, bulk density, rock fragments, soil type, which although not new [Reyna-Bowen, 2020] reminds the large uncertainty when determining SOC for a given situation. This might have relevant implications for initiatives, such as the 4 X 1000 initiative [Minasny et al., 2017] to ensure that agriculture plays a relevant role in mitigating and adapting to climate change, which will require certification by some empirical technique. Chapters 4 and 5, which provide a modest contribution to the global pool of experimental data on SOC stocks in soil, suggest that it is going to be quite complicated, if possible, to achieve the required accuracy to determine this increment, or differences among similar systems with the required accuracy. Improvement of VIS-NIR techniques, such as the ones studied in Chapter 6, might enhance our capabilities providing more affordable, and robust, technologies to measure a large number of samples with the required accuracy, albeit it is less clear how to deal with other major sources of variability such as soil depth, soil type, bulk density and rock content. An alternative for a better understanding of the response of SOC in agricultural systems to soil management are detailed experiments, such as those described in chapters 4 and 5. A comprehensive set of these experiments can provide a good understanding of the dynamic of SOC in an given agricultural system, dehesa in this thesis, that might lead to conceptual or numerical simulations models which, can reduced (if the required accuracy is achieved) the need of large scale sampling to verify evolution of the increase of SOC into the soil as expected. In the case of dehesa, the contribution of this thesis has been to clarify the non-detectable effect of substituting mature low tree density dehesas into high density dehesas to increase SOC stock in the medium term under the environmental and management conditions typical of Southern Spain. Also the high impact of mature trees on modifying SOC concentration in their surroundings, which suggest that they might be retained when changing tree density. Another result is that these studies in dehesa has also shown a non-existing effect of changing grazing density on SOC stock in the top 30 cm of the soil within the usual range of sustainable grazing density used in the region. This thesis also presented, to our knowledge the first study of SOC among the four functional groups defined by Six et al. [2002] indicating also a dominance of the non-protected fraction in all the situations studied in chapters 4 and 5, which might provide a good overview of typical situations in dehesa, how the biochemically protected fraction has the lowest share of the overall stock, and the lack of effect on the distribution among functional groups of increasing tree density after 22 years or the modification of grazing intensity.

Bibliography

State of the world's forests: enhancing the socioeconomic benefits from forests. *Choice Reviews Online*, 53(01):53–0033–53–0033, aug 2015. doi: 10.5860/choice.191454.

M. Abdalla, A. Hastings, D. Chadwick, D. Jones, C. Evans, M. Jones, R. Rees, and P. Smith. Critical review of the impacts of grazing intensity on soil organic carbon storage and other soil quality indicators in extensively managed grasslands. *Agriculture, Ecosystems & Environment*, 253:62–81, feb 2018. doi: 10.1016/j.agee.2017.10.023.

S. W. Alexandrowicz. Outlines of geology of the babia góra range. in b.w. wołoszyn, a. jaworski, j. szwagrzyk (eds). *The Nature of the Babiogórski National Park. Kraków: Komitet Ochrony Przyrody PAN, Babiogórski Park Narodowy*, 1, 2004.

Andalucia. Plan director de las dehesas de andalucía. In *Anexos, Junta de Andalucia*. Junta de Andalucia, 2017.

J. M. Andrade-Garda, A. Carlosena-Zubieta, R. Boqué-Martí, and J. Ferré-Baldrich. CHAPTER 5. partial least-squares regression. In *RSC Analytical Spectroscopy Series*, pages 280–347. Royal Society of Chemistry, 2013. doi: 10.1039/9781849739344-00280.

F.-C.-J. C. S. N. A. F. . B. M. Arrouays, D. Estimation de stocks de carbone organique des sols à différentes échelles d ' espace et de temps. *Et Gestion Des Sols.*, 1(1), 2003.

M. S. Askari, S. M. O'Rourke, and N. M. Holden. A comparison of point and imaging visible-near infrared spectroscopy for determining soil organic carbon. *Journal of Near Infrared Spectroscopy*, 26(2):133–146, apr 2018. doi: 10.1177/0967033518766668.

D. J. Augustine and S. J. McNaughton. Interactive effects of ungulate herbivores, soil fertility, and variable rainfall on ecosystem processes in a semi-arid savanna.

Ecosystems, 9(8):1242–1256, dec 2006. doi: 10.1007/s10021-005-0020-y.

E. Ayres, H. Steltzer, S. Berg, M. D. Wallenstein, B. L. Simmons, and D. H. Wall. Tree species traits influence soil physical, chemical, and biological properties in high elevation forests. *PLoS ONE*, 4(6):e5964, jun 2009. doi: 10.1371/journal.pone.0005964.

E. Bahr, D. C. Zaragocin, and F. Makeschin. Soil nutrient stock dynamics and land-use management of annuals, perennials and pastures after slash-and-burn in the southern ecuadorian andes. *Agriculture, Ecosystems & Environment*, 188:275–288, apr 2014. doi: 10.1016/j.agee.2014.03.005.

J. A. Baldock, B. Hawke, J. Sanderman, and L. M. Macdonald. Predicting contents of carbon and its component fractions in australian soils from diffuse reflectance mid-infrared spectra. *Soil Research*, 51(8):577, 2013. doi: 10.1071/sr13077.

T. Bardelli, M. Gómez-Brandón, J. Ascher-Jenull, F. Fornasier, P. Arfaioli, D. Francioli, M. Egli, G. Sartori, H. Insam, and G. Pietramellara. Effects of slope exposure on soil physico-chemical and microbiological properties along an altitudinal climosequence in the italian alps. *Science of The Total Environment*, 575:1041–1055, jan 2017. doi: 10.1016/j.scitotenv.2016.09.176.

R. Baritz, G. Seufert, L. Montanarella, and E. V. Ranst. Carbon concentrations and stocks in forest soils of europe. *Forest Ecology and Management*, 260(3):262–277, jun 2010. doi: 10.1016/j.foreco.2010.03.025.

R. J. Barnes, M. S. Dhanoa, and S. J. Lister. Standard normal variate transformation and de-trending of near-infrared diffuse reflectance spectra. *Applied Spectroscopy*, 43(5):772–777, jul 1989. doi: 10.1366/0003702894202201.

E. B.-L. Barrezueta, Salomon; Luna-Romero. Carbon storage in several soils planted with cocoa in el oro province, ecuador. *Revista Científica Agroecosistemas*, 6 (1) (May):154–161, 2018.

S. Barrezueta-Unda and A. Paz-González. Estudio comparativo de la estructura elemental de materia orgánica de suelo y mantillo cultivados de cacao en El Oro, Ecuador. *Revista Agroecosistemas*, 5(3):2–9, 2017.

N. Batjes. Total carbon and nitrogen in the soils of the world n. *European Journal of Soil Science*, 65(1):1–1, jan 2014. doi: 10.1111/ejss.12120.

A. G. Bengough, B. M. McKenzie, P. D. Hallett, and T. A. Valentine. Root elongation, water stress, and mechanical impedance: a review of limiting stresses and beneficial root tip traits. *Journal of Experimental Botany*, 62(1):59–68, jan 2011. doi: 10.1093/jxb/erq350.

R. Berhongaray, G.; Alvarez. Evaluation of the ipcc tool to estimate soil organic carbon changes in pampean soils. *XXIII CONGRESO ARGENTINO DE LA CIENCIA DEL SUE*, 2012.

M. Bernoux, M. da Conceição Santana Carvalho, B. Volkoff, and C. C. Cerri. Brazil's soil carbon stocks. *Soil Science Society of America Journal*, 66(3):888, 2002. doi: 10.2136/sssaj2002.8880.

R. Bhattacharyya, V. Prakash, S. Kundu, A. K. Srivastva, H. S. Gupta, and S. Mitra. Long term effects of fertilization on carbon and nitrogen sequestration and aggregate associated carbon and nitrogen in the indian sub-himalayas. *Nutrient Cycling in Agroecosystems*, 86(1):1–16, mar 2009. doi: 10.1007/s10705-009-9270-y.

A. A. A.-S. K. Bhavya V., Kumar and M. Shivanna. Carbon sequestration under different cropping systems with different depth and its impact on climate change. *International Journal of Pure and Applied*, 6:1612–1616, 2018.

E. Błońska, J. Lasota, and P. Gruba. Effect of temperate forest tree species on soil dehydrogenase and urease activities in relation to other properties of soil derived from loess and glaciofluvial sand. *Ecological Research*, 31(5):655–664, jun 2016. doi: 10.1007/s11284-016-1375-6.

G. Bongiorno, E. K. Bünemann, C. U. Oguejiofor, J. Meier, G. Gort, R. Comans, P. Mäder, L. Brussaard, and R. de Goede. Sensitivity of labile carbon fractions to tillage and organic matter management and their potential as comprehensive soil quality indicators across pedoclimatic conditions in europe. *Ecological Indicators*, 99:38–50, apr 2019. doi: 10.1016/j.ecolind.2018.12.008.

S. A. Bowers and R. J. Hanks. REFLECTION OF RADIANT ENERGY FROM SOILS. *Soil Science*, 100(2):130–138, aug 1965. doi: 10.1097/00010694-196508000-00009.

B. A. R. C. D. M. H. R. H. N. Bravo, C; Torres. Soil structure and carbon sequestration as ecosytem services under different land uses in the ecuadorian amazon region. *International Conference Series on Multidisciplinary Sciences*, 3:1–8, 2017. doi: 10.3390/mol2net-03-xxxx.

C. Bronick and R. Lal. Soil structure and management: a review. *Geoderma*, 124 (1-2):3–22, jan 2005. doi: 10.1016/j.geoderma.2004.03.005.

G. Börjesson, M. A. Bolinder, H. Kirchmann, and T. Kätterer. Organic carbon stocks in topsoil and subsoil in long-term ley and cereal monoculture rotations. *Biology and Fertility of Soils*, 54(4):549–558, apr 2018. doi: 10.1007/s00374-018-1281-x.

F. B. R. P. M. G. R. P. P. B. S. O. P. D. P. V. P. F. G. S. A. I. I. Caballero, Rafael Fernández-gonzález. Grazing systems and biodiversity in mediterranean areas: Spain, italy and greece. *Grass and Forage Science*, 39(1):9–152, 2009. doi: 10.1111/j.1365-2494.2011.00820.x.

D. R. Cameron, M. V. Oijen, C. Werner, K. Butterbach-Bahl, R. Grote, E. Haas, G. B. M. Heuvelink, R. Kiese, J. Kros, M. Kuhnert, A. Leip, G. J. Reinds, H. I. Reuter, M. J. Schelhaas, W. D. Vries, and J. Yeluripati. Environmental change impacts on the c- and n-cycle of european forests: a model comparison study. *Biogeosciences*, 10(3): 1751–1773, mar 2013. doi: 10.5194/bg-10-1751-2013.

P. Campos, L. Huntsinger, J. L. O. Pro, P. F. Starrs, M. Diaz, R. B. Standiford, and G. Montero, editors. *Mediterranean Oak Woodland Working Landscapes*. Springer Netherlands, 2013. doi: 10.1007/978-94-007-6707-2.

M. E. S. S.-E. Canadell, J.-J. A global analysis of root distributions for terrestrial biomes. *Oecologia*, pages 389–411, 1996.

C. Cappai, A. R. Kemanian, A. Lagomarsino, P. P. Roggero, R. Lai, A. E. Agnelli, and G. Seddaiu. Small-scale spatial variation of soil organic matter pools generated by cork oak trees in mediterranean agro-silvo-pastoral systems. *Geoderma*, 304: 59–67, oct 2017. doi: 10.1016/j.geoderma.2016.07.021.

M. Carbonero and P. Fernández-Rebollo. Dehesas de encinas. influencia de la meteorología en la producción de bellotas. *Revista Ecosistemas*, 23(2):55–63, 2014. doi: Doi.:10.7818/ECOS.2014.23-2.08.

R. Cardinael, B. Guenet, T. Chevallier, C. Dupraz, T. Cozzi, and C. Chenu. High organic inputs explain shallow and deep SOC storage in a long-term agroforestry system &ndash combining experimental and modeling approaches. *Biogeosciences*, 15(1):297–317, jan 2018. doi: 10.5194/bg-15-297-2018.

L. R. Castro, A. J. Otiniano, and M. C. Sáenz. Zonificación del sistema agrosilvopastoril de la primera etapa del proyecto de riego carrizal-chone, provincia de manabí, ecuador. *La Técnica*, (14):30–39, 2016.

M. S.-C. Charts. Munsell soil-color charts with genuine munsell color chips. *Grand Rapids. Michigan, Etats-Unis*, 2010.

J. Chen and H. Tang. Effect of grazing exclusion on vegetation characteristics and soil organic carbon of leymus chinensis grassland in northern china. *Sustainability*, 8(1):56, jan 2016. doi: 10.3390/su8010056.

S. Chen, S. Li, W. Ma, W. Ji, D. Xu, Z. Shi, and G. Zhang. Rapid determination of soil classes in soil profiles using vis-NIR spectroscopy and multiple objectives mixed support vector classification. *European Journal of Soil Science*, 70(1):42–53, sep 2018. doi: 10.1111/ejss.12715.

R. N. Clark, T. V. V. King, M. Klejwa, G. A. Swayze, and N. Vergo. High spectral resolution reflectance spectroscopy of minerals. *Journal of Geophysical Research*, 95(B8):12653, 1990. doi: 10.1029/jb095ib08p12653.

R. Corral-Fernández, L. Parras-Alcántara, and B. Lozano-García. Stratification ratio of soil organic c, n and c:n in mediterranean evergreen oak woodland with conventional and organic tillage. *Agriculture, Ecosystems & Environment*, 164: 252–259, jan 2013. doi: 10.1016/j.agee.2012.11.002.

M. A. F. R. E. Costa, J.C. *Dehesas de Andalucia Caracterización Ambiental*. 2006. ISBN 849632981X.

F. Cubbage, G. Balmelli, A. Bussoni, E. Noellemeyer, A. N. Pachas, H. Fassola, L. Colcombet, B. Rossner, G. Frey, F. Dube, M. L. de Silva, H. Stevenson, J. Hamilton, and W. Hubbard. Comparing silvopastoral systems and prospects in eight regions of the world. *Agroforestry Systems*, 86(3):303–314, feb 2012. doi: 10.1007/s10457-012-9482-z.

J. A. Daniel, K. Potter, W. Altom, H. Aljoe, and R. Stevens. LONGTERM GRAZING DENSITY IMPACTS ON SOIL COMPACTION. *Transactions of the ASAE*, 45(6), 2002. doi: 10.13031/2013.11442.

L. Deng, G. yu Zhu, Z. sheng Tang, and Z. ping Shangguan. Global patterns of the effects of land-use changes on soil carbon stocks. *Global Ecology and Conservation*, 5:127–138, jan 2016. doi: 10.1016/j.gecco.2015.12.004.

S. Díaz, S. Lavorel, S. McIntyre, V. Falczuk, F. Casanoves, D. G. Milchinas, C. Skapre, G. Rusch, M. Sterberg, I. Noy-Meir, J. Landberg, W. Zang, H. Clark, and B. D. Campnell. Plant trait responses to grazing ? a global synthesis. *Global Change Biology*, 13(2):313–341, feb 2007. doi: 10.1111/j.1365-2486.2006.01288.x.

R. K. Dixon, A. M. Solomon, S. Brown, R. A. Houghton, M. C. Trexier, and J. Wisniewski. Carbon pools and flux of global forest ecosystems. *Science*, 263(5144): 185–190, jan 1994. doi: 10.1126/science.263.5144.185.

A. Don, J. Schumacher, M. Scherer-Lorenzen, T. Scholten, and E.-D. Schulze. Spatial and vertical variation of soil carbon at two grassland sites — implications for measuring soil carbon stocks. *Geoderma*, 141(3-4):272–282, oct 2007. doi: 10.1016/j.geoderma.2007.06.003.

N. T. Donkor, J. V. Gedir, R. J. Hudson, E. W. Bork, D. S. Chanasyk, and M. A. Naeth. Impacts of grazing systems on soil compaction and pasture production in alberta. *Canadian Journal of Soil Science*, 82(1):1–8, feb 2002. doi: 10.4141/s01-008.

J. W. Doran and L. N. Mielke. A rapid, low-cost method for determination of soil bulk density1. *Soil Science Society of America Journal*, 48(4):717, 1984. doi: 10.2136/sssaj1984.03615995004800040004x.

R. Douglas, S. Nawar, M. Alamar, A. Mouazen, and F. Coulon. Rapid prediction of total petroleum hydrocarbons concentration in contaminated soil using vis-NIR spectroscopy and regression techniques. *Science of The Total Environment*, 616-617: 147–155, mar 2018. doi: 10.1016/j.scitotenv.2017.10.323.

J. J. Drewry, K. C. Cameron, and G. D. Buchan. Pasture yield and soil physical property responses to soil compaction from treading and grazing—a review. *Soil Research*, 46(3):237, 2008. doi: 10.1071/sr07125.

J. A. J. Dungait, R. Bol, and R. P. Evershed. Quantification of dung carbon incorporation in a temperate grassland soil following spring application using bulk stable carbon isotope determinations. *Isotopes in Environmental and Health Studies*, 41 (1):3–11, mar 2005. doi: 10.1080/10256010500053516.

. P. J. Egli, M. Soils of mountainous landscapes. *International Encyclopedia of Geography: People, the Earth, Environment and Technology*, (1):1–10, 2016. doi: https://doi.org/10.1002/9781118786352.

M. P. Eichhorn, P. Paris, F. Herzog, L. D. Incoll, F. Liagre, K. Mantzanas, M. Mayus, G. Moreno, V. P. Papanastasis, D. J. Pilbeam, A. Pisanelli, and C. Dupraz. Silvoarable systems in europe – past, present and future prospects. *Agroforestry Systems*, 67(1):29–50, apr 2006. doi: 10.1007/s10457-005-1111-7.

C. Feller and M. Beare. Physical control of soil organic matter dynamics in the tropics. *Geoderma*, 79(1-4):69–116, sep 1997. doi: 10.1016/s0016-7061(97)00039-6.

E. Fernández-Ondoño, L. R. Serrano, M. N. Jiménez, F. B. Navarro, M. Díez, F. Martín, J. Fernández, F. J. Martínez, A. Roca, and J. Aguilar. Afforestation improves soil fertility in south-eastern spain. *European Journal of Forest Research*, 129(4): 707–717, mar 2010. doi: 10.1007/s10342-010-0376-1.

B. A. A. J.-L. M. P. . C. M. D. Fernandez Rebollo, P. Efecto del pastoreo con ganado ovino y el laboreo en las propiedades físicas y químicas de un suelo de textura arenosa de d. in pastos y ganadería extensiva. *XLIV Reunión Científica de la Sociedad Española para el Estudio de los Pastos*, 2004.

M. L. Fernández-Romero, B. Lozano-García, L. Parras-Alcántara, C. D. Collins, and J. M. Clark. Effects of land management on different forms of soil carbon in olive groves in mediterranean areas. *Land Degradation & Development*, 27(4): 1186–1195, nov 2014. doi: 10.1002/ldr.2327.

N. Ferreiro-Domínguez, A. Rigueiro-Rodríguez, K. Rial-Lovera, R. Romero-Franco, and M. Mosquera-Losada. Effect of grazing on carbon sequestration and tree growth that is developed in a silvopastoral system under wild cherry (prunus avium l.). *CATENA*, 142:11–20, jul 2016. doi: 10.1016/j.catena.2016.02.002.

C. Fissore, B. Dalzell, A. Berhe, M. Voegtle, M. Evans, and A. Wu. Influence of topography on soil organic carbon dynamics in a southern california grassland. *CATENA*, 149:140–149, feb 2017. doi: 10.1016/j.catena.2016.09.016.

R. Francaviglia, G. Renzi, L. Doro, L. Parras-Alcántara, B. Lozano-García, and L. Ledda. Soil sampling approaches in mediterranean agro-ecosystems. influence on soil organic carbon stocks. *CATENA*, 158:113–120, nov 2017. doi: 10.1016/j.catena.2017.06.014.

A. Franzluebbers. Soil organic matter stratification ratio as an indicator of soil quality. *Soil and Tillage Research*, 66(2):95–106, jul 2002. doi: 10.1016/s0167-1987(02) 00018-1.

J. M. C. S.-L. K. C. Galantini, J.A.; Iglesias. Sistemas de labranza en el sudoeste bonaerense. efectos de largo plazo sobre las fraccionesorgánicas y el espacio poroso del suelo. *RIA. Revista de Investigaciones Agropecuarias*, 35:15–30, april 2006.

A. Gallardo. Effect of tree canopy on the spatial distribution of soil nutrients in a mediterranean dehesa. *Pedobiologia*, 47(2):117–125, jan 2003. doi: 10.1078/ 0031-4056-00175.

M. Gandariasbeitia, G. Besga, I. Albizu, S. Larregla, and S. Mendarte. Prediction of chemical and biological variables of soil in grazing areas with visible- and near-infrared spectroscopy. *Geoderma*, 305:228–235, nov 2017. doi: 10.1016/j.geoderma.2017.05.045.

A. M. García-Moreno, M. D. Carbonero-Muñoz, M. Serrano-Moral, and P. Fernández-Rebollo. Grazing affects shoot growth, nutrient and water status of quercus ilex l. in mediterranean open woodlands. *Annals of Forest Science*, 71(8):917–926, jun 2014. doi: 10.1007/s13595-014-0397-x.

M. L. Gatti, A. T. A. Torales, P. A. Cipriotti, and R. A. Golluscio. Effects of defoliation frequency and nitrogen fertilization on the production and potential for persistence of dactylis glomerata sown in multispecies swards. *Grass and Forage Science*, 72 (3):489–501, sep 2016. doi: 10.1111/gfs.12245.

H. K. Gibbs, A. S. Ruesch, F. Achard, M. K. Clayton, P. Holmgren, N. Ramankutty, and J. A. Foley. Tropical forests were the primary sources of new agricultural land in the 1980s and 1990s. *Proceedings of the National Academy of Sciences*, 107(38): 16732–16737, aug 2010. doi: 10.1073/pnas.0910275107.

B. Glina, A. Bogacz, and M. Gulyás. The effect of long-term forestry drainage on the current state of peatland soils: A case study from the central sudetes, sw poland. *Mires and Peat*, (18):1–11, Oct 2016. ISSN 1819-754X. doi: 10.19189/MaP.2016.OMB.239.

C. Gomez, R. A. V. Rossel, and A. B. McBratney. Soil organic carbon prediction by hyperspectral remote sensing and field vis-NIR spectroscopy: An australian case study. *Geoderma*, 146(3-4):403–411, aug 2008. doi: 10.1016/j.geoderma.2008.06.011.

J.-R. Gong, Y. Wang, M. Liu, Y. Huang, X. Yan, Z. Zhang, and W. Zhang. Effects of land use on soil respiration in the temperate steppe of inner mongolia, china. *Soil and Tillage Research*, 144:20–31, dec 2014. doi: 10.1016/j.still.2014.06.002.

I. G. González, J. M. G. Corbí, A. F. Cancio, R. J. Ballesta, and M. R. G. Cascón. Soil carbon stocks and soil solution chemistry in quercus ilex stands in mainland spain. *European Journal of Forest Research*, 131(6):1653–1667, apr 2012. doi: 10.1007/s10342-012-0623-8.

. M. B. Grabherr, G. An overview of the world's mountain environments. *Austrian MAB Committee*, 1(8):8–14, 2011.

P. Grassini, K. M. Eskridge, and K. G. Cassman. Distinguishing between yield advances and yield plateaus in historical crop production trends. *Nature Communications*, 4(1), dec 2013. doi: 10.1038/ncomms3918.

K. L. Greenwood and B. M. McKenzie. Grazing effects on soil physical properties and the consequences for pastures: a review. *Australian Journal of Experimental Agriculture*, 41(8):1231, 2001. doi: 10.1071/ea00102.

R. B. Grossman and T. G. Reinsch. 2.1 bulk density and linear extensibility. In J. H. Dane and C. G. Topp, editors, *Methods of Soil Analysis: Part 4 Physical Methods*. Soil Science Society of America, 2002. doi: 10.2136/sssabookser5.4.c9.

C. E. Guillén, M. J. Dávila, J.-M. Gilliot, and E. Vaoudour. Aporte de la espectroscopia a la estimación de carbono orgánico de los suelos de la planicie de versalles, francia. *Revista Geográfica Venezolana*, 54(1):85–98, 2013.

L. B. Guo and R. M. Gifford. Soil carbon stocks and land use change: a meta analysis. *Global Change Biology*, 8(4):345–360, apr 2002. doi: 10.1046/j.1354-1013.2002.00486.x.

N. Haghdoost, M. Akbarinia, and S. M. Hosseini. Land-use change and carbon stocks: A case study, noor county, iran. *Journal of Forestry Research*, 24(3):461–469, jan 2013. doi: 10.1007/s11676-013-0340-2.

S. G. Haile, V. D. Nair, and P. K. R. Nair. Contribution of trees to carbon storage in soils of silvopastoral systems in florida, USA. *Global Change Biology*, 16(1):427–438, jan 2010. doi: 10.1111/j.1365-2486.2009.01981.x.

B. B. C. J. C.-M. P. G. Hao, X. Soi density and porosity. in: Carte, m.r., and gregorich, e.g. (eds.), soil sampling and methods of analysis. *Canadian Society of Soil Science*, pages 743–759, 2008.

N. P. He, Y. H. Zhang, Q. Yu, Q. S. Chen, Q. M. Pan, G. M. Zhang, and X. G. Han. Grazing intensity impacts soil carbon and nitrogen storage of continental steppe. *Ecosphere*, 2(1):art8, jan 2011. doi: 10.1890/es10-00017.1.

Y. He, M. Huang, A. García, A. Hernández, and H. Song. Prediction of soil macronutrients content using near-infrared spectroscopy. *Computers and Electronics in Agriculture*, 58(2):144–153, sep 2007. doi: 10.1016/j.compag.2007.03.011.

M. Heimann and M. Reichstein. Terrestrial ecosystem carbon dynamics and climate feedbacks. *Nature*, 451(7176):289–292, jan 2008. doi: 10.1038/nature06591.

A. Henry, L. Mabit, R. E. Jaramillo, Y. Cartagena, and J. P. Lynch. Land use effects on erosion and carbon storage of the Rio Chimbo watershed, Ecuador. *Plant and Soil*, 367(1-2):477–491, 2013. ISSN 0032079X. doi: 10.1007/s11104-012-1478-y.

A. Hernández, L. Vera, C. Naveda, F. Véliz, A. Guzmán, M. Vivar, T. Zambrano, F. Mesias, and K. Ormanza. Impactos Del Cambio De Uso De La Tierra En La Microcuenca Membrillo, Manabí, Ecuador. *Espamciencia*, 4(2):59–66, 2013.

S. Hobbs. Climate change 1992: The supplementary report to the IPCC scientific assessment. *Journal of Atmospheric and Terrestrial Physics*, 58(10):1189, jul 1996. doi: 10.1016/s0021-9169(96)90059-8.

J. L. Holechek. An approach for setting the stocking rate. *Rangelands*, 10:10–14, feb 1988.

J. Holland. The environmental consequences of adopting conservation tillage in europe: reviewing the evidence. *Agriculture, Ecosystems & Environment*, 103(1): 1–25, jun 2004. doi: 10.1016/j.agee.2003.12.018.

M. Hosseini, S. R. Agereh, Y. Khaledian, H. J. Zoghalchali, E. C. Brevik, and S. A. R. M. Naeini. Comparison of multiple statistical techniques to predict soil phosphorus. *Applied Soil Ecology*, 114:123–131, jun 2017. doi: 10.1016/j.apsoil.2017.02.011.

D. J. Houlbrooke, R. J. Paton, R. P. Littlejohn, and J. D. Morton. Land-use intensification in new zealand: effects on soil properties and pasture production. *The Journal of Agricultural Science*, 149(3):337–349, nov 2010. doi: 10.1017/s0021859610000821.

D. S. Howlett, G. Moreno, M. R. M. Losada, P. K. R. Nair, and V. D. Nair. Soil carbon storage as influenced by tree cover in the dehesa cork oak silvopasture of central-western spain. *Journal of Environmental Monitoring*, 13(7):1897, 2011. doi: 10.1039/c1em10059a.

C. Huang, L. Han, X. Liu, and L. Ma. The rapid estimation of cellulose, hemicellulose, and lignin contents in rice straw by near infrared spectroscopy. *Energy Sources, Part A: Recovery, Utilization, and Environmental Effects*, 33(2):114–120, nov 2010. doi: 10.1080/15567030902937127.

J. Huang, B. Minasny, A. B. McBratney, J. Padarian, and J. Triantafilis. The location- and scale- specific correlation between temperature and soil carbon sequestration across the globe. *Science of The Total Environment*, 615:540–548, feb 2018. doi: 10.1016/j.scitotenv.2017.09.136.

C. O. Hunt. Ruined landscapes. *Ecology*, 83(1):295–296, jan 2002. doi: 10.1890/0012-9658(2002)083[0295:rl]2.0.co;2.

L. J. Ingram, P. D. Stahl, G. E. Schuman, J. S. Buyer, G. F. Vance, G. K. Ganjegunte, J. M. Welker, and J. D. Derner. Grazing impacts on soil carbon and microbial communities in a mixed-grass ecosystem. *Soil Science Society of America Journal*, 72(4):939–948, jul 2008. doi: 10.2136/sssaj2007.0038.

IPCC. Definitions and methodological options to inventory emissions from direct human-induced degradation of forests and devegetation of other vegetation types. *National Greenhouse Gas Inventories Programme*, 2003. doi: 10.1016/B978-0-12-375067-9.00128-5.

O. Jadan, M. Cifuentes, B. Torres, D. Selesi, D. Veintimilla, and S. Guenter. Influence of Tree Cover on Diversity, Carbon Sequestration and Productivity of Cocoa Systems in the Ecuadorian Amazon. *Bois Et Forets Des Tropiques*, 325(325):35–47, 2015.

R. Jandl, M. Lindner, L. Vesterdal, B. Bauwens, R. Baritz, F. Hagedorn, D. W. Johnson, K. Minkkinen, and K. A. Byrne. How strongly can forest management influence soil carbon sequestration? *Geoderma*, 137(3-4):253–268, jan 2007. doi: 10.1016/j.geoderma.2006.09.003.

H. Jenny. *The soil resource: Origin and behaviour. Ecological Studies*. Springer New York, 1980.

W. Ji, S. Li, S. Chen, Z. Shi, R. A. V. Rossel, and A. M. Mouazen. Prediction of soil attributes using the chinese soil spectral library and standardized spectra recorded at field conditions. *Soil and Tillage Research*, 155:492–500, jan 2016. doi: 10.1016/j.still.2015.06.004.

K. Jindo, C. Chocano, J. M. de Aguilar, D. González, T. Hernandez, and C. García. Impact of compost application during 5 years on crop production, soil microbial activity, carbon fraction, and humification process. *Communications in Soil Science and Plant Analysis*, jul 2016. doi: 10.1080/00103624.2016.1206922.

R. Joffre and S. Rambal. How tree cover influences the water balance of mediterranean rangelands. *Ecological Society of America*, 74(2):570–582, 1993.

R. Joffre, J. Vacher, C. de los Llanos, and G. Long. The dehesa: an agrosilvopastoral system of the mediterranean region with special reference to the sierra morena area of spain. *Agroforestry Systems*, 6(1):71–96, feb 1988. doi: 10.1007/bf02220110.

P. A. . S. Z. Jonczak, J. Distribution of carbon and nitrogen forms in histosols of headwater areas – a case study from the valley of the kamienna creek (northern poland). *Journal of Elementology*, 20(1), 2015a. doi: https://doi.org/10.5601/jelem.2014.19.4.398.

P. A. . S. Z. Jonczak, J. The content and profile distribution patterns of cu, ni and zn in histosols of headwater areas in the valley of kamienna creek (northern poland). *Journal of Ecology and Protection of the Coastline*, 18(1), 2015b. doi: https://doi.org/10.5601/jelem.2014.19.4.398.

G. B. Jones and B. F. Tracy. Persistence and productivity of orchardgrass and orchardgrass/alfalfa mixtures as affected by cutting height. *Grass and Forage Science*, 73(2):544–552, sep 2017. doi: 10.1111/gfs.12309.

R. J. A. Jones, R. Hiederer, E. Rusco, and L. Montanarella. Estimating organic carbon in the soils of europe for policy support. *European Journal of Soil Science*, 56(5): 655–671, oct 2005. doi: 10.1111/j.1365-2389.2005.00728.x.

S. juan Gao, J. sheng Gao, W. dong Cao, C. qin Zou, J. Huang, J. shun Bai, and F. gen Dou. Effects of long-term green manure application on the content and structure of dissolved organic matter in red paddy soil. *Journal of Integrative Agriculture*, 17 (8):1852–1860, aug 2018. doi: 10.1016/s2095-3119(17)61901-4.

A. Kacprzak, P. Migoń, and Ł. Musielok. Using soils as indicators of past slope instability in forested terrain, kamienne mts., SW poland. *Geomorphology*, 194: 65–75, jul 2013. doi: 10.1016/j.geomorph.2013.04.014.

O. Kara, İ. Bolat, K. Çakıroğlu, and M. Öztürk. Plant canopy effects on litter accumulation and soil microbial biomass in two temperate forests. *Biology and Fertility of Soils*, 45(2):193–198, aug 2008. doi: 10.1007/s00374-008-0327-x. URL `https://doi.org/10.1007/s00374-008-0327-x`.

J. Kowalska, B. Kajdas, and T. Zaleski. Variability of morphological, physical and chemical properties of soils derived from carbonate-rich parent material in the pieniny mountains (south poland). *Soil Science Annual*, 68(1):27–38, mar 2017. doi: 10.1515/ssa-2017-0004.

J. Krug, M. Koehl, and D. Kownatzki. Revaluing unmanaged forests for climate change mitigation. *Carbon Balance and Management*, 7(1), nov 2012. doi: 10.

1186/1750-0680-7-11.

B. Kulli. Visualizing soil compaction based on flow pattern analysis. *Soil and Tillage Research*, 70(1):29–40, mar 2003. doi: 10.1016/s0167-1987(02)00121-6.

I. Kurz, C. D. O'Reilly, and H. Tunney. Impact of cattle on soil physical properties and nutrient concentrations in overland flow from pasture in ireland. *Agriculture, Ecosystems & Environment*, 113(1-4):378–390, apr 2006. doi: 10.1016/j.agee.2005.10.004.

B. H. Kusumo, Sukartono, and Bustan. The rapid measurement of soil carbon stock using near-infrared technology. *IOP Conference Series: Earth and Environmental Science*, 129:012023, mar 2018a. doi: 10.1088/1755-1315/129/1/012023.

B. H. Kusumo, S. Sukartono, and B. Bustan. Rapid measurement of soil carbon in rice paddy field of lombok island indonesia using near infrared technology. *IOP Conference Series: Materials Science and Engineering*, 306:012014, feb 2018b. doi: 10.1088/1757-899x/306/1/012014.

M. Köchy, R. Hiederer, and A. Freibauer. Global distribution of soil organic carbon – part 1: Masses and frequency distributions of SOC stocks for the tropics, permafrost regions, wetlands, and the world. *SOIL*, 1(1):351–365, apr 2015. doi: 10.5194/soil-1-351-2015.

A. Łajczak and B. Spyt. Differentiation of vertical limit of forest at the babia góra mt., the western carpathian mountains. *Geographia Polonica*, 91(2):217–242, 2018. doi: 10.7163/gpol.0118.

R. Lal. Soil carbon sequestration to mitigate climate change. *Geoderma*, 123(1-2):1–22, nov 2004. doi: 10.1016/j.geoderma.2004.01.032.

W. F. Laurance. Have we overstated the tropical biodiversity crisis? *Trends in Ecology & Evolution*, 22(2):65–70, feb 2007. doi: 10.1016/j.tree.2006.09.014.

S. Leavitt. Biogeochemistry, an analysis of global change. *Eos, Transactions American Geophysical Union*, 79(2):20–20, 1998. doi: 10.1029/98eo00015.

M. LeRoy. Soil chemical analysis: advanced course: a manual of methods useful for instruction and research in soil chemistry, physical chemistry of soils, soil fertility, and soil genesis. *Retrieved from http://www.sidalc.net*, 1969.

J. Li, Y. Wen, X. Li, Y. Li, X. Yang, Z. Lin, Z. Song, J. M. Cooper, and B. Zhao. Soil labile organic carbon fractions and soil organic carbon stocks as affected by long-term organic and mineral fertilization regimes in the north china plain. *Soil and Tillage Research*, 175:281–290, jan 2018. doi: 10.1016/j.still.2017.08.008.

X. Li, C. Zhang, H. Fu, D. Guo, X. Song, C. Wan, and J. REN. Grazing exclusion alters soil microbial respiration, root respiration and the soil carbon balance in grasslands of the loess plateau, northern china. *Soil Science and Plant Nutrition*, 59(6):877–887, dec 2013. doi: 10.1080/00380768.2013.862157.

C. Liao, Y. Luo, C. Fang, and B. Li. Ecosystem carbon stock influenced by plantation practice: Implications for planting forests as a measure of climate change mitigation. *PLoS ONE*, 5(5):e10867, may 2010. doi: 10.1371/journal.pone.0010867.

Y. Liu, Y. Liu, Y. Chen, Y. Zhang, T. Shi, J. Wang, Y. Hong, T. Fei, and Y. Zhang. The influence of spectral pretreatment on the selection of representative calibration samples for soil organic matter estimation using vis-NIR reflectance spectroscopy. *Remote Sensing*, 11(4):450, feb 2019. doi: 10.3390/rs11040450.

G.-R. a. V.-Y.-O. P. J. S. O. O. L. . P. M. J. Lloveras, J. Sustainable grassland productivity. sustainable grassland productivity. *Proceedings of the 21st General Meeting of the European Grassland Federation*, 11(1), apr 2006.

C. R. Lobsey, R. A. V. Rossel, P. Roudier, and C. B. Hedley. rs-localdata-mines information from spectral libraries to improve local calibrations. *European Journal of Soil Science*, 68(6):840–852, nov 2017. doi: 10.1111/ejss.12490.

C. López-Carrasco, A. López-Sánchez, A. S. Miguel, and S. Roig. The effect of tree cover on the biomass and diversity of the herbaceous layer in a mediterranean dehesa. *Grass and Forage Science*, 70(4):639–650, feb 2015. doi: 10.1111/gfs.12161.

B. Lozano-García, L. Parras-Alcántara, and E. C. Brevik. Impact of topographic aspect and vegetation (native and reforested areas) on soil organic carbon and nitrogen budgets in mediterranean natural areas. *Science of The Total Environment*, 544:963–970, feb 2016. doi: 10.1016/j.scitotenv.2015.12.022.

M. S. Luce, N. Ziadi, B. J. Zebarth, C. A. Grant, G. F. Tremblay, and E. G. Gregorich. Rapid determination of soil organic matter quality indicators using visible near infrared reflectance spectroscopy. *Geoderma*, 232-234:449–458, nov 2014. doi: 10.1016/j.geoderma.2014.05.023.

M. S. Luce, N. Ziadi, B. Gagnon, and A. Karam. Visible near infrared reflectance spectroscopy prediction of soil heavy metal concentrations in paper mill biosolid- and liming by-product-amended agricultural soils. *Geoderma*, 288:23–36, feb 2017. doi: 10.1016/j.geoderma.2016.10.037.

E. Lugato, P. Panagos, F. Bampa, A. Jones, and L. Montanarella. A new baseline of organic carbon stock in european agricultural soils using a modelling approach. *Global Change Biology*, 20(1):313–326, aug 2013. doi: 10.1111/gcb.12292.

J. Makovníková, M. Širáň, B. Houšková, B. Pálka, and A. Jones. Comparison of different models for predicting soil bulk density. case study – slovakian agricultural soils. *International Agrophysics*, 31(4):491–498, oct 2017. doi: 10.1515/intag-2016-0079.

M. B. . M.-A. B. Malone, B. P. Using r for digital soil mapping. basel, switzerland. *Springer International Publishing*, 2017.

R. . S. B. Manojlovic, M. Soil organic carbon in serbian mountain soils: Effects of land use and altitude. *Journal of Environmental Studies*, 4:977–986, jan 2011.

M. A. Marinho, M. W. Pereira, E. V. Vázquez, M. Lado, and A. P. González. Depth distribution of soil organic carbon in an oxisol under different land uses: Stratification indices and multifractal analysis. *Geoderma*, 287:126–134, feb 2017. doi: 10.1016/j.geoderma.2016.09.021.

V. N. J. T. S. C. R. Marmette, Marie-christine Adamchuk. Comparison of the performance of two vis-nir spectrometers in the prediction of various soil properties. *Proceedings of the 14th International Conference on Precision Agriculture June 24 – June 27, 2018, Montreal, Quebec, Canada*, 223-225:1–12, jun 2018.

. N. T. Martens, H. Assessment, validation and choice of calibration method. *Multivariate Calibration*, pages 237–266, 1989.

J. R. Martín, J. Álvaro-Fuentes, J. Gonzalo, C. Gil, J. Ramos-Miras, J. G. Corbí, and R. Boluda. Assessment of the soil organic carbon stock in spain. *Geoderma*, 264: 117–125, feb 2016. doi: 10.1016/j.geoderma.2015.10.010.

S. A. Materechera, A. R. Dexter, and A. M. Alston. Penetration of very strong soils by seedling roots of different plant species. *Plant and Soil*, 135(1):31–41, aug 1991. doi: 10.1007/bf00014776.

M. E. McSherry and M. E. Ritchie. Effects of grazing on grassland soil carbon: a global review. *Global Change Biology*, 19(5):1347–1357, feb 2013. doi: 10.1111/gcb.12144.

E. Medina-Roldán, J. Paz-Ferreiro, and R. D. Bardgett. Grazing exclusion affects soil and plant communities, but has no impact on soil carbon storage in an upland grassland. *Agriculture, Ecosystems & Environment*, 149:118–123, mar 2012. doi: 10.1016/j.agee.2011.12.012.

J. MEERSMANS, B. V. WESEMAEL, F. D. RIDDER, M. F. DOTTI, S. D. BAETS, and M. V. MOLLE. Changes in organic carbon distribution with depth in agricultural soils in northern belgium, 1960â2006. *Global Change Biology*, 15(11):2739–2750, nov 2009. doi: 10.1111/j.1365-2486.2009.01855.x.

I. C. Meier, F. Knutzen, L. M. Eder, H. Müller-Haubold, M.-O. Goebel, J. Bachmann, D. Hertel, and C. Leuschner. The deep root system of fagus sylvatica on sandy soil: Structure and variation across a precipitation gradient. *Ecosystems*, 21(2): 280–296, may 2017. doi: 10.1007/s10021-017-0148-6.

J. Mikola, H. Setälä, P. Virkajärvi, K. Saarijärvi, K. Ilmarinen, W. Voigt, and M. Vestberg. Defoliation and patchy nutrient return drive grazing effects on plant and soil properties in a dairy cow pasture. *Ecological Monographs*, 79(2):221–244, may 2009. doi: 10.1890/08-1846.1.

B. Minasny, B. P. Malone, A. B. McBratney, D. A. Angers, D. Arrouays, A. Chambers, V. Chaplot, Z.-S. Chen, K. Cheng, B. S. Das, D. J. Field, A. Gimona, C. B. Hedley, S. Y. Hong, B. Mandal, B. P. Marchant, M. Martin, B. G. McConkey, V. L. Mulder, S. O'Rourke, A. C. R. de Forges, I. Odeh, J. Padarian, K. Paustian, G. Pan, L. Poggio, I. Savin, V. Stolbovoy, U. Stockmann, Y. Sulaeman, C.-C. Tsui, T.-G. Vågen, B. van Wesemael, and L. Winowiecki. Soil carbon 4 per mille. *Geoderma*, 292:59–86, apr 2017. doi: 10.1016/j.geoderma.2017.01.002.

S. Minu and A. Shetty. Prediction accuracy of soil organic carbon from ground based visible near-infrared reflectance spectroscopy. *Journal of the Indian Society of Remote Sensing*, 46(5):697–703, jan 2018. doi: 10.1007/s12524-017-0744-0.

S.-C.-N. H. S. S. Mohanty, A. Soil carbon sequestration potential of different land use and land cover system in easterm planteau and hills agroclimatic zone of india. *Fresenius Environmental Bulletin*, (26):8263–8269, January 2017.

J. L. Moir, G. R. Edwards, and L. N. Berry. Nitrogen uptake and leaching loss of thirteen temperate grass species under high n loading. *Grass and Forage Science*, 68(2):313–325, aug 2012. doi: 10.1111/j.1365-2494.2012.00905.x.

P. H. M. Monroe, E. F. Gama-Rodrigues, A. C. Gama-Rodrigues, and J. R. B. Marques. Soil carbon stocks and origin under different cacao agroforestry systems in southern bahia, brazil. *Agriculture, Ecosystems & Environment*, 221:99–108, apr 2016. doi: 10.1016/j.agee.2016.01.022.

A. Morellos, X.-E. Pantazi, D. Moshou, T. Alexandridis, R. Whetton, G. Tziotzios, J. Wiebensohn, R. Bill, and A. M. Mouazen. Machine learning based prediction of soil total nitrogen, organic carbon and moisture content by using VIS-NIR spectroscopy. *Biosystems Engineering*, 152:104–116, dec 2016. doi: 10.1016/j.biosystemseng.2016.04.018.

G. Moreno and J. J. Obrador. Effects of trees and understorey management on soil fertility and nutritional status of holm oaks in spanish dehesas. *Nutrient Cycling in Agroecosystems*, 78(3):253–264, feb 2007. doi: 10.1007/s10705-007-9089-3.

G. Moreno and F. J. Pulido. The functioning, management and persistence of dehesas. In *Advances in Agroforestry*, pages 127–160. Springer Netherlands, 2009. doi: 10.1007/978-1-4020-8272-6_7.

G. Moreno, J. Obrador, E. Cubera, and C. Dupraz. Fine root distribution in dehesas of central-western spain. *Plant and Soil*, 277(1-2):153–162, dec 2005. doi: 10.1007/s11104-005-6805-0.

M. Muñoz-Rojas, A. Jordán, L. M. Zavala, D. D. la Rosa, S. K. Abd-Elmabod, and M. Anaya-Romero. Organic carbon stocks in mediterranean soil types under different land uses (southern spain). *Solid Earth*, 3(2):375–386, nov 2012. doi: 10.5194/se-3-375-2012.

M.-R.-M. C. e. a. Myers, N. Biodiversity hotspots for conservation priorities. *Nature*, 403:853–858, 2000. doi: doi:10.1038/35002501.

G. A. M. . M.-L. M. R. Nair, P. K. R. Agroforestry. in encyclopedia of ecology. *Current Opinion in Environmental Sustainability*, 20008. doi: doi.org/10.1016/B978-008045405-4.00038-0.

R. Nair and V. D. Nair. 'solid–fluid–gas': the state of knowledge on carbon-sequestration potential of agroforestry systems in africa. *Current Opinion in Environmental Sustainability*, 6:22–27, feb 2014. doi: 10.1016/j.cosust.2013.07.014.

C. I. Nicholls and M. A. Altieri. Plant biodiversity enhances bees and other insect pollinators in agroecosystems. a review. *Agronomy for Sustainable Development*, 33(2):257–274, jun 2012. doi: 10.1007/s13593-012-0092-y.

X. Nie, T. Zhao, and X. Qiao. Impacts of soil erosion on organic carbon and nutrient dynamics in an alpine grassland soil. *Soil Science and Plant Nutrition*, 59(4): 660–668, aug 2013. doi: 10.1080/00380768.2013.795475.

X. Nie, L. Yang, F. Li, F. Xiong, C. Li, and G. Zhou. Storage, patterns and controls of soil organic carbon in the alpine shrubland in the three rivers source region on the qinghai-tibetan plateau. *CATENA*, 178:154–162, jul 2019. doi: 10.1016/j.catena.2019.03.019.

B. Obrebska Starkel. Climate of babia góra range. in monografia przyrodnicza, krak w: Wydawnictwo i drukarnia towarzystwa sowak w w polsce;. *The Nature of the Babiogórski National Park; University of Agriculture in Krakow*, 1(1), 2004.

J. J. Ojeda, O. P. Caviglia, and M. G. Agnusdei. Vertical distribution of root biomass and soil carbon stocks in forage cropping systems. *Plant and Soil*, 423(1-2): 175–191, nov 2017. doi: 10.1007/s11104-017-3502-8.

S. Omran. Rapid soil analyses using modern sensing technology: Toward a more sustainable agriculture. *In The Handbook of Environmental Chemistry*, pages 41–53, 2017. doi: https://doi.org/10.1007/698.

Y. Ostovari, S. Ghorbani-Dashtaki, H.-A. Bahrami, M. Abbasi, J. A. M. Dematte, E. Arthur, and P. Panagos. Towards prediction of soil erodibility, SOM and CaCO 3 using laboratory vis-NIR spectra: A case study in a semi-arid region of iran. *Geoderma*, 314:102–112, mar 2018. doi: 10.1016/j.geoderma.2017.11.014.

W. Ouerghemmi, C. Gomez, S. Naceur, and P. Lagacherie. Applying blind source separation on hyperspectral data for clay content estimation over partially vegetated surfaces. *Geoderma*, 163(3-4):227–237, jul 2011. doi: 10.1016/j.geoderma.2011.04.019.

Y. Pan, R. A. Birdsey, J. Fang, R. Houghton, P. E. Kauppi, W. A. Kurz, O. L. Phillips, A. Shvidenko, S. L. Lewis, J. G. Canadell, P. Ciais, R. B. Jackson, S. W. Pacala, A. D. McGuire, S. Piao, A. Rautiainen, S. Sitch, and D. Hayes. A large and persistent carbon sink in the world's forests. *Science*, 333(6045):988–993, jul 2011. doi: 10.1126/science.1201609.

V. P. Papanastasis, S. Bautista, D. Chouvardas, K. Mantzanas, M. Papadimitriou, A. G. Mayor, P. Koukioumi, A. Papaioannou, and R. V. Vallejo. Comparative assessment of goods and services provided by grazing regulation and reforestation in degraded mediterranean rangelands. *Land Degradation & Development*, 28(4):1178–1187, mar 2015. doi: 10.1002/ldr.2368.

L. Parras-Alcántara, L. Díaz-Jaimes, B. Lozano-García, P. F. Rebollo, F. M. Elcure, and M. D. C. Muñoz. Organic farming has little effect on carbon stock in a mediterranean dehesa (southern spain). *CATENA*, 113:9–17, feb 2014. doi: 10.1016/j.catena.2013.09.002.

L. Parras-Alcántara, B. Lozano-García, E. Brevik, and A. Cerdá. Soil organic carbon stocks assessment in mediterranean natural areas: A comparison of entire soil profiles and soil control sections. *Journal of Environmental Management*, 155: 219–228, may 2015. doi: 10.1016/j.jenvman.2015.03.039.

K. Paul, P. Polglase, J. Nyakuengama, and P. Khanna. Change in soil carbon following afforestation. *Forest Ecology and Management*, 168(1-3):241–257, sep 2002. doi: 10.1016/s0378-1127(01)00740-x.

B. Peco, E. Navarro, C. Carmona, N. Medina, and M. Marques. Effects of grazing abandonment on soil multifunctionality: The role of plant functional traits. *Agriculture, Ecosystems & Environment*, 249:215–225, nov 2017. doi: 10.1016/j.agee.2017.08.013.

H. Å. Persson. The distribution and productivity of fine roots in boreal forests. *Plant and Soil*, 71(1-3):87–101, feb 1983. doi: 10.1007/bf02182644.

G. Piñeiro, J. M. Paruelo, M. Oesterheld, and E. G. Jobbágy. Pathways of grazing effects on soil organic carbon and nitrogen. *Rangeland Ecology & Management*, 63 (1):109–119, jan 2010. doi: 10.2111/08-255.1.

É. Pinheiro, M. Ceddia, C. Clingensmith, S. Grunwald, and G. Vasques. Prediction of soil physical and chemical properties by visible and near-infrared diffuse reflectance spectroscopy in the central amazon. *Remote Sensing*, 9(4):293, mar 2017. doi: 10.3390/rs9040293.

D. A. Poeplau, C. Sensitivity of organic carbon stocks and fractions to different lad use changes across europe. *Geoderma*, 192:189–201, 2013.

W. M. Post and K. C. Kwon. Soil carbon sequestration and land-use change: processes and potential. *Global Change Biology*, 6(3):317–327, mar 2000. doi: 10.1046/j.

1365-2486.2000.00308.x.

A. Premrov, T. Cummins, and K. A. Byrne. Assessing fixed depth carbon stocks in soils with varying horizon depths and thicknesses, sampled by horizon. *CATENA*, 150:291–301, mar 2017. doi: 10.1016/j.catena.2016.11.030.

J. Pretty and Z. P. Bharucha. Sustainable intensification in agricultural systems. *Annals of Botany*, 114(8):1571–1596, oct 2014. doi: 10.1093/aob/mcu205.

M. Pulido, S. Schnabel, J. F. L. Contador, J. Lozano-Parra, Á. Gómez-Gutiérrez, E. C. Brevik, and A. Cerdà. Reduction of the frequency of herbaceous roots as an effect of soil compaction induced by heavy grazing in rangelands of SW spain. *CATENA*, 158:381–389, nov 2017. doi: 10.1016/j.catena.2017.07.019.

M. Pulido-Fernández, S. Schnabel, J. F. Lavado-Contador, I. M. Mellado, and R. O. Pérez. Soil organic matter of iberian open woodland rangelands as influenced by vegetation cover and land management. *CATENA*, 109:13–24, oct 2013. doi: 10.1016/j.catena.2013.05.002.

C. Quintana, M. Girardello, and H. Balslev. Balancing plant conservation and agricultural production in the ecuadorian dry inter-andean valleys. *PeerJ*, 7:e6207, feb 2019. doi: 10.7717/peerj.6207.

D. P. Rasse, C. Rumpel, and M.-F. Dignac. Is soil carbon mostly root carbon? mechanisms for a specific stabilisation. *Plant and Soil*, 269(1-2):341–356, feb 2005. doi: 10.1007/s11104-004-0907-y.

M. Rawlik, M. Kasprowicz, A. M. Jagodziński, K. Rawlik, and C. Kaźmierowski. Slope exposure and forest stand type as crucial factors determining the decomposition rate of herbaceous litter on a reclaimed spoil heap. *CATENA*, 175:219–227, apr 2019. doi: 10.1016/j.catena.2018.12.008.

M. Reina, L.; Julca A.; Cantos. Agrosilvopastoral zoning system of the first stage of carrizal-chone irrigation project, manabi province, ecuador. *La Tecnica*, 14(1-2): 30–39, 2015.

F.-r. P. . G. J. A. Reyna-Bowen, L. Influence of tree and occasional cropping on soil organic carbon and its fractionation in dehesa system. *Catene*, feb 2020.

L. Reyna-Bowen, L. Vera-Montenegro, and L. Reyna. Soil-organic-carbon concentration and storage under different land uses in the carrizal-chone valley in ecuador. *Applied Sciences*, 9(1):45, dec 2018. doi: 10.3390/app9010045.

M. V.-M. L. Reyna-Bowen, L.; Reyna-Bowen. Zoning of the territory to apply conservation tillage mechanics using the evaluation approach. *Rev. Cienc. Téc. Agropecu*, 114(2):1571–1596, jan 2017.

C. A. Roberts, J. W. Jr., J. B. R. III, D. F. Malley, P. D. Martin, and E. Ben-Dor. Application in analysis of soils. In *Near-Infrared Spectroscopy in Agriculture*. American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, 2004. doi: 10.2134/agronmonogr44.c26.

J. J. Rochon, C. J. Doyle, J. M. Greef, A. Hopkins, G. Molle, M. Sitzia, D. Scholefield, and C. J. Smith. Grazing legumes in europe: a review of their status, management, benefits, research needs and future prospects. *Grass and Forage Science*, 59(3): 197–214, sep 2004. doi: 10.1111/j.1365-2494.2004.00423.x.

A. Román-Sánchez, T. Vanwalleghem, A. Peña, A. Laguna, and J. Giráldez. Controls on soil carbon storage from topography and vegetation in a rocky, semi-arid landscapes. *Geoderma*, 311:159–166, feb 2018. doi: 10.1016/j.geoderma.2016.10.013.

J. Romanyà, J. Cortina, P. Falloon, K. Coleman, and P. Smith. Modelling changes in soil organic matter after planting fast-growingPinus radiataon mediterranean agricultural soils. *European Journal of Soil Science*, 51(4):627–641, dec 2000. doi: 10.1111/j.1365-2389.2000.00343.x.

J. Romanyà, P. Rovira, B. Duguy, R. Vallejo, and A. R. Sánchez. C sequestration issues in the mediterranean soils. *Greenhouse-gas budget of soils under changing climate and land use (BurnOut) ? COST 639*, pages 15–22, 2010. URL `http://oa.upm.es/48636/`.

R. V. Rossel and T. Behrens. Using data mining to model and interpret soil diffuse reflectance spectra. *Geoderma*, 158(1-2):46–54, aug 2010. doi: 10.1016/j.geoderma.2009.12.025.

R. V. Rossel, D. Walvoort, A. McBratney, L. Janik, and J. Skjemstad. Visible, near infrared, mid infrared or combined diffuse reflectance spectroscopy for simultaneous assessment of various soil properties. *Geoderma*, 131(1-2):59–75, mar 2006. doi: 10.1016/j.geoderma.2005.03.007.

R. V. Rossel, T. Behrens, E. Ben-Dor, D. Brown, J. Demattê, K. Shepherd, Z. Shi, B. Stenberg, A. Stevens, V. Adamchuk, H. Aïchi, B. Barthès, H. Bartholomeus, A. Bayer, M. Bernoux, K. Böttcher, L. Brodský, C. Du, A. Chappell, Y. Fouad, V. Genot, C. Gomez, S. Grunwald, A. Gubler, C. Guerrero, C. Hedley, M. Knadel,

H. Morrás, M. Nocita, L. Ramirez-Lopez, P. Roudier, E. R. Campos, P. Sanborn, V. Sellitto, K. Sudduth, B. Rawlins, C. Walter, L. Winowiecki, S. Hong, and W. Ji. A global spectral library to characterize the world's soil. *Earth-Science Reviews*, 155: 198–230, apr 2016. doi: 10.1016/j.earscirev.2016.01.012.

S. M. O. Rourke and N. M. Holden. Optical sensing and chemometric analysis of soil organic carbon - a cost effective alternative to conventional laboratory methods? *Soil Use and Management*, 27(2):143–155, may 2011. doi: 10.1111/j.1475-2743.2011.00337.x.

C. Rumpel and I. Kögel-Knabner. Deep soil organic matter—a key but poorly understood component of terrestrial c cycle. *Plant and Soil*, 338(1-2):143–158, may 2010. doi: 10.1007/s11104-010-0391-5.

W. H. Schlesinger and J. A. Andrews. Soil respiration and the global carbon cycle. *Biogeochemistry*, 48(1):7–20, 2000. doi: 10.1023/a:1006247623877.

D. W.-E. B. Schoeneberger, P.J. and S. S. Staff. *Field book for describing and sampling soils, Version 3.0. Natural Resources Conservation Service, National Soil Survey Center*. National Soil Survey Center, Lincoln, NE, september 2012.

G. Seddaiu, S. Bagella, A. Pulina, C. Cappai, L. Salis, I. Rossetti, R. Lai, and P. P. Roggero. Mediterranean cork oak wooded grasslands: synergies and trade-offs between plant diversity, pasture production and soil carbon. *Agroforestry Systems*, apr 2018. doi: 10.1007/s10457-018-0225-7.

P. C. Selmants, S. C. Hart, S. I. Boyle, and J. M. Stark. Red alder (alnus rubra) alters community-level soil microbial function in conifer forests of the pacific northwest, USA. *Soil Biology and Biochemistry*, 37(10):1860–1868, oct 2005. doi: 10.1016/j.soilbio.2005.02.019.

. S. I. P. Siirt, M. B. Visible and near infrared spectroscopy techniques for determination of some physical and chemical properties in kazova ... visible and near infrared spectroscopy techniques for determination of some physical and chemical properties in kazova watershed. *Advances in Environmental Biology*, 10:61–72, may 2016.

N. Simón, F. Montes, E. Díaz-Pinés, R. Benavides, S. Roig, and A. Rubio. Spatial distribution of the soil organic carbon pool in a holm oak dehesa in spain. *Plant and Soil*, 366(1-2):537–549, sep 2012. doi: 10.1007/s11104-012-1443-9.

J. Six, R. T. Conant, E. A. Paul, and K. Paustian. Stabilization mechanisms of soil organic matter: Implications for c-saturatin of soils. *Plant and Soil*, 241(2): 155–176, 2002. doi: 10.1023/a:1016125726789.

A. Slepetiene, K. Amaleviciute-Volunge, J. Slepetys, I. Liaudanskiene, and J. Volungevicius. The status of pachiterric histosol properties as influenced by different land use. In *Peat*. InTech, sep 2018. doi: 10.5772/intechopen.74151.

L. N. Soucémarianadin, L. Cécillon, B. Guenet, C. Chenu, F. Baudin, M. Nicolas, C. Girardin, and P. Barré. Environmental factors controlling soil organic carbon stability in french forest soils. *Plant and Soil*, 426(1-2):267–286, mar 2018. doi: 10.1007/s11104-018-3613-x.

J.-F. Soussana and G. Lemaire. Coupling carbon and nitrogen cycles for environmentally sustainable intensification of grasslands and crop-livestock systems. *Agriculture, Ecosystems & Environment*, 190:9–17, jun 2014. doi: 10.1016/j.agee.2013.10.012.

C. Steiner, W. G. Teixeira, J. Lehmann, T. Nehls, J. L. V. de Macêdo, W. E. H. Blum, and W. Zech. Long term effects of manure, charcoal and mineral fertilization on crop production and fertility on a highly weathered central amazonian upland soil. *Plant and Soil*, 291(1-2):275–290, feb 2007. doi: 10.1007/s11104-007-9193-9.

B. Stenberg, R. A. V. Rossel, A. M. Mouazen, and J. Wetterlind. Visible and near infrared spectroscopy in soil science. In *Advances in Agronomy*, pages 163–215. Elsevier, 2010. doi: 10.1016/s0065-2113(10)07005-7.

C. E. Stewart, K. Paustian, R. T. Conant, A. F. Plante, and J. Six. Soil carbon saturation: Implications for measurable carbon pool dynamics in long-term incubations. *Soil Biology and Biochemistry*, 41(2):357–366, feb 2009. doi: 10.1016/j.soilbio.2008.11.011.

O. Stock and N. K. Downes. Effects of additions of organic matter on the penetration resistance of glacial till for the entire water tension range. *Soil and Tillage Research*, 99(2):191–201, jun 2008. doi: 10.1016/j.still.2008.02.002.

E. Tamburini, F. Vincenzi, S. Costa, P. Mantovi, P. Pedrini, and G. Castaldelli. Effects of moisture and particle size on quantitative determination of total organic carbon (TOC) in soils using near-infrared spectroscopy. *Sensors*, 17(10):2366, oct 2017. doi: 10.3390/s17102366.

R. Tárrega, L. Calvo, Á. Taboada, S. García-Tejero, and E. Marcos. Abandonment and management in spanish dehesa systems: Effects on soil features and plant species richness and composition. *Forest Ecology and Management*, 257(2):731–738, jan 2009. doi: 10.1016/j.foreco.2008.10.004.

F. S. Terra, J. A. Demattê, and R. A. V. Rossel. Spectral libraries for quantitative analyses of tropical brazilian soils: Comparing vis–NIR and mid-IR reflectance data. *Geoderma*, 255-256:81–93, oct 2015. doi: 10.1016/j.geoderma.2015.04.017.

J. Tian, Y. Lou, Y. Gao, H. Fang, S. Liu, M. Xu, E. Blagodatskaya, and Y. Kuzyakov. Response of soil organic matter fractions and composition of microbial community to long-term organic and mineral fertilization. *Biology and Fertility of Soils*, 53(5): 523–532, apr 2017. doi: 10.1007/s00374-017-1189-x.

C.-C. Tsui, Z.-S. Chen, and C.-F. Hsieh. Relationships between soil properties and slope position in a lowland rain forest of southern taiwan. *Geoderma*, 123(1-2): 131–142, nov 2004. doi: 10.1016/j.geoderma.2004.01.031.

M. Upson, P. Burgess, and J. Morison. Soil carbon changes after establishing woodland and agroforestry trees in a grazed pasture. *Geoderma*, 283:10–20, dec 2016. doi: 10.1016/j.geoderma.2016.07.002.

C. Uribe, R. Inclán, L. Hernando, M. Román, M. A. Clavero, S. Roig, and H. V. Miegroet. Grazing, tilling and canopy effects on carbon dioxide fluxes in a spanish dehesa. *Agroforestry Systems*, 89(2):305–318, dec 2014. doi: 10.1007/s10457-014-9767-5.

F. van der Meer. Near-infrared laboratory spectroscopy of mineral chemistry: A review. *International Journal of Applied Earth Observation and Geoinformation*, 65: 71–78, mar 2018. doi: 10.1016/j.jag.2017.10.004.

E. I. Vanguelova, T. R. Nisbet, A. J. Moffat, S. Broadmeadow, T. G. M. Sanders, and J. I. L. Morison. A new evaluation of carbon stocks in british forest soils. *Soil Use and Management*, 29(2):169–181, jan 2013. doi: 10.1111/sum.12025.

J. Vicente-Vicente, B. Gómez-Muñoz, M. Hinojosa-Centeno, P. Smith, and R. Garcia-Ruiz. Carbon saturation and assessment of soil organic carbon fractions in mediterranean rainfed olive orchards under plant cover management. *Agriculture, Ecosystems & Environment*, 245:135–146, jul 2017. doi: 10.1016/j.agee.2017.05.020.

U. Vitharana, U. Mishra, and R. Mapa. National soil organic carbon estimates can improve global estimates. *Geoderma*, 337:55–64, mar 2019. doi: 10.1016/j.

geoderma.2018.09.005.

B. D. Vos, N. Cools, H. Ilvesniemi, L. Vesterdal, E. Vanguelova, and S. Carnicelli. Benchmark values for forest soil carbon stocks in europe: Results from a large scale forest soil survey. *Geoderma*, 251-252:33–46, aug 2015. doi: 10.1016/j.geoderma.2015.03.008.

A. Walkley. A crititcal examination of a rapid method for determining organic carbo in soils —Efect of variation in digestion conditions and of inorganic soil constituents. *Soil Science*, 63(4):251–264, apr 1947. doi: 10.1097/00010694-194704000-00001.

N. Wang, B. Quesada, L. Xia, K. Butterbach-Bahl, C. L. Goodale, and R. Kiese. Effects of climate warming on carbon fluxes in grasslands— a global meta-analysis. *Global Change Biology*, 25(5):1839–1851, mar 2019. doi: 10.1111/gcb.14603.

L. Wei, H. Hai-Zhou, Z. Zhi-Nan, and W. Gao-Lin. Effects of grazing on the soil properties and c and n storage in relation to biomass allocation in an alpine meadow. *Journal of soil science and plant nutrition*, 11(4):27–39, 2011. doi: 10.4067/s0718-95162011000400003.

A. Weiss. Topographic position and landforms analysis andrew d. weiss, the nature conservancy. *The Nature Conservancy, Northwest Division*, 68(1):1–200, mar 2017. doi: https://doi.org/http://www.jennessent.com/downloads/TPI-poster-TNC_18x22.pdf.

J. Wenjun, S. Zhou, H. Jingyi, and L. Shuo. In situ measurement of some soil properties in paddy soil using visible and near-infrared spectroscopy. *PLoS ONE*, 9(8):e105708, aug 2014. doi: 10.1371/journal.pone.0105708.

J. P. Wight, F. L. Allen, A. J. Ashworth, D. D. Tyler, N. Labbé, and T. G. Rials. Comparison of near infrared reflectance spectroscopy with combustion and chemical methods for soil carbon measurements in agricultural soils. *Communications in Soil Science and Plant Analysis*, 47(6):731–742, feb 2016. doi: 10.1080/00103624.2016.1146750.

S. Wold, M. Sjöström, and L. Eriksson. PLS-regression: a basic tool of chemometrics. *Chemometrics and Intelligent Laboratory Systems*, 58(2):109–130, oct 2001. doi: 10.1016/s0169-7439(01)00155-1.

D. Xu, W. Ma, S. Chen, Q. Jiang, K. He, and Z. Shi. Assessment of important soil properties related to chinese soil taxonomy based on vis–NIR reflectance spectroscopy. *Computers and Electronics in Agriculture*, 144:1–8, jan 2018. doi: 10.1016/j.compag.2017.11.029.

F. Yang, J. Tian, J. Meersmans, H. Fang, H. Yang, Y. Lou, Z. Li, K. Liu, Y. Zhou, E. Blagodatskaya, and Y. Kuzyakov. Functional soil organic matter fractions in response to long-term fertilization in upland and paddy systems in south china. *CATENA*, 162:270–277, mar 2018a. doi: 10.1016/j.catena.2017.11.004.

Y. Yang, Y. Chen, Z. Li, and Y. Chen. Land-use/cover conversion affects soil organic-carbon stocks: A case study along the main channel of the tarim river, china. *PLOS ONE*, 13(11):e0206903, nov 2018b. doi: 10.1371/journal.pone.0206903.

S. Zhang, D. Chen, D. Sun, X. Wang, J. L. Smith, and G. Du. Impacts of altitude and position on the rates of soil nitrogen mineralization and nitrification in alpine meadows on the eastern qinghai–tibetan plateau, china. *Biology and Fertility of Soils*, 48(4):393–400, nov 2011. doi: 10.1007/s00374-011-0634-5.

G. Zhou, X. Zhou, Y. He, J. Shao, Z. Hu, R. Liu, H. Zhou, and S. Hosseinibai. Grazing intensity significantly affects belowground carbon and nitrogen cycling in grassland ecosystems: a meta-analysis. *Global Change Biology*, 23(3):1167–1179, sep 2016. doi: 10.1111/gcb.13431.

Y. Zhou, T. W. Boutton, and X. B. Wu. Soil carbon response to woody plant encroachment: importance of spatial heterogeneity and deep soil storage. *Journal of Ecology*, 105(6):1738–1749, apr 2017. doi: 10.1111/1365-2745.12770.

M. Zhu, Q. Feng, Y. Qin, J. Cao, H. Li, and Y. Zhao. Soil organic carbon as functions of slope aspects and soil depths in a semiarid alpine region of northwest china. *CATENA*, 152:94–102, may 2017. doi: 10.1016/j.catena.2017.01.011.

M. Zhu, Q. Feng, M. Zhang, W. Liu, Y. Qin, R. C. Deo, and C. Zhang. Effects of topography on soil organic carbon stocks in grasslands of a semiarid alpine region, northwestern china. *Journal of Soils and Sediments*, 19(4):1640–1650, dec 2018. doi: 10.1007/s11368-018-2203-0.

S. A. Zimov. CLIMATE CHANGE: Permafrost and the global carbon budget. *Science*, 312(5780):1612–1613, jun 2006. doi: 10.1126/science.1128908.