Diversified Arable Cropping Systems and Management Schemes in Selected European Regions Have Positive Effects on Soil Organic Carbon Content

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Abstract: In the last few decades, various crop diversification strategies and management practices have been promoted to improve or at least maintain environmental quality and agroecosystem services. We conducted a data-analysis to evaluate the effectiveness of alternatives for crop diversification and environmentally friendly farming management for arable crops in four selected European pedoclimatic regions and typical cropping systems in the Atlantic, Boreal, Mediterranean North, and Mediterranean South regions. The dataset was retrieved from 38 references and included data on site-specific environmental conditions, soil tillage, crop rotation, fertilization, and final soil organic carbon content (SOC). No tillage (NT) was more effective (7%) in increasing SOC content than minimum tillage (MT) across the studied depths (from 5 to 40 cm). Conservation tillage as whole, including NT, MT, and rotational tillage (RT) positively affected SOC content in the top 10 cm (28%) in comparison with conventional tillage (CT). Compared to monoculture, longer crop rotations (3–5 years) and the introduction of legumes resulted in higher increases in SOC contents (18%), that were higher in semiarid conditions (11%) than under humid and sub-humid climates (3.2%). The effect of fertilization on SOC contents was higher in the Mediterranean North region (28%), and organic fertilization showed the highest increases (25%) compared to the control with mineral fertilization. Higher increases in SOC contents with tillage and fertilization management were found in sites with lower SOC contents in the control treatment (conventional tillage and mineral fertilization respectively). The data analysis indicated that various European arable agroecosystems benefit both from diversified cropping systems and the adoption of environmentally friendly farming management and are thereby capable to increase SOC contents.

Keywords: crop diversification; tillage; fertilization; soil organic carbon

1. Introduction

Agricultural land accounts for almost half (47%) of the total land area in the European Union [1], with about 60% of agricultural land covered by arable, mostly annual crops, 33% by perennial grassland and meadows, and less than 7% by permanent crops [2]. Over the last years, traditional agroecosystems have been progressively simplified and are now highly dependent on external inputs of energy and
agrochemicals [3]. However, negative impacts on agroecosystem services and on the environment have emerged [4], such as soil degradation, depletion of soil organic carbon (SOC) stocks, water contamination and eutrophication, and loss of biodiversity [5]. In Europe, present efforts to reduce negative environmental impact of intensive agriculture [6] are mostly dedicated to reducing external inputs (e.g., inorganic fertilizers) and the consumption of non-renewable resources (e.g., fossil fuels). Various cropping strategies and management practices—such as diversification of cropping systems by crop rotation, conservation tillage, and the use of cover crops—have been promoted by several authors to enhance crop productivity and ecosystem services [7].

Adopting proper soil tillage or fertilization practices can alleviate problems related to degradation of the environment, but the effects can vary depending on, e.g., soil type or climate zone [8]. In addition to savings in energy and labor, reduced tillage intensity can also protect soil from erosion or losses of organic matter in the topsoil [9]. Moreover, a more complex management change with diverse rotation and permanent soil cover coupled with conservation tillage may reduce the environmental impact of agriculture [10,11].

Crop diversification is an attempt to add complexity to agroecosystems to improve their sustainability, i.e., the maintenance of their economic, biological and physical components over long time frames [12]. Multispecies systems are a step towards more natural systems where, e.g., plant interactions provide mechanisms for increased yield stability and resilience, i.e., the ability of agroecosystems to resist a disturbance or recover to the initial state following a perturbation due to changes in climate (temperature stress or water availability), pests, and other natural disturbances [13]. Increased diversity of species can be implemented through crop rotations (alternating crops in different years), multiple cropping, and intercropping. Multiple cropping refers both to growing crops in multiple seasons and growing more than one crop in the same season [14] but it is also possible to grow multiple crops simultaneously on the same land, referred to as intercropping [15].

Organic amendments, if used as an alternative or in addition to mineral fertilizers, have the capacity to increase SOC content [16,17]. Crop residue management (e.g., incorporated into the soil or used as mulch) may play a key role in the crop nutrient cycle, and is an important measure to maintain or increase soil organic matter (SOM) levels, especially in rainfed cropping systems [18].

To our knowledge, this is the first comprehensive study to assess SOC contents coupling crop diversification, tillage, and fertilization management at a wide European scale. Many studies have assessed SOC sequestration rates, in Mediterranean cropping systems. Recommended management practices (RMPs) to increase SOC stocks were studied in arable and woody crops by Aguilera et al. [19] and in woody crops by Vicente-Vicente et al. [20,21]. Francaviglia et al. [22] assessed the achievement of 4% objective to increase SOC stocks within the “Soils for Food Security and Climate” initiative launched by French Government in 2015 during the United Nations Framework Convention on Climate Change 21st Conference of the Parties in Paris. The specific effect of soil tillage on SOC dynamics was studied worldwide by Luo et al. [23], by Virto et al. [24] as a function of carbon (C) inputs from crops, and with a data mining approach by Francaviglia et al. [25] in the Mediterranean basin. SOC stocks in soils under arable, permanent crops, grasslands, pasture and forests was studied at national level in France [26,27], in Spain for arable crops under conservation agriculture [28] and in Germany for agricultural soils [29]. Finally, other worldwide studies on different factors affecting C sequestration rates were specific for tillage and crop rotations [30], crop rotation diversification [31], and cover crops [32].

The present study is the result of the on-going activities carried out within the H2020 Diverfarming project (www.diverfarming.eu). The objective of this work was to identify the diversified cropping systems and crop associations with low-input practices and strategies tailored to various pedoclimatic European regions, to be thereafter tested and validated in field experiments within the project. Thus, we conducted a data-analysis to evaluate the effectiveness of the existing alternatives for crop diversification and environmentally-sound farming practices for the main arable cropping systems in
four European pedoclimatic regions (Atlantic, Boreal, Mediterranean North, Mediterranean South) addressing fodder grains, leys and mixtures, autumn–winter and spring–summer cereals.

Accordingly, the specific aims of the present study were to: (i) collate studies providing environmental data and soil variables from field experiments in four pedoclimatic regions of Europe, and (ii) evaluate the effect of tillage management, crop diversification, and fertilization management on SOC content across soil depths.

2. Materials and Methods

2.1. Data Collection

A data collection of field studies was performed for four pedoclimatic regions of Europe [33,34]: Atlantic (Belgium, parts of France and Germany, Netherlands), Boreal (Finland, Latvia, Lithuania, parts of Norway and Sweden), Mediterranean North (Italy), and Mediterranean South (Greece, Spain). Furthermore, field studies in Morocco were also included as Mediterranean South, despite not being an EU country, to give more consistency to the data-analysis of this region. The following cropping systems were addressed: fodder grains in the Atlantic region (AtFodG), fodder leys and mixtures (e.g., wheat/oats+pea/barley/ley) in the Atlantic and Boreal regions (AtFodMix, BorFodMix), autumn–winter cereals in the Mediterranean North and South regions (MedNCerAW, MedSCerAW), and spring–summer cereals in the North Mediterranean region (MedNCerSS).

Data were derived from a web search of scientific literature with SCOPUS and Google Scholar, specifically searching the title, abstract, and keywords of the reference for: “diversification”, AND/OR “crop rotation”, AND/OR “intercropping”, AND/OR “multiple cropping”, AND/OR “soil organic carbon”, coupled with the country or region name. Outcomes from European/national projects and related databases, as well specific national reports were also considered where available. Literature cited in the selected articles was further examined for collecting more studies only if they met the adopted criteria. The studies to be included in the analysis were selected based on the study length (at least one full year) and the type of the experiment (only field experiments). The final selection included 304 comparisons from a total of 38 references [35–72]. Moreover, studies providing SOC content by soil depths under tillage management were also considered, with 167 comparisons from 10 references [39,69,73–80].

This search was carried out from May to August 2017, and data were collected in online excel spreadsheets. The spreadsheet template contained general site, crop and soil information. General site characteristics included: region/crop, country, province, experiment site, elevation (m), mean annual temperature (°C), mean total annual rainfall (mm) and aridity class derived from the De Martonne aridity index [34]. Crop information included fertilization management (mineral, mixed, organic, and rates of application), tillage management (e.g., conventional tillage, no tillage, and tillage depth), crop diversification (monoculture, rotation, intercropping, or multiple cropping), residues management (e.g., mulched, incorporated), cultivation of cover crops and presence of legumes in the rotation. Soil characteristics included texture group according to USDA classification, soil sampling depth that ranged from 5 to 60 cm in the different studies and SOC content at the end of the experiment (%). Additional information included experiment length (from 2 to 50 years).

2.2. Data Evaluation

The diversified (D) treatments of each experiment were evaluated based on the percentage change of the considered response variable—i.e., SOC content (%)—from that of the control (C). Since we used the ratio between the difference (D–C) and the C treatment, already adopted by Francaviglia and Di Bene [81], we could eliminate the differences deriving from the different soil analytical methods among studies.

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\text{Percentage change (SOC)} = 100 \times \frac{(\text{SOC}_D - \text{SOC}_C)}{\text{SOC}_C}
\]
Considering that SOC content can be affected by different factors (e.g., tillage management, crop diversification, and fertilization management) we analyzed each effect separately. Thus, we compared the effects of crop diversification (e.g., rotation vs. monoculture system), tillage (e.g., no tillage vs. conventional tillage), and fertilization management (e.g., organic fertilization vs. mineral fertilization). SOC was evaluated considering the concentration values in % at the end of the experiment reported in the studies and calculating their percentage change (Equation (1)) by comparing the diversified and the control treatment of each experiment. We did not calculate and compare SOC stocks, since most studies did not report measurements of bulk density, and using pedotransfer functions was not considered appropriate due to the high heterogeneity of soils, treatments, and environmental conditions.

SOC content changes by soil depths \([39,69,73–80]\) were represented using the depth range reported in the studies and analyzed by groups, i.e., 0–10, 10–20, 20–30, and 30–60 cm.

Changes in SOC contents (Equation (1)) were firstly analyzed evaluating the effects of management, i.e., tillage, crop diversification and fertilization management, and further by environmental (e.g., climate) or soil parameters (e.g., texture). Data are represented by box and whisker plots (central point means, and 95% confidence interval CI) following the method reported in previous data-analyses \([25,81]\). Responses were considered significantly different if their 95% CIs did not overlap, and significantly different from the controls if the 95% CIs did not overlap with zero \([19,20,82]\). Furthermore, correlation statistics for SOC content changes were performed based on a non-parametric Spearman rank analysis \([22,25]\) including 26, 22, and 23 qualitative and quantitative predictors derived from the data-set for tillage management, crop diversification, and fertilization management respectively (Table S1). Data were additionally analyzed by comparing age groups, e.g., 2–10 years, 11–20 years, and >20 years in relation to the duration of the experiment (Table S2). Statistical analyses were performed using Statistica 7.0 (Statsoft, Tulsa, OK, USA).

3. Results

3.1. Tillage Management

Compared to CT, SOC content increases were significantly higher only with NT (7%) (Figure 1a) when considering the references providing SOC content only for a single layer \([35–72]\). Significant SOC content increases by conservation tillage management were found with crop rotation (5%, Figure 1b) and organic and mixed fertilization (27 and 5% respectively, Figure 1c). Spearman rank correlation analysis among SOC content changes and the selected predictive variables for tillage management are illustrated in Figure 2a. Significant negative coefficients were reported for SOC content in the control (−0.41), indicating higher SOC content changes in sites with lower SOC content in the control treatment, cover crop (−0.23), residue incorporation (−0.30), subhumid climates (−0.24), clay (−0.19), and clay-loam (−0.30) textures. SOC content changes were positively and significantly correlated with no tillage (0.17), organic fertilization (0.31), residue mulching (0.20), humid climates (0.18), as well as loam and silty-loam soil textures (0.25). No significant correlation was found between SOC content changes and the duration of the experiment.

Results by soil depths \([39,69,73–80]\) showed an average SOC content increase by 28% in the 0–10 cm layer with conservation tillage management (including no tillage, minimum tillage, and rotational tillage) compared to conventional deep tillage (Figure 3a). Conversely, SOC contents decreased by 10%, 6%, and 13% in the layers 10–20, 20–30, and 30–60 cm respectively. No tillage (Figure 3b) had a significant effect (12%) in increasing SOC contents compared to CT across all depths (0–60 cm) when considering the references providing SOC content by depth ranges \([39,69,73–80]\). In addition, both NT and MT systems were significantly different in comparison with CT in the 0–10 cm depth range (Figure 3c), with SOC content increases by 36 and 25% respectively.
Figure 1. Mean plot of soil organic carbon content change (%) of conservation tillage vs. conventional deep tillage grouped by: (a) tillage management across all depths [35–72] (see Figure 3a for the depth increments), (b) crop diversification, (c) fertilization group. Box and whisker plots represent central point means, and 95% confidence interval. Numbers in brackets represent the number of comparisons used in each category.
Figure 2. Cont.
Figure 2. Spearman rank correlation coefficients (rs) among SOC content changes and the predictive variables. (a) Tillage management, (b) crop diversification, (c) fertilization management. The asterisks (*) indicate both positive and negative correlations with significant coefficients at $p < 0.05$ above $rs = |0.15|$. 

Figure 3. Cont.
3.2. Crop Diversification

Crop diversification with long rotations (at least 3 years up to 5 years) and the introduction of legume crops significantly increased SOC content changes by 18% compared with the control treatment with monoculture and no legumes in the rotation (Figure 4). Conversely, SOC content changes decreased by 6% in long rotations without legumes, and by 3–5% in short rotations, even with the introduction of legumes in the rotation. In addition, average increases of SOC with crop diversification were significantly higher in semiarid conditions (11%) than in other climates (Figure 5).
Figure 4. Mean plot of soil organic carbon content change (%) of crop diversification vs. control management (monoculture) with and without the presence of legumes. Box and whisker plots represent central point means, and 95% confidence interval. Numbers in brackets represent the number of comparisons used in each category. Short + leg and short-leg indicate short rotations (2-years) with and without the introduction of legumes in the rotation respectively. Long + leg and long-leg indicate long rotation (3–5 years) with and without the introduction of legumes in the rotation respectively.

Figure 5. Mean plot of soil organic carbon content change (%) of crop diversification vs. control management (monoculture) grouped by aridity class. Box and whisker plots represent central point means, and 95% confidence interval. Numbers in brackets represent the number of comparisons used in each category.

Spearman rank correlation analysis among SOC content changes and the selected predictive variables for crop diversification are illustrated in Figure 2b. Significant negative coefficients were reported for the duration of the experiment (−0.45), indicating higher SOC content changes in sites where crop diversification was established more recently, coupled with no tillage (−0.32), residue incorporation (−0.31) and mulching (−0.33), mixed fertilization (−0.16), in cropping systems with autumn–winter cereals of the Mediterranean South region (−0.45), and in clay (−0.28) and loam (−0.41) soil textures. SOC content changes were positively and significantly correlated with SOC content in the control (0.20), indicating higher SOC content changes in sites with higher SOC content in the control treatment, crop rotations ≥3 years (0.61) and the presence of legumes in rotations (0.60), conventional
tillage (0.22) and the consequent removal of crop residues (0.58), in semiarid climates (0.16), in cropping systems with autumn–winter cereals of the Mediterranean North region (0.17) and in sandy clay loam soil textures (0.61).

3.3. Fertilization Management

Compared to the control treatment with mineral fertilization, organic fertilization showed the higher SOC content increases (25%) (Figure 6), particularly with fertilization managements that included manure (39%), slurry (37%), mineral + slurry (18%), compost (13%), and crop residue management (11%) (Figure 7). Fertilization average effect on SOC contents differed among regions and crops and was significantly higher for spring–summer cereal crops in the North Mediterranean region (28%) and fodder crops in the Boreal area (15%) compared to fodder crops in the Atlantic region (11%) (Figure 8).

The average amounts of nitrogen and phosphorus added during the field experiments included in the data-analysis were 99 and 55 kg ha\(^{-1}\) respectively; however, nitrogen fertilization differed by crops and pedoclimatic regions. Higher amounts of nitrogen were added as average in the Mediterranean regions for corn in Italy (160 kg N ha\(^{-1}\)) and winter wheat and barley in Italy and Spain (108 kg N ha\(^{-1}\)). Comparable amounts of nitrogen were used in the Atlantic region on fodder grains and monocropping and rotations with cereal mixtures (86 kg N ha\(^{-1}\)). The lowest nitrogen fertilization was applied in the Boreal region for fodder grains and cereal mixtures.

![Figure 6. Mean plot of soil organic carbon content change (%) of fertilization management vs. control management grouped by fertilization group. Box and whisker plots represent central point means, and 95% confidence interval. Numbers in brackets represent the number of comparisons used in each category.](image-url)
Figure 7. Mean plot of soil organic carbon content change (%) of fertilization management vs. control management grouped by fertilization group. Box and whisker plots represent central point means, and 95% confidence interval. Numbers in brackets represent the number of comparisons used in each category.

Figure 8. Mean plot of soil organic carbon content change (%) of fertilization management vs. control management (mineral or unfertilized treatment) grouped by region/crop. Box and whisker plots represent central point means, and 95% confidence interval. Numbers in brackets represent the number of comparisons used in each category. At_FodG, Atlantic fodder grains; At_FodMix, Atlantic fodder mixtures; Bor_FodMix, Boreal fodder mixtures; MedN_CerAW, Mediterranean North autumn–winter cereals; MedN_CerSS, Mediterranean North spring–summer cereals; MedS_CerAW, Mediterranean South autumn–winter cereals.

Spearman rank correlation analysis among SOC content changes and the selected predictive variables for fertilization management are illustrated in Figure 2c. Significant negative coefficients were reported for SOC content in the control (−0.33), indicating higher SOC content changes by fertilization in sites with lower SOC content in the control treatment, no tillage (−0.17), crop rotations with ≥3 years (−0.20), residue mulching (−0.17), in cropping systems with fodder grains of the Atlantic region (−0.39), and sandy (−0.46) soil textures. SOC content changes by fertilization were positively and significantly correlated with the duration of the experiment in years (0.44), organic fertilization (0.19), residue
incorporation (0.18), in cropping systems with autumn–winter cereals of the Mediterranean South region (0.23), and loam (0.32) soil textures.

4. Discussion

4.1. Tillage Management

Changes in SOC content decreased in the order NT > MT > Rotational tillage compared to the control treatment with CT, but the analysis of many more studies would have probably allowed to indicate significant differences among tillage systems in relation to regional characteristics such as soil types and climatic conditions. However, when conservation tillage was coupled with organic and mixed fertilization, the change in SOC contents was significant. This is not surprising since CT is associated with higher SOM mineralization compared to conservation tillage [25]. SOC content changes by layer depth were positive in the top 10 cm, but negative below this depth up to 60 cm. Thus, changes in SOC contents under conservation tillage actually reflect the localization of C derived from crop residues and roots, with a C gain near the soil surface but a C loss at lower depths [23]. Conversely, under CT plant residues are distributed throughout the tillage zone and can show C contents similar or even higher than NT for a given depth especially below the top 10 cm [83,84]. However, when considering the SOC content increases by depth ranges (Figure 3a–c), results could have been influenced by the low number of data available for some tillage management (e.g., rotational tillage).

No significant correlation was found between SOC content changes and the duration of the experiment (see Section 3.1 and Figure 2a), however higher changes in SOC contents compared to conventional tillage were found in the period 11–20 years (8%) after the adoption of conservation tillage (Table S2).

The fact that NT or MT practices alone unlikely increase the SOC content in the whole soil profile suggests that other management strategies should be applied in connection with them, i.e., conservation agriculture, that includes retaining crop residues, crop rotations, and cover crops, instead of merely conservation tillage [85,86]. Our results show that organic fertilization together with conservation tillage improves the SOC content, but still the SOC content would only increase in the upper layers; thus, better results would derive by the implementation of crop rotations that allocate C input to deeper layers. Our results (Figure 1b) support the implementation of crop rotations together with conservation tillage, as the changes in SOC contents compared to monoculture did not present negative values and confidence limits were narrow. However, a more thorough analysis should be conducted to elucidate whether, e.g., the root depth of the species in the rotations defines how much SOC is allocated to the deeper layers.

4.2. Crop Diversification

Changes in SOC content with longer crop rotations (3–5 years) were positive as average in the Mediterranean North region (14.7%) and in the Atlantic and Boreal regions (11.8%). In the Mediterranean North region, SOC content changes in more diverse rotations compared to the simple rotations (2 years) ranged from −9.1% in Central Italy [40] to 24.9% in Southern Italy [55], and in the Boreal and Atlantic conditions from −12.5% in southwestern Finland [65] to 61.9% in The Netherlands respectively [70]. The benefits of diverse rotations regarding SOC contents are likely due to yield improvements or improvements in pest and disease control as well soil health, i.e., the improvement of soil quality though a better soil management [87]. However, yield improvements are not always connected to increased crop residues. For example, in Central Italy [40] the increase of yield did not correspond to a SOC content increase due to the inability of the summer cereal (i.e., maize in rotation with wheat) to produce a sufficiently high amount of crop residues under rainfed conditions. When crop diversification included legume crops, SOC content changes averaged 12.4% at Mediterranean North sites, and 14.8% in Atlantic and Boreal regions. Root residues have a higher potential to increase SOC contents than aboveground residues due to their poorer decomposability and better contact with
soil resulting to physical and chemical protection [88]. In the case of legume crops in longer crop rotations, roots are likely the reason for the observed effect on the increase in SOC changes contents. A study performed in The Netherlands included in the data-analysis [70] proved that implementing temporary legume grass leys in rotation with fodder maize was highly effective in increasing SOC contents through enhanced root C input and soil biota diversity as indicated by other research [31,89].

A significant correlation was found between the changes in SOC contents and the duration of the experiment (see Section 3.2 and Figure 2b), and higher SOC content changes (28%) were found in the first period (2–10 years) after the adoption of crop diversification (Table S2). Conversely, changes in SOC contents decreased to 6% after 11–20 years and were negative (−6%) when the experiment duration was very long (>20 years), indicating that a steady-state condition can be reached very fast.

4.3. Fertilization Management

In this study, the benefits of organic fertilizers were clear since SOC contents decreased in the order of manure > slurry > mineral + slurry > compost. Manures usually are found to increase SOC contents compared to mineral fertilizer [61,62,66,68,72] and the study by Heinzj et al. in Germany [48] indicated that application of high rates of manure over 10 years (equivalent to up to 210 kg N ha⁻¹) increased SOC content in a sandy soil under humid climatic conditions. However, to increase SOC contents at country scale would require using other types of organic fertilizers as well, since manures typically are already utilized as fertilizers, although often not with optimal geographic spread. Based on our data, farmyard manure and slurry amendments did not differ with respect to changes in SOC content. In addition, this study partially supports the findings that materials that have undergone a longer decomposition process, such as composts, would have a higher impact on SOC content [90]. However, Alluvione et al. [35] indicated that compost application in Italy significantly affected SOC content in soils with a low clay content, which are more susceptible to native SOM and compost mineralization, but the effect was lower where clay content was high. However, there were too few studies to compare the groups of organic fertilizers that typically have highly varying compositions.

Similarly to crop diversification, a significant correlation was found between the changes in SOC contents and the duration of the experiment (see Section 3.3 and Figure 2c), however SOC content changes (Table S2) decreased with the duration of the experiment in the order >20 years > 11–20 years > 2–10 years (29%, 14%, and 11% respectively). This finding is not surprising and is due to the continuous addition of C inputs from different sources, e.g., manures, slurry and crop residues.

5. Conclusions

The data analysis of various European arable systems showed that conversion from the traditional monocropping systems with intensive tillage and mineral fertilization to more diversified cropping systems through the use of crop rotations together with no tillage and organic fertilization has a positive effect on SOC contents. However, in the case of NT the positive effect was only observed in the upper soil layers which points to the need to develop conservation tillage systems closer to conservation agriculture systems to increase the SOC content of the deeper soil layers. Higher increases in SOC contents with tillage and fertilization management were found in sites with lower SOC contents in the control treatment, suggesting that the highest benefits in terms of SOC content increases would be achieved by targeting management changes to fields with low initial SOC contents. Conversely, with crop diversification, higher changes in SOC contents were found at sites with higher SOC contents in the control treatment as a consequence of the previous cropping history that already included crop rotations in many sites. In addition, the study indicated that longer crop rotations and the presence of legumes increased SOC contents. The duration of the experiment positively affected the changes in SOC contents in the first years after the adoption of crop diversification, and over longer time periods with fertilization management. The results also pointed out many regional differences that should be considered when targeting measures aiming at improving soil quality through the adoption of crop diversification, tillage, and fertilization management.
Supplementary Materials: The following are available online at http://www.mdpi.com/2077-0472/9/12/261/s1, Table S1: Predictive variables considered in the Spearman rank correlation analysis; Table S2: SOC changes (%) analysis by age groups (duration of the experiment in years and number of data).


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Conflicts of Interest: The authors declare no conflict of interest.

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