## Soil Organic Carbon Under Conservation Agriculture In Mediterranean And Humid Subtropical Climates: Global Meta-Analysis

Short running title: Meta-Analysis of Soil Organic Carbon Under Conservation Agriculture

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## Abstract

Conservation agriculture (CA) is an agronomic system based on minimum soil disturbance (notillage, NT), permanent soil cover and species diversification. The effects of NT on soil organic carbon (SOC) changes have been widely studied, showing somewhat inconsistent conclusions, especially in relation to the Mediterranean and humid subtropical climates. These areas are highly vulnerable and predicted climate change is expected to accentuate desertification and, for these reasons, there is a need for clear agricultural guidelines to preserve or increment SOC. We quantitively summarized the results of 47 studies all around the world in these climates investigating the sources of variation in SOC responses to CA, such as soil characteristics, agricultural management, climate and geography.

Within the climatic area considered, the overall effect of CA on SOC accumulation in the plough layer (0-0.3 m) was 12% greater in comparison to conventional agriculture. On average this result corresponds to a carbon increase of 0.48 Mg C ha<sup>-1</sup> year<sup>-1</sup>. However, the effect was variable depending on the SOC content under conventional agriculture: it was 20% in soils which had  $\leq$  40 Mg C ha<sup>-1</sup>, the ile it was only 7% in soils that had > 40 Mg C ha<sup>-1</sup>. We proved that 10 years of CA impact the most on soil with SOC  $\leq$  40 Mg C ha<sup>-1</sup>. For soils with less than 40 Mg C ha<sup>-1</sup>, increasing the proportion of crops with bigger residue biomasses in a CA rotation was a solution to increase SOC. The effect of CA on SOC depended on clay content only with more than 40 Mg C ha<sup>-1</sup> and become null with a SOC/clay index of 3.2. Annual rainfall (ranged between 331-1850 mm yr<sup>-1</sup>) and geography had specific effects on SOC depending on its content under conventional agriculture. In conclusion, SOC increments due to CA application can be achieved especially in agricultural soils with less than 40 Mg C ha<sup>-1</sup> and located in the middle latitudes or in the dry conditions of Mediterranean and humid subtropical climates.

## Keywords

SOC, no-till, conservation agriculture, C sequestration, Mediterranean and humid subtropical climates, meta-analysis

### Highlights

- The results of 47 studies were quantitively summarized by using a meta-analysis
- SOC accumulation due to CA was 12% greater compared to conventional agriculture
- SOC increment due to CA can be reached to 20% in soils having less than 40 Mg C ha<sup>-1</sup>
- The impacts of pedo-climatic factors and agronomic management practices were studied

## 1. Introduction

Soil organic carbon (SOC), the major component of soil organic matter, has a great impact in all soil processes. SOC dynamics are regulated by climatic variables, geographical characteristics, soil physico-chemical properties, quantity and quality of C inputs into the soil and management practices, as well their interactions (Haddaway et al., 2017; Lal, 2004; Lorenz and Lal, 2018; Ogle et al., 2019; Paustian et al., 1997; West and Post, 2002). Playing an important role in modifying the SOC dynamics (Lorenz and Lal, 2018), conservation agriculture (CA) is being promoted by FAO as an approach for achieving sustainable land management, environmental protection and climate change adaptation and mitigation (Pisante et al., 2015). Conservation agriculture utilizes three agronomic principles: [1] minimum soil disturbance, avoiding soil inversion, i.e., no-tillage (NT) or minimum tillage or vertical tillage; [2] permanent soil cover that is guaranteed by retaining crop residues or by cover crop adoption; and [3] the integration of crop rotations involving at least three different crops (FAO, 2017b).

Since the 2000s, the effects of NT on SOC changes have been summarized in nine meta-analyses conducted mostly on a global scale, but with a few on regional or national scales (Table 1). Most of

them revealed a range of SOC sequestration increases attributable to NT from 8 to 18 % in the 0-0.25/0.3 m soil layer. In contrast, for temperate climates, Mondal et al. (2020) reported a 40% and a 5% of SOC increases in the 0-5 and 5-10 cm soil depths, respectively, while there was a significant decrease in deeper soil layers, resulting in a slight overall increase of 1.1% in the 0-0.6 m soil layer (Table 1). Haddaway et al. (2017) found a SOC sequestration almost twice as large as that reported by Luo et al. (2010) and by West and Post (2002). Moreover, Li et al. (2020) reported that conservation tillage practices increased SOC stock in humid or perhumid (Thornthwaite, 1948) climate conditions, but not in semi-humid. Mondal et al. (2020) highlighted that in tropical and subtropical climates, the effect was positive and significant only up to 10-cm depth, whereas in temperate climates, changes were significant but negative further down the profile. However, Sun et al., (2020) stated that CA adoption is recommended in arid regions, while other meta-analyses did not report a significant effect of climate (Haddaway et al., 2017; Luo et al., 2010; Virto et al., 2012). In addition, different meta-analyses demonstrated inconsistent outcomes for the effects soil texture on SOC accumulation under NT management (Table 1). For example, the effect of NT varied from study to study: increasing effects were only reported in silty and sandy soils within the 0-0.3 m layer (Li et al., 2020), or in loamy and clay soils within the 0-0.1 m layer (Mondal et al., 2020). Sometimes mere were no effects of the texture class (Haddaway et al., 2017; Sun et al., 2020).

On the other hand, the impact of carbon input into soil, which has been considered as a key factor involved in SOC accumulation, was confirmed by many meta-analyses (Table 1). It is also common to refer to the effect of experiment duration to explain the variability of SOC responses across the studies. In this case, five out of nine meta-analyses demonstrated the importance of the NT management duration on SOC accumulation, finding greater effects when the duration of the experiment was longer. Nevertheless, Luo et al. (2010) and Sun et al. (2020) found no consistent relationships with the duration of NT practice.

The reason of such inconsistencies mainly stems from the different methodologies or even erroneous approaches applied in the nine meta-analyses. For instance, three meta-analyses included studies in which bulk densities were not originally measured but were estimated from pedotransfer functions to compute SOC stocks from SOC concentrations. The potential uncertainty which can arise by applying a pedotransfer function developed in a particular area and which is then applied on different sites can seriously impact the final results. In fact, in recent works, Schillaci et al. (2021) and Nasta et al. (2020) tested the performance in predicting BD of new and previous developed pedotransfer functions, respectively. They found that the associated error (RMSE %, Root Mean Square Error of prediction) of the pedotransfer ranged on average between 19% and 26%, respectively.

This review on the existing meta-analyses highlighted that the climate effect is highly variable or influences the SOC sequestration in interaction/addition with other factors. In addition, it has been highlighted a knowledge gap for a specific climate, in particular for areas characterized by mild winters and hot summers (Bouma, 2005; Hernandez-Ochoa and Asseng, 2018), since only one meta-analysis summarizing 33 studies on herbaceous crops was conducted in similar climatic conditions (Aguilera et al., 2013). This type of climate is found in areas mainly characterized by temperate and wediterranean climates and it can be identified with the Cfa, Csa, Csb Köppen sub-types areas (Bouma 2005; Kottek et al., 2006). These areas belong to the warm temperate climates and they are all univocal identified as areas with the temperature of the coldest month ( $T_{min}$ ) ranged between -3 C° <  $T_{min} < +18$  C°, and a hot (where  $T_{max}$ , the monthly mean temperature is  $T_{mon} \ge +10$  °C) summer (Kottek et al., 2006). This characterization identifies areas with high SOC mineralization rates due to of the high temperature during the summer season (Álvaro-Fuentes and Paustian 2011; Pravalie et al., 2021), and with soils having quite low (usually between 0.7 and 1% SOC content depending on the textural class) SOC content (FAO, 2017a; Jones et al., 2005). Thus, these areas are highly

vulnerable and predicted climate change is expected to accentuate human-induced desertification processes like intensive use of agricultural lands, poor irrigation practices and deforestation (Ruiz et al., 2020; Spinoni et al., 2015; Underwood et al., 2009). In addition, an increase of extreme of event and especially drought, is expected in parts of the Mediterranean area and even in some humid areas (Mihailescu and Bruno Soares, 2020) where agricultural practices are also affecting soil fertility since the production system (based on winter wheat and maize, soybean and sunflower during summer) is based on intensive traditional plough-based crop production systems (Mazzoncini et al., 2016).

Due to the similarities of this climate regions and their common problems, it is important to treat these threatened areas as a whole for a broader comprehension of the climate change impact on the soils organic carbon stock. Thus, to update the findings and define clear guidelines under this specific climate condition, the present work aims to conduct a robust meta-analytic approach that highlights the possibility of CA adoption to mitigate soil C depletion. A strong scientific comprehension of the CA practices is needed to support the new policies that will be applied in the future. For instance, the new UE common agricultural policy (2023-27) will consider conservation agriculture as a potential agricultural practice to increase carbon (European Commission, 2021).

Therefore, the present study aims to summarize studies on the effects of CA on SOC sequestration capability in the plough layer (0-0.3 m) in Mediterranean and humid subtropical climates (from all over the world) by using a weighted meta-analysis. We used a rigorous approach that relies on including studies with measured bulk density (when carbon stock was not already reported) and utilizing no pedotransfer functions to compute the carbon stock. We examined the sources of variation in SOC responses to CA across the studies, such as climate, geography, soil characteristics (clay and sand content, pH, SOC/clay index and SOC stock amount under conventional tillage) and agricultural management (N fertilization levels, duration of CA practice, crop diversification, proportion of high-residue crops in rotation, legume presence in the rotation).

|  | Li et al. (2020)  | Sun et al. (2020)   | Mondal et al.<br>(2020)   | Aguilera et al.<br>(2013)                         | Haddaway et al.<br>(2017)       | Luo et al. (2010)         | West and Post<br>(2002)                               | Virto et al. (2012)        | Angers and<br>Eriksen-Hame<br>(2008) |
|--|---|---|---|---|---------------------------------|---------------------------|---|----------------------------|--------------------------------------|
| Origin                                 | global  | global  | global  | Mediterranean                                     | global                          | mostly USA and<br>Canada  | global  | global                     | global                               |
| Type of effect size                    | log response ratio  | log response ratio  | log response ratio  | log response ratio                                | mean difference                 | mean difference           | mean difference                                       | non-standard<br>metrics    | non-standard<br>metrics              |
| No till<br>overall effect <sup>1</sup> | $^{+13\%}_{(\S 3.1)^2}$   | + 8%<br>(Fig. 4)  | +1.1%<br>(Fig. S6)  | +18.2%<br>(§ 3)                                   | + 16%<br>(Fig. 35) <sup>2</sup> | + 2.8%<br>(p. 228)        | + 16%<br>(p. 1943)                                    | + 7%<br>(p. 21)            | + 5%<br>(p. 1373)                    |
| oil depth range (m)                    | 0-0.3   | 0-0.3   | 0-0.6   | 0-0.25  | 0-0.3                           | 0-1                       | 0-0.3   | 0-0.3                      | 0-0.9                                |
| Crops                                  | herbaceous crop   | herbaceous crop   | cereal and legume   | cereals, horticulture                             | herbaceous crop                 | herbaceous crop           | herbaceous crop                                       | herbaceous crop            | mainly cerea                         |
| Bulk density                           | measured  | pedotransfer  | measured  | pedotransfer                                      | measured                        | pedotransfer              | measured  | measured                   | measured                             |
| Climate                                | no effect in semi-<br>humid climate<br>(Fig. 7)   | positive effect in dry<br>region (Fig. 4)                             | the effect dependent<br>on climate and soil<br>depth interaction<br>(Table 3)   | -   | no effect<br>(p. 22)            | no effect<br>(Fig. 5a, b) | -   | no effect<br>(Fig. 1a/b/c) | -                                    |
| Soil                                   | positive effect with<br>sandy and silty soils<br>(Fig. 6)   | no effect of clay and<br>sand<br>(Fig. 1)                             | loamy and clay<br>texture positively<br>affects only 0-0.1 m<br>layer (Table 3) | -   | no effect<br>(p. 22)            | -                         | -   | no effect<br>(Fig. 1d)     |                                      |
| C input                                | positive effect<br>regardless of tillage<br>intensity (§ 3.1)   | the most important<br>management factor<br>(decision tree, Fig.<br>1) | effect only in upper<br>layers (§ 4)  | positive (§ 4)                                    | -                               | -                         | -   | positive<br>(Fig. 2)       |                                      |
| Experiment duration                    | Experiment<br>durationpositive<br>(§ 3.4)no effect<br>(Fig. 1)positive<br>0-0not declared for<br>(Value to table)not<br>(243 in total)not<br>(243 in total) |   | positive effect only<br>0-0.1 m layer (Table<br>3)                              | ositive effect only<br>0.1 m layer (Table -<br>3) |                                 | no effect<br>(Fig. 5c)    | peak sequestration<br>rates in 5 to 10 yr<br>(Fig. 5) | -                          | positive<br>(Fig. 2)                 |
| N° of studies <sup>3</sup>             |   |   | not declared for<br>SOC under no till<br>(522 in total)                         | 33  | 29                              | 69                        | 74  | 37                         | 24                                   |

## 2. Materials and Methods

#### 2.1 Systematic search and data extraction from literature

The database creation for the final statistical analysis involved three different steps: (1) primary studies collection from different online database resources, (2) selection of studies with several inclusion criteria to match the research purpose and (3) data extraction.

#### 2.1.1 Studies collection

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All the screening process followed the PRISMA checklists (Page et al., 2021) for evaluating research synthesis in systematic reviews and meta-analysis. Figure S1 reports the flow diagram of literature search and screening adapted from the PRISMA checklists (Page et al., 2021).

We found the articles by searching for keywords with a nested query (Supplementary file 1) in Web of Science and Scopus databases. The query was based on four different parts related to conservation agriculture, conventional tillage, SOC and the list of the 67 countries that belong to Mediterranean and humid subtropical climates (i.e., that have at least one correspondence with the Cfa, Csa, Csb sub-types) of the Köppen classification (Chen and Chen, 2013; Peel, 2007). Finally, the Bibliometrix R package (Aria and Cuccurullo, 2017) was utilized to merge all the results in a unique dataset (n = 911), excluding the duplicated studies. After this first step, the resulting 911 studies were evaluated with a first screening that allowed to exclude a large part of the database. There were many reasons why this first screening was needed, for instance, because studies were not peer-reviewed or belonged to modelling studies. The outcome of this first screening ended up with 186 studies that were further screened based on the inclusion criteria listed in the next paragraph. The final database used for the meta-analysis was composed of 47 studies.

#### 2.1.2 Inclusion criteria

To be included in the final database, a study had to meet the following criteria:

- 1. the study was conducted on herbaceous field crops;
- 2. the study coordinates belong to Cfa, Csa or Csb;
- the study had an appropriate control group (conventional agriculture): inversion/mixing tillage (moldboard/disk plowing, disk harrow or chisel plowing) in spring, autumn or in both, residues incorporated and no cover crop utilization. Within the single study, the rotation with the least number of crops was selected;
- 4. the study had an appropriate treatment group (conservation agriculture): no tillage management, residues retained on the top of the soil (chopped or not) and with or without cover crops. Within the single study, the rotation with the largest number of crops was selected;
- the study assessed the effect of CA on SOC stock or concentration in the plough layer reported either for a single soil layer (e.g., 0-0.30 m or 0-0.2 m) or for multiple soil layers (e.g., 0-0.15 m and 0.15-0.30 m);
- 6. along with SOC concentration, the study reported bulk density (BD) measured separately for control and treatment;
- 7. at the end of experiment, SOC was recorded as means for treatment (CA) and control (conventional agriculture), with sample sizes and standard deviations (SD) or standard errors (SE), or statistical analysis references (e.g., P(F) or LSD value from the ANOVA table) to compute SD.

#### 2.1.3 Data extraction

The data extraction method is crucial to deal with the non-independence of the observations that can lead to underestimates of the standard error of the mean effect and therefore liberal evaluations of the statistical significance of effects (Gurevitch and Hedges, 1999; Nakagawa et al., 2017).

To avoid problems with non-independence of the effect sizes, only one pair comparison corresponding to the last sampling date was extracted from a study. If an article reported results from different experimental sites with different pedo-climatic characteristics, those sites were considered as independent studies and were included in the database. However, if several articles referred to the same experimental site with the same pedological characteristics, the article with the longest experimental duration was chosen.

Several articles treated factorial experiments, in which tillage treatments were studied in combination with different fertilization or cover crops. In the case of different fertilization levels, the second one from the top was chosen for both control and treatment. Legume cover crops were selected as a first choice when available.

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Data were extracted from tables and digitized from figures using WebPlotDigitizer software (Rohatgi, 2020). Standard errors were converted to standard deviations ( $se = \frac{SD}{\sqrt{n}}$  where n is the number of replicates) where necessary. When no measure of variability was provided, we extracted the SD from the ANOVA table using the EX-TRACT tool (Acutis et al., 2021; Acutis et al., 2022). This tool allows the estimation of the experimental error (i.e., standard deviation and standard error of treatments mean) associated to statistical analysis results of published articles (i.e., estimated from the LSD, P(F) values or even from the letters assignment indicating differences among means based on the results of a multiple comparison test).

#### 2.2 Database creation

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The final database used for this study consisted of 47 studies published in 41 articles in peerreviewed scientific journals (Table S1, Fig. S2). Entire database for meta-analysis is available in Zenodo (Database 1, <u>https://doi.org/10.5281/zenodo.7404592</u>).

No restrictions were set about the articles' publication date: those selected were published between 1998 and 2020. The final number of studies was only 5% of that obtained by searching for keywords.

Studies were located in North America (n=19), South America (n=9), Europe (n=10), Asia (n=8) and Africa (n=1) between 23° and 36° S and 19° to 45° N of latitude (Table S1, Fig. S2). The soils mainly belonged to the clay, loam and silt loam texture classes, and annual precipitation ranged from 331 to 1850 mm. The major climate sub-types were Cfa (n = 33) and Csa (n = 13), while only one study referred to Csb.

The soil management of the controls included different soil inversion techniques of which the fall/spring moldboard ploughing was the most frequent (71% of the studies). In all cases, control and treatments included nitrogen fertilization, weed control (without soil mechanical disturbance for the treatments) and no grazing. These main agronomic features were kept the same during the entire duration of experiments, ranging from 2 to 51 years. At least three different crops in the rotation of the treatment were reported in 11 studies, and monocropping in 9 studies. Four studies did not report any information on crop rotation.

Most of the studies (39) report the bulk density while the standard deviation was not always reported: 27 studies did not report SD or SE. For the remaining articles (20) no additional computation was required to obtain a measure of variability.

#### 2.3 Soil organic carbon computation

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The results for SOC changes in the plough layer for controls and treatments were reported as stock (Mg ha<sup>-1</sup> or kg m<sup>-2</sup>) in 37 studies or as concentration (g kg<sup>-1</sup> or %) and bulk density (BD) that were converted to stock in 10 studies. Studies that reported C concentrations with no measured BD were excluded from the final database. Moreover, to avoid false computation due to ignoring the differences in soil BD between treatments (Du et al., 2017; Toledo et al., 2013), only studies with BD measured separately for treatment and control were considered. SOC was reported for a single topsoil layer (e.g., 0-0.30 m or 0-0.2 m) in 29 studies and for multiple soil layers (e.g., 0-0.15 m and 0.15-0.30 m) in 18 studies (Database 2, https://doi.org/10.5281/zenodo.7404592).

Nowadays, to assess different agronomic practices, it is usually required to report SOC as mass per unit area (Mg ha<sup>-1</sup> or kg m<sup>-2</sup>) for a single soil layer (e.g., 0-0.3) and the associated standard deviation. Nevertheless, sometimes data were not directly reported as mass per unit area, or multiple layers were included. To deal with these kinds of data, we developed a specific methodology to compute a mean of SOC for a single soil layer and its SD from a product of two normally distributed variables (C concentration and BD, being correlated variables) or from a multiple correlated layer sum, when SOC results for several layers were reported (Tadiello et al., 2022). This method allows considering the correlation coefficient between C concentration and BD or/and between multiple sub-soil layers for a better estimation of the SD of the single stock soil layer. Within the countries considered in this study, the total CA area under the Cfa, Csa and Csb Köppen climate zones has been retrieved from Chen and Chen (2013). Then, within this area, the difference between the total agricultural land and the agricultural land under CA areas has been used to compute the potential increase of C sequestration if all the agricultural land were under CA. The complete dataset and statistical approach are reported in Table S2.

#### 2.4 Explanatory variables

To explain the variation in SOC stock due to the CA application in the plough layer, we included pedo-climatic and management-related explanatory variables (moderators) listed in Table 2.

Latitude and longitude were expressed as decimal degrees; latitude moderator was expressed as the absolute value (e.g.,  $30^{\circ}$  and  $-30^{\circ}$  refer to the same latitude, indicating an equal distance from the equator).

| Туре        | Moderator   | Group or range  |  |  |  |
|-------------|---|---|--|--|--|
| Climate     | Köppen classification   | Cfa, Csa, Csb   |  |  |  |
|             | Rainfall (mm year <sup>-1</sup> )                               | 331 - 1850  |  |  |  |
|             | Average annual temperature (°C)                                 | 14 - 25   |  |  |  |
| Geography   | Continent   | Asia, Africa, Europe, North<br>America, South America |  |  |  |
|             | Longitude (degree)  | -121 - 139  |  |  |  |
|             | Latitude (degree absolute value)                                | 19 - 45   |  |  |  |
|             | SOC stock under conventional agriculture (Mg ha <sup>-1</sup> ) | 18 - 102  |  |  |  |
|             | SOC stock under conventional agriculture (Mg ha <sup>-1</sup> ) | SOC $\leq 40$ , SOC $> 40^1$                          |  |  |  |
| Sail        | Clay (%)  | 7 - 76  |  |  |  |
| 5011        | Sand (%)  | 3 - 78  |  |  |  |
|             | pH (H <sub>2</sub> O)   | 4.5 - 8.5   |  |  |  |
|             | SOC/clay index  | 0.3 -7.1  |  |  |  |
|             | N fertilization level (kg N year <sup>-1</sup> )                | 0 - 390   |  |  |  |
| Aguanamia   | Experiment duration (years)                                     | 2 - 51  |  |  |  |
| Agronomic   | Number of crops in treatment rotation                           | <3,>=3  |  |  |  |
| management  | Proportion of crops with high residue biomass $(\%)^2$          | 0 - 100   |  |  |  |
|             | Legumes presence  | yes, no   |  |  |  |
| 1 1 7 4 4 1 |   |   |  |  |  |

Table 2. Categorical and continuous explanatory variables (moderators) included in the meta-analysis.

<sup>1</sup> based on Lugato et al. (2014); <sup>2</sup> grain maize, sorghum, cotton, rice.

If annual average annual rainfall or temperature were missing in a study, we used the value available from World Bank Group (World Bank Group, Climate change knowledge Portal, 2021). When only the textural class of the soil was available, we used the central values of clay and sand of the given textural class as continuous moderators. The pH was extracted from the

articles in 24 out of 47 articles. When not reported the World Soil Information Service WoSIS (https://www.isric.org/explore/wosis) was used to retrieve data. The SOC/clay index was computed between the SOC stock under control and the clay percentage. This kind of index has been widely adopted (as a ratio between SOC and clay concentrations in Proud et al, 2022; Proud et al., 2021) to describe soil physical properties such as bulk density, water retention characteristics or clay dispersibility rather than using the SOC or clay total contents (Dexter et al., 2008).

Based on the SOC in control ( $SOC_{ctrl}$ ) in the plough layer (0-0.3 m) measured at the end of the experiment, we created two different studies groups (i.e., " $SOC \le 40$  Mg ha<sup>-1</sup>" and "SOC > 40 Mg ha<sup>-1</sup>" groups). A single study (x) was then assigned to one of the groups following this formula:

$$\begin{array}{ll} if (SOC_{ctrl}/layer \, depth) * 0.3 \leq 40 \, Mg \, ha^{-1} & Then \ x \in "SOC \leq 40" \, group \\ if (SOC_{ctrl}/layer \, depth) * 0.3 > 40 \, Mg \, ha^{-1} & Then \ x \in "SOC > 40" \, group \\ \end{array}$$

where layer depth is expressed in meters.

The threshold was selected based on the paper by Lugato et al. (2014), who reported that, in the Mediterranean area, frequently the topsoil SOC stock values were below 40 Mg C ha<sup>-1</sup>. We included the number of crops in a treatment rotation as an explanatory variable, because the presence of at least three different crops is one of the three CA principles defined by FAO (2016). Moreover, we included the proportion of crops with high residue biomass (grain maize, sorghum, cotton, rice) in a rotation as an indicator of C input to the soils.

#### 2.5 Meta-analysis

Meta-analyses were conducted in R (R Core Team, 2018) with the "metafor" package (Viechtbauer, 2010, see Supplementary file 2 for the code used).

Quantitative meta-analysis involves calculating an effect size (i.e., the magnitude of the treatment effect) that can be averaged across independent studies. Since two experimental groups have been compared, the response ratio (r) was computed for the response variables as an index of the effect size:

$$r = \bar{X}_{CA} / \bar{X}_C \tag{1}$$

where  $\bar{X}_{CA}$  and  $\bar{X}_{C}$  represent the means for treatments (conservation agriculture) and for control (conventional agriculture), respectively, averaged for experimental replicates or samples. Since the distribution of *r* is skewed, performing statistical analyses in the metric of the natural logarithm of *r* is usually preferred due to its much more normal distribution in small samples than that of *r* (Hedges et al., 1999):

$$\ln(r) = \ln(\bar{X}_{CA}/\bar{X}_{C}) = \ln(\bar{X}_{CA}) - \ln(\bar{X}_{C})$$
[2]

We calculated the variance of  $\ln(r)$  as:

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$$V_{\ln(r)} = \frac{(SD_{CA})^2}{n_{CA}(\bar{X}_{CA})^2} + \frac{(SD_C)^2}{n_C(\bar{X}_C)^2}$$
[3]

where  $SD_{CA}$  and  $SD_{C}$  are the corresponding standard deviations, and *n* is the sample size.

We assumed that studies do not share the same effect sizes and consequently we used a random effects model to combine estimates across the studies. The application of this kind of model accounts for experimental method differences between studies (that are considered only a random sample of possible effect sizes) which may introduce variability ("heterogeneity",  $\tau^2$ ) among the true effects.

We calculated the weighted mean of the log response ratio for all studies as:

$$\overline{\ln(r)} = \frac{\sum_{i=1}^{n} w_i \ln r_i}{\sum_{i=1}^{n} w_i}$$
[4]

where  $lnr_i$  is the log response ratio for study *i*, *n* is the number of studies, and  $w_i$  is the weight for study *i*, defined as (Borenstein et al., 2009):

$$w_i = \frac{1}{V_i + \tau^2} \tag{5}$$

where  $V_i$  is the variance of the study *i* and  $\tau^2$  denotes the amount of residual heterogeneity (between-study variance). Because the variance of the effect sizes is a function of the sample size (Eq. 3), studies with a larger sample size had lower variances and received heavier weights.

The  $\tau^2$  parameter is considered the variance of the true effect size. Since is it not possible to compute it from the entire population of the effect size, the  $\tau^2$  is an estimation from the observed effect:

$$\tau^2 = \frac{(Q-df)}{C} \tag{6}$$

where

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$$Q = \sum_{i=1}^{k} w_i (Y_i - M)^2, df = n - 1, C = \sum w_i - \frac{\sum w_i^2}{\sum w_i}$$

where  $w_i$  is the study weight,  $Y_i$  is the study effect size, M is the summary effect, and n is the number of studies. The  $\tau^2$  coefficient is in the same metric (squared) as the effect size itself and reflects the absolute amount of variation in that scale (Borenstein et al., 2009). To describe the distribution of the effect size it is more useful to use its "standard deviation" measurement expressed as:

$$\tau = \sqrt{\tau^2} \tag{7}$$

that is on the same scale as the effect size itself but, while  $\tau^2$  is a squared value,  $\tau$  is not. The *rma* function has been used to compute the random model and the maximum-likelihood estimator ("ML") to estimate the amount of heterogeneity (Raudenbush, 2009). When a moderator was taken into account in the model to explain at least part of the total heterogeneity, a mixed-effect model was fitted. The Cochran's Q-test (Hedges and Olkin, 1985) was used to test the null hypothesis  $H_0: \tau^2 = 0$  that examines the between-group heterogeneity, while an omnibus test (that excludes the intercept  $\beta_0$ ) of all the model coefficients was conducted when moderators were included in the model (test of moderator, model (Q<sub>M</sub>) heterogeneities). Weighted meta-regressions were run to study the effect of continuous explanatory variables, with ln(*r*) as the dependent variable and the continuous variables as independent ones.

For the outliers' identification we used the backward search algorithm specifically developed for meta-analysis (Mavridis et al., 2017). Backward search algorithms start with the full data set and remove sequentially outlying observations until all outliers have been removed. This method can be useful when there are a few outlying studies (Mavridis et al., 2017).

Moreover, the descriptive  $l^2$  statistic (%) was reported for the overall effect size. This coefficient is useful to explain the estimated amount of heterogeneity as the inconsistency across the studies (Borenstein et al., 2009), and it is expressed as the ratio between the true heterogeneity and the total variance:

$$I^2 = \frac{\tau^2}{\tau^2 + V_Y} * 100$$
 [8]

where  $V_Y$  is the within study variance.

As an additional parameter, the metafor package also computed the  $R^2$  statistic (Raudenbush, 2009) as the ratio:

$$R^{2} = \frac{\hat{\tau}_{RE}^{2} + \hat{\tau}_{ME}^{2}}{\hat{\tau}_{RE}^{2}}$$
[9]

where  $\hat{\tau}_{RE}^2$  refers to the random model  $\tau^2$  (total amount of heterogeneity) while  $\hat{\tau}_{ME}^2$  to the estimated value of  $\tau^2$  based on the mixed-effect model. The  $R^2$  coefficient defines the amount of heterogeneity accounted for by the moderator inclusion in the model. This coefficient does

not take into account the within-study variance and, for this reason, it cannot be compared to the classical R<sup>2</sup> referred to *OLS (ordinary least square)* regression.

Results were back-transformed, except for meta-regression, and reported in the text and figures as percentage changes from the controls:

$$Response(\%) = [EXP(\ln(r)) - 1] * 100$$
[10]

The percentage difference between the control and the treatment is a straightforward way to show the increment/decrement of SOC due to the conservation agriculture technique.

The p-value and the 95%CI were used to identify the significant effect of continuous and categorical moderators, respectively (Hedges et al., 1999).

To detect possible publication bias in the meta-analysis we first used a graphical method based on two funnel plots (Nakagawa et al., 2021; Nakagawa et al., 2017; Sterne and Egger, 2001). The x-axis displays the  $\ln(r)$ , and the y-axis is the sample size and the standard error respectively in the two funnel plots. When the standard error (SE) was used as the vertical axis, it had the zero placed at the top (i.e., standard error 0 at the top). In this way, the largest studies have the smallest standard errors, and they are placed at the top of the graph. When present, the diagonal lines show the expected 95% confidence intervals around the summary effect. Moreover, we checked the funnel plots asymmetry with the Egger's regression test for the mixed-effects model reported by Viechtbauer (2010) and implemented in the "regtest" function.

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To assess the robustness of the observed effects the fail-safe number (Nfs) has been computed for both "SOC  $\leq$  40 Mg ha<sup>-1</sup>" and "SOC > 40 Mg ha<sup>-1</sup>" groups to estimates the number of nonsignificant, unpublished, or missing studies need to be added to a meta-analysis to change its results from significant to non-significant. Specifically, we used the Rosenthal method (Rosenthal, 1979) that estimates how many missing studies we would need to retrieve and incorporate in the analysis before the p-value became non-significant (Borenstein et al., 2009).

#### 3. Results

#### 3.1 Overall effect and SOC stock amount

For the entire database, the CA effects on SOC was highly variable, ranging from -9% to 99% compared to the controls (Median of 43 Mg C ha<sup>-1</sup>), with a weighted summarized effect of 12% (95% CI 8-17%, n = 47, Fig. 1). The summarized effect can be translated to an annual carbon stock increase of 0.48 Mg C ha<sup>-1</sup> yr<sup>-1</sup>, based on the weighted average duration of experiments of 11 years.

#### [FIGURE 1]

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Across 47 studies, the SOC stock under conventional agriculture (control) were highly variable, ranging between 18 and 102 Mg C ha<sup>-1</sup> in the plough layer. Unequal controls may cause a "noise" in meta-analysis, confounding the effects of explanatory variables, such as pedoclimatic factors and management practices. Therefore, we ran a weighted meta-regression between SOC stock and the response to CA. The meta-regression indicated that increasing SOC stock reduced linearly the response to CA, and an increase of 1 Mg ha<sup>-1</sup> in SOC stock was associated with a 0.22% decrease in the response ( $R^2 = 13.4$ ;  $Q_M = 5.98$ , P = 0.014, n = 47; Fig. 2). For example, when soils had 30 Mg C ha<sup>-1</sup> the application of CA increased the SOC amount by 16% ( $\ln(r) = 0.15$ ). However, on more fertile soils that had 60 Mg C ha<sup>-1</sup>, the SOC increase due to CA was only 9% ( $\ln(r) = 0.08$ ) and no response can be expected on soils reaching 100 Mg C ha<sup>-1</sup>.

[FIGURE 2]

To eliminate the effect of unequal controls, we subdivided the database into two groups with a threshold value of 40 Mg C ha<sup>-1</sup>. Compared to the controls, the weighted summarized effect was a 20% (12-28%, n = 22) of SOC increase in the "SOC  $\leq$  40 Mg ha<sup>-1</sup>" group, and only a 7% (95% CI 3-11%, n = 25) in the "SOC  $\geq$  40 Mg ha<sup>-1</sup>" group (Fig. 1). However, the first group had a larger variability of responses ( $\tau$  = 0.14), compared to the latter ( $\tau$  = 0.08).

Since within each group no associations between SOC stock and the response to CA were found (P = 0.93 and P = 0.70 for the "SOC  $\leq 40$  Mg ha<sup>-1</sup>" and "SOC > 40 Mg ha<sup>-1</sup>" groups, respectively), this allowed us to study the effects of pedo-climatic factors and management practices within each group.

#### 3.2 The source of variation across studies

The effects of five different moderators were significant in at least one of the two SOC stock groups (Table 3). The  $R^2$  coefficient varied significantly from one moderator to another, indicating that the SOC response variability is strictly dependent on specific moderators. For instance, within the continuous moderator group the largest  $R^2$  was recorded for rainfall (43%) and latitude (39%), while the smallest was the pH (1%).

| Variable   | Explanatory variables                                      | SOC ≤ 40 Mg ha <sup>-1</sup> |                    |        |            |        |                        | SOC > 40 Mg ha <sup>-1</sup> |                    |        |                         |        |                        |
|--|--|------------------------------|--------------------|--------|------------|--------|------------------------|------------------------------|--------------------|--------|-------------------------|--------|------------------------|
| category   |  | n                            | R <sup>2</sup> (%) | р (Qм) | df<br>(QM) | Figure | Outliers<br>(study ID) | n                            | R <sup>2</sup> (%) | р (Qм) | df<br>(Q <sub>M</sub> ) | Figure | Outliers<br>(study ID) |
| AGRONOMIC PEDO-CLIMATIC AND<br>MANAGEMENT GEOGRAPHICAL | Clay (%)   | 21                           | 0.00               | 0.980  | 1          | 3A     | -                      | 23                           | 16.35              | 0.047  | 1                       | 3A     | 14                     |
|  | Sand (%)   | 21                           | 0.00               | 0.829  | 1          | -      | -                      | 24                           | 4.86               | 0.312  | 1                       | -      | -                      |
|  | SOC/clay index   | 21                           | 5.76               | 0.452  | 1          | 3B     | -                      | 23                           | 29.35              | 0.006  | 1                       | 3B     | 14                     |
|  | pH (H <sub>2</sub> O)                                      | 22                           | 1.03               | 0.776  | 1          | 3C     | 29, 42                 | 22                           | 37.32              | 0.021  | 1                       | 3C     | 10, 46                 |
|  | Rainfall (mm year-1)                                       | 21                           | 43.07              | 0.007  | 1          | 4A     | 42                     | 23                           | 33.28              | 0.021  | 1                       | 4A     | 10, 40                 |
|  | Temperature  | 22                           | 6.28               | 0.092  | 1          | -      | -                      | 25                           | 3.10               | 0.431  | 1                       | -      | -                      |
|  | Latitude<br>(degree absolute value)                        | 21                           | 24.91              | 0.018  | 1          | 4B     | 29                     | 23                           | 39.31              | 0.007  | 1                       | 4B     | 10, 40                 |
|  | Longitude (degree)   | 22                           | 5.32               | 0.310  | 1          | -      | -                      | 25                           | 0.01               | 0.991  | 1                       | -      | -                      |
|  | Continent  | 22                           | 3.28               | 0.839  | 3          | 5A     | -                      | 24                           | 4.88               | 0.807  | 3                       | 5B     | -                      |
|  | Köppen climate   | 21                           | 0.00               | 0.75   | 1          | 5C     | -                      | 25                           | 0.63               | 0.793  | 1                       | 5D     | -                      |
|  | Experiment duration (years)                                | 22                           | 17.76              | 0.020  | 1          | 6A     |                        | 24                           | 17.46              | 0.047  | 1                       | 6A     | 47                     |
|  | Proportion of crops with high residue biomass (%)          | 19                           | 35.06              | 0.013  | 1          | 6B     | 16, 42                 | 22                           | 0.00               | 0.999  | 1                       | 6B     | -                      |
|  | Number of crops in rotation (treatment)                    | 22                           | 8.25               | 0.175  | 1          | 5E     | -                      | 25                           | 0.56               | 0.746  | 1                       | 5F     | -                      |
|  | Nitrogen fertilization<br>level (kg N year <sup>-1</sup> ) | 12                           | 4.45               | 0.476  | 1          | -      | -                      | 15                           | 4.13               | 0.411  | 1                       | -      | -                      |
|  | Legumes presence   | 22                           | 4.61               | 0.3621 | 1          | 5G     | -                      | 25                           | 1.03               | 0.7646 | 1                       | 5H     | -                      |

**Table 3**. The effect of pedo-climatic and geographical factors and management practices on SOC changes due to conservation agriculture in soils with SOC  $\leq$  40 Mg ha<sup>-1</sup> and > 40 Mg ha<sup>-1</sup> under conventional agriculture

"n" is the number of studies; " $R^2$ " estimates the amount of heterogeneity accounted for by the moderators included in the model; " $p(Q_M)$ " is the P-value of the heterogeneity test; " $df(Q_M)$ " are the degrees of freedom of the residual heterogeneity test. P-values highlighted in bold are significant (P < 0.05). Outliers was identified by backward search algorithm (Mavridis et al., 2017).

#### 3.2.1 Pedo-climatic and geographical factors

In the "SOC  $\leq$  40 Mg ha<sup>-1</sup>" group, a change in clay or sand percentage (within the range of this study) did not lead to a different response to the CA adoption, while in the "SOC > 40 Mg ha<sup>-1</sup>" group an increased clay content was positively correlated with the magnitude of the response (Table 3 and Fig. 3A). For example, at 60% of clay the SOC increase due to CA adoption was 12% (ln(*r*) = 0.10) compared to conventional agriculture, while no response was found (ln(*r*) = 0.0) at 8% of clay. The results obtained with the clay moderator reflected what found considering the SOC/clay index (Table 3 and Fig. 3B). In fact, if the "SOC  $\leq$  40 Mg ha<sup>-1</sup>" group did not report a significant regression, while in the "SOC > 40 Mg ha<sup>-1</sup>" group the result was significant. The lower the SOC/clay index the higher the CA impact on SOC compared to conventional agriculture. The pH was also considered in the analysis even though just the "SOC > 40 Mg ha<sup>-1</sup>" group was significant (Fig. 3C). The greatest effect size was found with the lowest pH value (around 5), while the impact of CA becomes null at pH equal to 7.3.

#### [FIGURE 3]

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The meta-regressions indicated that rainfall was an important factor governing the response to CA in both groups ( $R^2 = 33\%$  and 43%, Table 3). The relationship was negative in the "SOC  $\leq 40 \text{ Mg ha}^{-1}$ " group, and positive in the "SOC  $> 40 \text{ Mg ha}^{-1}$ " group (Fig. 4A). In both groups, an increase of 100 mm of rainfall was associated with a 1% change in the SOC response to CA. The temperature cannot explain the groups variability (P > 0.05, Table 3), with a  $R^2$  always lower than 10%.

Table 3 shows that the effect of CA strongly depended on geographical locations as indicated by the large proportion of heterogeneity (25-39%) accounted for by latitude (degrees absolute value). In "SOC  $\leq$  40 Mg ha<sup>-1</sup>" group, with increasing latitude the impact of CA sharply increased, whereas, in "SOC > 40 Mg ha<sup>-1</sup>" group, CA had an opposite trend (Fig. 4B).

#### [FIGURE 4]

The SOC responses to CA did not reveal any differences across continents (Fig. 5A/B). However, the number of studies in South America and in Asia for the "SOC  $\leq$  40 Mg ha<sup>-1</sup>" group and in Europe for the "SOC > 40 Mg ha<sup>-1</sup>" group was limited, and thus, the 95% CIs were large and overlapping.

No differences in the effect of CA adoption were found between Csa and Cfa Köppen climate categories, since the 95% CI were large and overlapping (Table 3; Fig. 5C/D). The Csb climate was excluded from the analysis since only one study was found.

#### [FIGURE 5]

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#### 3.2.2 Agronomic management practices

In most of the studies, the duration of experiments ranged from 2 to 30 years. Fig. 6A displays the significant positive relationship between experiment duration and SOC response due to CA adoption in both SOC stock groups (Table 3). The regression slope was greater in the "SOC  $\leq$  40 Mg ha<sup>-1</sup>" group; in particular, after ten years of CA implementation, the percentage change from conventional agriculture was 20% in the "SOC  $\leq$  40 Mg ha<sup>-1</sup>" group (ln(*r*) = 0.17), and 5% in the "SOC > 40 Mg ha<sup>-1</sup>" group (ln(*r*) = 0.04).

The proportion of crops with high residue biomass in a rotation significantly affected C sequestration under CA only in the "SOC  $\leq$  40 Mg ha<sup>-1</sup>" group (Table 3 and Fig. 6B). If all the crops in a rotation produced a large amount of residue, the SOC increase was about 29% (ln(*r*)

= 0.25). In a rotation in which only half of the crops left a high amount of residue on the soil, the SOC increase due to CA adoption was about 20% ( $\ln(r) = 0.18$ ) compared to conventional agriculture. In the "SOC > 40 Mg ha<sup>-1</sup>" group, the amount of residues did not modify the SOC response to CA (Table 3, Fig. 6B).

#### [FIGURE 6]

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In both groups, the introduction of three or more crops in a treatment rotation (Fig. 5E/F), the presence of legumes in the rotation (Fig.5G/H) and the nitrogen fertilization level did not have a significant impact (Table 3).

#### 3.3 Publication bias

Although the funnel plots indicated some asymmetry, the Egger's regression test did not indicate a significant asymmetry (P = 0.64) when the sample size is present on the y-axis (Fig. S3A). When the SE appeared in the y-axis (Fig. S3B), some points fell outside the 95% CI, but the regression test still confirmed a non-significant asymmetry (P = 0.051). We concluded that our research does not suffer from publication bias.

In addition, the fail-safe number indicated that the results are robust for both SOC groups. In fact, the fail-safe number is 280 and 62 for the "SOC  $\leq$  40 Mg ha<sup>-1</sup>" and "SOC > 40 Mg ha<sup>-1</sup>" groups respectively, suggesting that there would need to be a consistent number of studies for each group before the cumulative effect would become statistically non-significant (Supplementary file 1).

#### 4. Discussion

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This meta-analysis summarizes the results of 47 studies published over a period of 20 years on the effects of CA practices on SOC sequestration in the plough layer under the Mediterranean and humid subtropical climates in five continents. Since our database included numerous different crops (n = 23) and rotations (n = 31) this allowed us to explore different agronomic conditions (Database 1, https://doi.org/10.5281/zenodo.7404592). Previous meta-analysis on similar topic and climatic conditions summarized 33 studies on herbaceous crops (Aguilera et al., 2013). Other meta-analyses reached greater number of studies including deeper soil layers (Luo et al., 2010, Mondal et al., 2020) or they were conducted on a global scale (e.g., Li et al., 2010, Sun et al., 2020, West and Post, 2002). Although our initial database was larger, many studies were not included to the database due to (1) the variability of management practices (e.g., minimum tillage in place of no-till, agronomic management change during the experiment's duration), or cover crop inclusion in control treatments; (2) incomplete and poor reporting of the results, such as missing means, SDs or sample sizes for controls and treatments; (3) utilization of pedotransfer functions or lack of reported bulk density. Despite such constrains, the number of studies included in the final database is comparable with other similar meta-analyses (Table 1). This number was sufficient to perform a robust, weighted metaanalysis to calculate a summarized effect size across the studies, as well as the means for the different categories of explanatory variables, and to determine the CIs around the means.

#### 4.1 Overall effect and the role of SOC stock

This meta-analysis clearly indicates that under Mediterranean and humid subtropical climates, CA adoption had an overall positive effect on SOC sequestration amounting to an overall mean increase of 12% in the plough layer compared to conventional agriculture. This result can also be translated as an annual C increase of about 0.48 Mg C ha<sup>-1</sup> (Table S2). This annual C increase

has been scaled across the 12 countries included in the present study, considering the agricultural area (under Cfa, Csa, and Csb) where CA can be potentially adopted. The result indicated that, in this area, 0.15 Pg C yr<sup>-1</sup> can be stored in the first 30 cm layer due to the CA adoption. This result shows how the recent literature, combined with the summarized result of the meta-analysis, can quantify the real impact of the CA adoption.

This meta-analysis also gives the first evidence that the magnitude of SOC gain due to CA adoption was strongly influenced by the SOC stock under conventional agriculture. In soils with SOC  $\leq$  40 Mg C ha<sup>-1</sup>, the impact of CA adoption was three times larger (20%, 95% CI 12-28%, n = 22) than in soils which stored the higher SOC stock (7%, 95% CI 3-11%, n = 25). In our database, the SOC stock under conventional agriculture varied from 18 to 102 Mg C ha<sup>-1</sup> in the plough layer, which includes the average value of 63.5 Mg ha<sup>-1</sup> reported by FAO (2020) for the warm temperate climate. The evidence of distinct responses between the two SOC stock groups (Fig. 2) became important to better understand the variability of CA impacts across Mediterranean and humid subtropical climates. As a rule, the carbon content achieved under conventional agriculture must be considered to estimate whether SOC sequestration rates have been found to be greater in soils that are far from their potential steady state (Tiefenbacher et al., 2021). On the contrary, when SOC is already close reaching to a steady state, the SOC gains are lower (Corsi, 2012; Kämpf et al., 2016).

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Previous meta-analyses on this topic which summarized studies in Mediterranean (Aguilera et al., 2013) and temperate climates (Ogle et al., 2005) did not explicitly consider the SOC stock under conventional tillage as a moderator: however, they demonstrated a 17-18% of SOC increase as an overall effect, which is somewhat larger than we found in this meta-analysis (12%) for the entire database. These larger responses very likely stem from the use of weighting by sample size or no weighting. In this meta-analysis however, the weighting by the inverse of

During the last two decades, no-till management has also been recommended as a practice to mitigate greenhouse gas emissions through soil C sequestration (Ogle et al., 2012) and lower fuel consumption (Aguilera et al., 2013). The present meta-analysis defines that, under Mediterranean and humid subtropical climates, CA produce positive carbon sequestration regardless of the initial carbon content (i.e., positive effect in both SOC groups). This finding confirms that CA must be considered by the lawmakers to mitigate greenhouse gas emissions. In addition, Powlson et al. (2016) suggested to evaluate the SOC increase considering the management practices involved (e.g., crop rotation, residue management, soil characteristic).

#### 4.2 Source of variation across studies

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The final distribution of the effects size (Fig. 1) describes a wide range of SOC change in response to conservation agriculture (from -9% to 99% change from the control). The heterogeneity of the effect size, as quantified by the high  $I^2$  value (94.6%, 95% CI 92.4-97.2%, n=47) justifies the random model utilization (since the different studies do not share a common effect size). Similar  $I^2$  value was reported by other agronomic (Kim et al., 2020; Tremblay et al., 2012; Zhao et al., 2020) or ecological meta-analyses (Senior et al., 2016). The large  $I^2$  allows us to investigate reasons for the variability, applying subgroup analysis or meta-regression (Nakagawa et al., 2017).

Our results proved that splitting the database up into two groups based on the amount of SOC under conventional agriculture (i.e., the "SOC  $\leq$  40 Mg ha<sup>-1</sup>" and "SOC > 40 Mg ha<sup>-1</sup>") allowed us to detect contrasting effects of the pedoclimatic and management factors on SOC sequestration. In fact, the analysis of the whole database would have masked the moderators'

impacts on SOC sequestration, since the effect of the two groups would have been averaged. Conversely, with this approach we can give agronomic explanations of the moderator impact, separately for the soils with different SOC stocks under conventional agriculture.

#### 4.2.1 Pedo-climatic and geographical factors

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Our results suggest a significant positive effect of CA due to the clay percentage only in soils with SOC > 40 Mg ha<sup>-1</sup> (Fig. 3A). Several authors acknowledge the positive effect of the clay percentage on the SOC adsorption by the mineral fraction and the resulting SOC accumulation (Du et al., 2017; Haddaway et al., 2017; Xu et al., 2016). This is related to the fact that clay soils exhibit strong aggregate formation and stability that prevent SOM decomposition (Lorenz and Lal, 2018). Since C sequestration is constrained mainly by the availability of reactive surfaces (Churchman et al., 2020), high C amount (i.e., "SOC > 40 Mg ha<sup>-1</sup>" group) still leads to increase in SOC response, if supported by greater clay content in the soil (Fig. 3A). In contrast, in the soils with SOC  $\leq$  40 Mg ha<sup>-1</sup> all the C available is likely to be adsorbed by the clay minerals, making this factor irrelevant for further SOC increase. It is interesting to note that this finding is confirmed by the SOC/clay index: is soil with already high SOC availability, the CA implementation does not automatically lead to a greater SOC sequestration. An optimal combination of SOC and clay has to be match in order to get a positive effect on the SOC sequestration (Fig. 3B). In our study, we identify a maximum SOC/clay index threshold of 3.2: once this value is overcome the CA adoption became useless to increase SOC sequestration (i.e., the effect size became zero). This finding indirectly confirms that soils with poor clay content are likely to be closer to the soil saturation limit, while soil with greater clay content are more likely to have a higher SOC sequestration potential.

The worth of mention is the role of pH. In the "SOC  $\leq$  40 Mg ha<sup>-1</sup>" group, the differences in pH did not lead to a greater impact on carbon sequestration due to CA, probably because

oscillations in the pH values does not trigger a greater mineralization when the organic matter is too low and barely available for the microbes. On the contrary, when the SOC stock is greater ("SOC > 40 Mg ha<sup>-1</sup>" group), a not favorable pH values for the mineralization process (pH < 5, Aciego and Brookes, 2008), leads to lower OM decomposition (Bot and Benites, 2005) thus a higher SOC sequestration (i.e., greater effect size, Fig. 3C).

In our study, the amount of rainfall showed a significant effect on SOC sequestration due to CA in both groups (Table 3, Fig. 4A), while some previous meta-analyses failed to detect the rainfall effect (Du et al., 2017; Luo et al, 2010). Sun et al. (2020) indicated that higher SOC gain with the CA adoption is expected with a decrement of the humidity index (ratio of annual mean precipitation to mean temperature). It is likely that, in our meta-analysis, the enhanced soil water retention due to CA practices (Lal, 2020) occurred in soils with SOC  $\leq$  40 Mg C ha<sup>-1</sup>, which gave a visible advantage only in dry conditions (the left side of the regression shown in Fig. 4A), while in geographical areas where water is not limited, C sequestration improvement had only a small increment. Another possible explanation for the good CA performance in C depleted areas is associated with the irrigation technique: in some agricultural regions the irrigation counterbalances the negative effect of scant precipitation on carbon sequestration (Lorenz and Lal, 2018). In contrast, we found a positive trend in the soil with SOC > 40 Mg ha<sup>-1</sup>, although the low slope indicated a weak impact of rainfall on CA effect. This finding agrees with the result by Post et al. (1982), who linked a high rainfall regime with SOC accumulation in soils.

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Our meta-regressions clearly indicate that the geographical location of an experiment determined to what extent CA influenced SOC sequestration. Moving from the lower latitudes towards the middle latitudes suggests an increasing advantage of CA in soils with initial SOC lower than 40 Mg ha<sup>-1</sup>, as indicated by the positive meta-regression in the "SOC  $\leq$  40 Mg ha<sup>-1</sup>" group (Fig. 4B). In this group, CA showed a small positive contribution at low latitude where

the high temperature hastens the mineralization of the limited SOC stock. Conversely, moving to middle latitudes with lower temperatures, the CA effect increased, probably, due to slower mineralization. From the agronomic point of view, conservation agriculture practices are not enough to increase C sequestration in conditions with low carbon content and a warm climate (i.e., low latitude absolute value). In soils with SOC > 40 Mg ha<sup>-1</sup>, we found an opposite trend (Fig. 4B). This suggests that the higher effect size is found at low latitudes which are characterized by high temperature, where SOC stock is not a limiting driver, and it can be mineralized without decreasing the SOC stock accumulation in soil. Conversely, with higher latitudes, probably the introduction of CA practices in soil with an already high SOC stock is not enough to lead to an increment in SOC stock accumulation. However, no significant effect of temperature was found for both SOC groups (Table 3), indicating that even if temperature is certainly a driver of the SOC mineralization, other factors can influence the SOC accumulation. Therefore, these findings highlight the fact that conservation agriculture is not a "standardized solution" to explain the carbon accumulation problems in agricultural soils, and the benefits of its application should be evaluated by considering other agronomic and climatic variables.

The peculiar behaviors of the "SOC  $\leq$  40 Mg ha<sup>-1</sup>" and "SOC > 40 Mg ha<sup>-1</sup>" groups provide a novel contribution about the CA effect on SOC sequestration at different latitudes, due to the lack of previous findings related to this topic or for results limited to specific soil layers. For example, Haddaway et al., (2017), who studied SOC response regardless of the C stock in conventional tillage (control), found that latitude was positively correlated to C stocks' mean differences in full profile C stocks.

Other geographical moderators were not useful to explain heterogeneity of the effect sizes across the studies. In fact, the continent moderator had no impact to explain the heterogeneity, probably, because the studies from different continents had the same climate conditions (i.e.,

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Cfa, Csa). Therefore, in the present meta-analysis, the evidence that Cfa and Csa subgroups of the Köppen climate classification did not show significant differences could be partially explained by the similar average temperature and rainfall during the year. Ogle et al. (2005) confirmed that large differences occur with contrasting climates, finding that no-till implementation led to the largest increases in SOC storage under tropical moist conditions and the smallest under temperate dry conditions.

#### 4.2.2 Agronomic management

Our results support the results of many other meta-analyses on the same topic (Angers and Eriksen-Hamel, 2008; Haddaway et al., 2017), which reported that experiment duration positively influences SOC accumulation in soil (Table 1). Moreover, our results indicate that in soils with SOC content  $\leq$  40 Mg ha<sup>-1</sup> there was a quick temporal response to CA (i.e., a greater response starting from the beginning of the CA implementation). In addition, the SOC stock accumulation in soils with low SOC content throughout the years was faster, as confirmed by the higher regression slope compared to that of the "SOC > 40 Mg ha<sup>-1</sup>" group.

Another critical aspect related to SOC sequestration is the crop residues management. Differences in the amounts of yields and, thus, crop residues, directly influence the amount of C inputs to the soil (Meurer et al., 2018; Poeplau and Don, 2015). On the other hand, when with ploughed soil the residues are expected to be in contact with deeper soil layers, CA management leaves them on the soil surface. In the latter case, CA leads to positive effects, such as soil temperature control, the limitation of soil erosion and the reduction of soil water evaporation, which are all associated with the reduction of SOC decomposition in soil (Duiker and Lal, 2000; Luo et al., 2010). In literature, the SOC stock in CA is known to positively respond to crop residues retention, as supported by the meta-analysis by Virto et al. (2012), who found a significant (P = 0.001, n = 35) relation between SOC accumulation in 0-0.3 m soil

depth and organic input (i.e., crop residue), considering NT (with residues) as a treatment and inversion tillage as a control. Our result supported this previous finding, but with a positive relationship limited to soils with scarce SOC stock. In fact, we found out that increasing the proportion of crops with high residue production in the rotation results in a SOC increase only in soils with SOC  $\leq$  40 Mg ha<sup>-1</sup>, reaching 29% change from the control when all the crops in the rotation produce high residue amounts.

Even if the positive relationship between SOC sequestration and the amount of crop residues retained on the soil has been highlighted in different studies, the different quality of the crop residue also plays a role in the C stock accumulation. For example, in the meta-analysis by Sun et al. (2020), they found that in most Mediterranean and temperate climates, SOC sequestration increased when crop residue retention and crop rotation are applied together. The number of crops in the rotation indeed plays a role in SOC accumulation, since monoculture produces the worst quality and quantity of dry matter (Copeland and Crookston, 1992). The study by González-Sánchez et al. (2012) demonstrated that, in general, the higher C soil fixation values were found in soils in which crops were rotated, with on average C sequestration rate 19% higher in the case of crop rotation and NT rather than monoculture. In the present meta-analysis, however, the number of crops in the CA treatment rotation did not significantly impact the SOC accumulation in the plough layer (Table 3). This result is likely due to the unbalanced number of studies between the two levels considered (i.e., rotations with three or more crops or with less than three, Fig. 5 E/F).

#### 4.3 Perspectives

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The current carbon stock data availability under our studies selection criteria did not allow us to obtain enough studies to consider the deeper layers (> 0.3 m depth). However, other authors highlighted the importance of also engaging the deeper layers for a complete evaluation of the

soil carbon storage (Kopittke et al., 2017; Meurer et al., 2018; Piccoli et al., 2016). Sun et al. (2020) noted that, for the cases when SOC under no-till relative to conventional tillage increased in the top 0.3 m, the 0.3-0.6 m layer was also likely to increase its SOC. However, Du et al. (2017) reported that no-till management showed slightly lower SOC storage rates against conventional till in the subsoil layers (> 0.4 m).

Further research synthesis should address the SOC response to CA in the deeper soil layers, focus on specific climatic zones and different management practices. In this sense, the present work is useful since a reliable and replicable procedure is clearly presented.

Lastly, we should note that within this topic, the information regarding irrigation was rarely reported. The final low number of studies handling irrigation did not allow us to include this moderator in the meta-analysis.

## 5. Conclusions

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The present meta-analysis evaluated the SOC response to CA practices in Mediterranean and humid subtropical climates. Limiting the analysis to specific climates offered the possibility to detect more precisely the effects of pedo-climatic and management practices, which otherwise would have been masked. Therefore, we have provided a novel contribution to understanding the actual impact of CA in the SOC stock accumulation.

The meta-analysis showed an overall positive effect of CA on SOC sequestration (12%). Scaling this result across the countries and the specific climates considered in the present study, 0.15 Pg C yr<sup>-1</sup> can be stored in the first 30 cm layer due to the CA adoption. By dividing the whole database into two separate groups based on the SOC stock (with 40 Mg C ha<sup>-1</sup> as the threshold) under conventional agriculture allowed us to better explain the variability of SOC responses to CA management. This meta-analysis highlighted that, under the climates

The effect of CA on SOC accumulation depended on clay content solely in soils with more than 40 Mg C ha<sup>-1</sup> under conventional agriculture, while it was not relevant in soils with less than 40 Mg C ha<sup>-1</sup>. This result was confirmed by the SOC/clay index analysis the revealed that to get a positive impact of CA on SOC sequestration a specific range of the SOC/clay index is required. In both soil groups, experiment duration positively impacted SOC sequestration, with a greater effect found in the soils with SOC  $\leq$  40 Mg C ha<sup>-1</sup>. In addition, in these soils, the retention of crop residues enhanced the CA positive contribution.

We conclude that in Mediterranean and humid subtropical climates, the most benefits from CA application in terms of SOC increase apply to agricultural soils with SOC content  $\leq 40$  C Mg ha<sup>-1</sup> and located in the middle latitudes and/or in dry areas. With a base annual increment of 0.48 Mg C ha<sup>-1</sup> year<sup>-1</sup>, we support the idea that a reasonable carbon gain can be enhanced with a long CA application. For instance, to get a reasonable 20% more carbon stock, it is required ten years of CA application with an initial carbon stock  $\leq 40$  Mg ha<sup>-1</sup>, while more than 30 years are required if the soil already has more than 40 C Mg ha<sup>-1</sup>. During this period, it is recommended at least to apply to continue no-tillage management, retain residues on the top of the soil (chopped or not), and include as many crops as possible in the rotation.

# Figures Figure 1. (TO BE PRINTED B&W)



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## Figure 2.

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## Figure 4.



#### Figure 5.

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## Figure 6.

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Figure 1. Forest plot showing the results of 47 studies examining the effect of conservation agriculture on SOC sequestration. The diamonds are centred on the summary effect, which was estimated for the "SOC  $\leq$  40 Mg ha<sup>-1</sup>" and "SOC > 40 Mg ha<sup>-1</sup>" groups separately. The overall effect diamond (n = 47) is also displayed at the bottom of the plot. Lateral tips of diamonds represent the 95% confidence intervals. Numbers in the right-hand column are summary effect estimates for each study [lower 95% CI, upper 95% CI]. Dotted vertical line indicates conventional agriculture (control).

Figure 2. Weighted meta-regression between SOC changes due to CA expressed as  $\ln(r)$  and the SOC stock under conventional agriculture (control) for the entire database. Point size represents study weight in the analysis as expressed in the eq. [5]. The dotted line represents the control. For back-transformation of  $\ln(r)$ , see Eq.10.

Figure 3. Weighted meta-regressions between SOC changes due to conservation agriculture expressed as  $\ln(r)$  and clay (A), SOC/clay index (B) and pH (C) in soils with SOC  $\leq$  40 Mg ha<sup>-1</sup> and SOC > 40 Mg ha<sup>-1</sup> under conventional agriculture. The point size represents the study weight in the analysis as expressed in eq. [5]. Statistics for meta-regressions and ID of studies identified as outliers (crosses) appear in Table 3. For back-transformation of  $\ln(r)$ , see Eq.10.

Figure 4. Weighted meta-regressions between SOC changes due to conservation agriculture expressed as  $\ln(r)$  and rainfall (A) and latitude (B) in soils with SOC  $\leq$  40 Mg ha<sup>-1</sup> and SOC > 40 Mg ha<sup>-1</sup> under conventional agriculture. The point size represents the study weight in the

analysis as expressed in eq. [5]. Statistics for meta-regressions and ID of studies identified as outliers (crosses) appear in Table 3. For back-transformation of  $\ln(r)$ , see Eq.10.

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Figure 5. SOC percentage changes due to conservation agriculture as effected by continents (A and B), Köppen climate groups (C and D), number of crops in treatment rotation (E and F) and the legume presence in the treatment rotation (G and H) in soils with SOC  $\leq$  40 Mg ha<sup>-1</sup> and SOC > 40 Mg ha<sup>-1</sup> under conventional agriculture. The symbols indicate weighted average with 95% confidence intervals (CIs) and "n" represents the number of studies. Numbers in the right columns are summary effect estimates [lower 95% CI, upper 95% CI]. The dashed line indicates conventional agriculture (control). The effect of conservation agriculture on SOC was considered significantly different from the control if the 95% CIs do not overlap with zero, and significantly different between the groups of explanatory variables if their 95% CIs do not overlap. Groups with only one study were excluded from the analysis.

Figure 6. Weighted meta-regressions between SOC changes due to conservation agriculture expressed as  $\ln(r)$  and experiment duration (A) and proportion of crop with high residue biomass (B) in soils with SOC  $\leq 40$  Mg ha<sup>-1</sup> and SOC > 40 Mg ha<sup>-1</sup> under conventional agriculture. The point size represents the study weight in the analysis as expressed in eq. [5]. Statistics for meta-regressions and ID of studies identified as outliers (crosses) appear in Table 3. For back-transformation of  $\ln(r)$ , see Eq.10.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Data availability statement

Databases are free to download in Zenodo (<u>https://doi.org/10.5281/zenodo.7404592</u>): Database 1. Meta-analysis database;

Database 2. Original raw data (soil organic carbon content and/or stock, bulk density) for each soil layer as reported in the articles, and SOC stock computation for a single soil layer (0-0.3 m)

## Supplementary material

1. Supplementary file 1.

Figure S1. Flow diagram of literature search and screening

Figure S2. Location of studies included in the meta-analysis

Figure S3. Funnel plots of the meta-analysis as the measure of uncertainty

Table S1. Studies included in the meta-analysis

Table S2. Dataset used to compute the C sequestration increase due to conservation agriculture Dataset used to compute the C sequestration increase due to conservation agriculture (CA) utilization across 12 countries included in the present study. Query for articles searching; Reference list of the articles included in the database; Failsafe number computation

2. Supplementary file 2: R codes used for the analyses.

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