



Use of soil respiration measurements and RothC modelling show effects of catch crops and precision and traditional agriculture on productivity and soil organic carbon dynamics in a 5 year study in Mediterranean climate

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10 **Abstract.** Finding agricultural managements able to increase soil organic carbon without a reduction in crop yields is important to: decrease soil erosion, protect soil ecosystem services, increase soil health, help to curb net CO₂ emissions toward the EU goal of carbon neutrality. Various studies have shown that catch crops, when managed in the proper way, may result in an increase in soil carbon stocks; however, recent studies have cast doubts on those findings, due to short study duration (3 years or less), few data points, and catch crops mismanagement. Model studies to estimate the potentials of catch crops for soil
15 carbon sequestration shown mixed results; however, in these studies, only the direct effects of catch crops (i.e. the input of carbon from crop inclusion in the soil) was accounted for. Here, we show the result of a study to compare two crop managements: traditional against catch crop together with precision agriculture. We measured agricultural productivity, soil organic carbon, soil respiration, and soil conditions in two different sites in Italy for a period of 4+ years, then we modelled the field managements using a modified version of RothC model, to account for both direct and indirect catch crop effects on
20 soil. The results show that catch crops and precision agriculture can result in an increase in soil organic carbon, with no effects, or, in some cases, an increase in crop production.

1 Introduction

Soils in Mediterranean climates are often depleted in soil organic carbon (SOC; de Brogniez et al., 2015); this can be seen as an opportunity for the sequestration of carbon into the soil using focused agricultural practices (Dimassi et al., 2014). Soil is
25 both a sink and a source of CO₂ due to anthropogenic and natural drivers (Committee on the Environment, Public Health and Food Safety, 2022); turning the soils into net sinks of CO₂ is fundamental to reach carbon neutrality and maintain the global temperature rise within 1.5 °C (Ipcc, 2022). Carbon enters the soil as organic matter and is then mineralized by the soil microorganisms (Lehmann & Kleber, 2015); SOC can thus be increased by increasing the input of organic matter and/or decreasing the rate of mineralization (Lal, 2004). Every agricultural practice aimed at increasing SOC should study both soil



30 inputs and outputs: some studies show that increasing the input of organic matter to the soil, for example, can result in a net increase in soil CO₂ emissions (C. Liu et al., 2014; Wang et al., 2019). Moreover, regenerative agricultural practices proposed for increasing SOC stocks should also demonstrate that they do not impact agricultural yields, in order to avoid increases in food prices (Bai & Cotrufo, 2022) or indirect land use changes (Balugani et al., 2022; Jones & Albanito, 2020).

Of the various agricultural practices aimed at increasing soil carbon, the use of catch crops and precision agriculture together
35 looks particularly promising (Schreefel et al., 2020). Precision agriculture is the application of technologies and principles to manage spatial and temporal variability associated with all aspects of agricultural production for the purpose of improving crop performance and environmental quality (Pierce & Nowak, 1999). Catch crops are crops that are cultivated between crops either spatially (crops cultivated in the inter-rows of other crops, Couëdel et al., 2018, also called relay cropping, Lamichhane et al., 2023) or temporally (after harvesting a crop and before planting the new one, also referred as cover crops; Vogeler et al., 2022). Catch crops can be incorporated into the soil instead of harvested, in order to provide nutrients (N, P, S) and organic
40 matter; in that case, it is referred to as “green manure”.

Catch crops, apart for the direct effect of increasing the input of organic matter into the soil, have been shown to have “indirect effects” such as (Young et al., 2021): decrease soil erosion, since they provide cover to otherwise bare soil (López-Vicente et al., 2021); increase total plant production (Hijbeek et al., 2017); increase N₂O emissions (Sandén et al., 2018); increase
45 macropores, and thus water infiltration (Haruna et al., 2020); change soil temperature (Blanco-Canqui & Ruis, 2020); either increase or have no effect on SOC (Poeplau & Don, 2015). However, the use of catch crops requires appropriate timing for planting and harvest/incorporation in the soil as green manure (Cherr et al., 2006); thus, the combined use of catch/cover crops and precision agriculture is particularly recommended. Various studies have recently shown the beneficial effects of catch crops in Mediterranean climate (Cerdà et al., 2022; Curto et al., 2015; Sanz-Cobena et al., 2017; Shackelford et al., 2019; Ventura et al., 2022); however, the increase in SOC stock due to catch and cover crop application was recently questioned (Chaplot & Smith, 2023). Various initiatives exist to study and foster soil carbon sequestration, and most of them took catch
50 crops into account; for example, the French “4 per 1000” initiative (Kon Kam King et al., 2018) and the CarboSeq project, <https://ejpsoil.eu/soil-research/carboseq>; in both cases the approach is to combine field experiments with modelling to evaluate the potential of various agricultural practices for carbon sequestration (Bruni et al., 2021; Seitz et al., 2022).

55 The use of field experiments to build, calibrate and validate soil models has been highlighted as the most effective approach to large scale, long term estimates of SOC dynamics (Paustian et al., 2019; Smith et al., 2020). The RothC model is one of the most used soil carbon dynamic models, due to its simple design and low requirement of data (Coleman & Jenkinson, 1996). The simple design of RothC, however, can also be a hurdle to estimate the potential of agricultural practices whenever these practices affect soil processes not explicitly modelled in RothC. For example, catch crops have been modelled in RothC by
60 accounting only for their direct effect, i.e. increase in organic carbon input into the soil; the indirect effects on soil temperature and water regime have been neglected even though they have a role in soil carbon dynamics (Constantin et al., 2015; Nieto et

al., 2013; Seitz et al., 2022). Not accounting for catch crops indirect effects may lead to incorrect model calibration, since the mineralization rate, in RothC, is affected by both soil temperature and soil water content.

Another problem of the RothC model is related to the ratio between measured parameters and calibrated ones, especially when the calibrated parameters are numerous and only SOC data are used as target for the calibration procedure. First, this approach requires long term experiments (around 10 years, Smith 2004) to obtain sufficient data to detect a significant trend in SOC useful for RothC calibration. Second, the over-parametrization problem could lead to equifinality and decrease of the reliability of the model projections (Menichetti et al., 2016). Cagnarini et al., 2019, has shown that RothC equifinality problem can be minimized by conducting a multi-objective calibration: instead of using only SOC data as a target for calibration, multiple timeseries of relevant soil variables could be used to constrain the model parameters. The use of high accuracy, high frequency timeseries data related with the direct and indirect effects of the specific agricultural practice to be assessed would solve multiple problems: equifinality, erroneous calibrations due to incorrect mineralization rates, and time required for the calibration of RothC. In the case of catch crops, this points to timeseries of soil temperature, soil moisture, and a similar timeseries directly related with soil carbon dynamics.

Soil respiration is a quantity that can be measured continuously in the field, with a frequency of one measurement every 30 minutes or hourly when using automated gas chambers (Sánchez-Cañete et al., 2017). Total soil respiration (R_s) can be thought as the sum of the respiration by autotrophic organisms (mostly plant roots) and the respiration by heterotrophic organisms (mostly microbes and fungi in the soil degrading dead organic matter). However, the influence of roots on the emission of CO_2 in the soil goes beyond root respiration: roots emit exudates that are quickly degraded, parts of the roots die continuously and are degraded by heterotrophs; the respiration from these two mechanisms can be referred to as rhizosphere respiration ($R_{rhizosphere}$), and microbes in the rhizosphere may degrade soil organic matter with a different rate than in non-rhizosphere soil, a process called “root priming” ($R_{priming}$, Kuzyakov, 2006). Thus, R_s can be described as the sum of its autotrophic component (R_a) and its heterotrophic component (R_h), or as the sum of root-induced respiration (R_r) and soil basal respiration (R_b).

Soil respiration has been already used to calibrate RothC with short timeseries in laboratory conditions, where roots are absent (Mondini et al., 2017); however, to be used in field conditions, there is the need to separate R_a from R_s , since RothC only estimates the CO_2 flux from the degradation of soil organic matter (including exudates and dead roots). Various partitioning methods exist for field conditions, for example trenching (Dondini et al., 2017); however, soil respiration from a trenched plot may not represent basal respiration with respect to the non-trenched plot, as discussed in Savage et al. (2018). Other methods exist (e.g. leaf clipping (Macdonald et al., 2004), tree girdling (Subke et al., 2004), root excision (Cheng et al., 2005), etc...); in a comprehensive review, Kuzyakov, (2006) indicates as a promising, yet rarely used method, the regression technique (Kucera & Kirkham, 1971), which correlates changes in R_r with changes in root biomass (Hill et al., 2004; Tomotsune et al., 2013).



The objective of this study was to compare combined catch crops and precision agriculture on one hand, and traditional
95 practices on the other, from the point of view of soil carbon sequestration potential and of agricultural productivity. To do so,
we: (a) conducted two field experiments with duration 4+ years where we measured agricultural productivity, SOC, soil micro-
climate, weather conditions, and CO₂ flux with gas chambers; (b) partitioned measured R_s into R_h and R_a using a method
inspired by the regression technique (Kucera & Kirkham, 1971); (c) conducted a multi-objective calibration of RothC using
as target variables SOC, soil heterotrophic respiration, soil water content and soil temperature, in this order of importance, to
100 test its ability to predict catch crop effects on SOC dynamics.

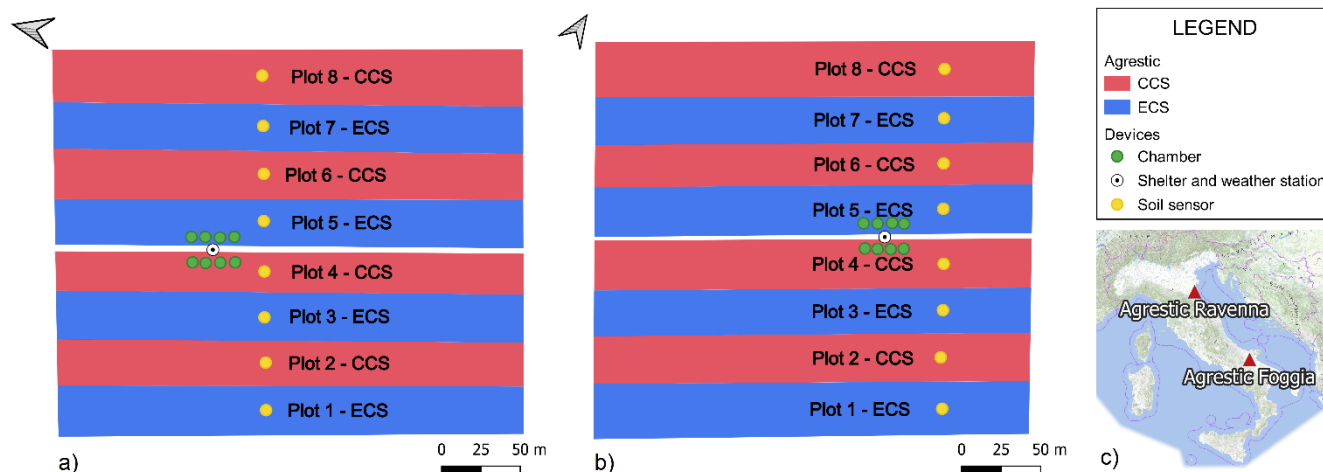
2 Materials and methods

2.1 Field sites

Two field experiments were set up at experimental farms in Ravenna (locality of Cà Bosco; 44° 29' 15." N, 12° 10' 44." E,
experimental area 7 hectares) and in Foggia (locality of San Giuseppe; 41° 29' 27" N, 15° 30' 14" E, experimental area 7,5
105 hectares), as shown in Figure 1. The two sites differ by soil types, water regimes, climate and land use; their differences are
meant to assure that the study results are representative for most conditions found in Italy. The Ravenna site is characterized
by: silty clay loam inceptisol in the west side and silty loam inceptisol in the east side; 4 m elevation a.m.s.l.; flat landscape;
humid sub-tropical climate (Cfa by Köppen-Geiger classification, Beck et al., 2018), with mean yearly temperature of 14.3
°C, cumulative annual rainfall of 659 mm, and rain events concentrated in spring and fall. The Foggia site is characterized by:
110 silty clay loam vertisol; 70 m elevation a.m.s.l.; flat landscape; cold semi-arid climate (BSk by Köppen-Geiger), with mean
yearly temperature of 16.9 °C and cumulative annual rainfall of 554 mm.

2.2 Field experiment setup and management

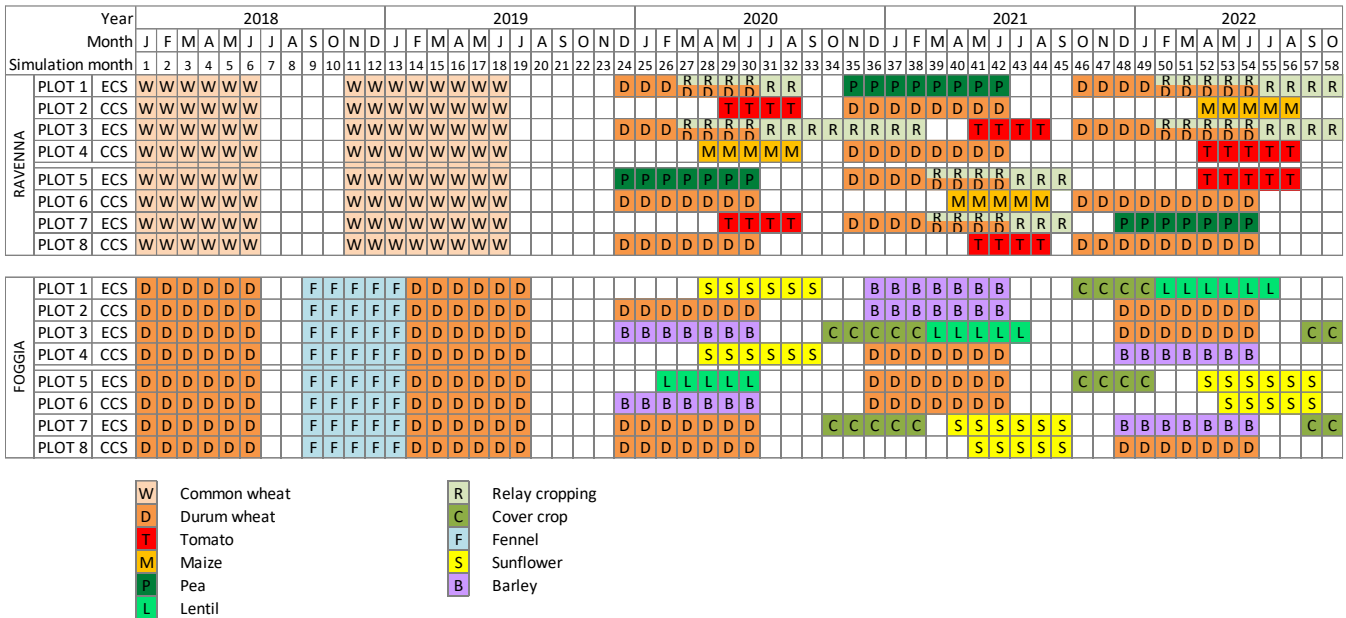
The field experiments were setup and managed by Horta Srl as part of the experimental fields of EU LIFE Project for
Agricultural GReenhouse gases EmiSsions Through Innovative Cropping systems (Agrestic, <https://www.agrestic.eu/en/>),
115 with the objective of promoting adoption of regenerative agricultural practices aimed at mitigating climate change by
decreasing emissions from soil to the atmosphere, increasing soil organic carbon, and increasing organic nitrogen availability.
The experimental sites were used to compare two management practices arranged in 8 plots (Figure 1): Conventional Cropping
System (CCS) and Efficient Cropping System (ECS); CCS uses fertilizers, pesticides, irrigation and soil tillage in line with
the practices followed generally in the surrounding farms; ECS uses fertilizers, pesticides, irrigation and seeding density as
120 suggested by a dedicated Decision Support System (DSS) developed by Horta Srl, while conservative soil tillage, relay crops
and catch crops are included and managed under expert agronomists advice. The arrangement of the field experiments and the
details of crop sequence are summarised in Figure 2.



125 **Figure 1: Plots and management practices subdivision, with the equipped devices (soil temperature sensors, soil gas chambers, and weather station), for the two experimental fields in a) Ravenna and b) Foggia, and c) the location of the experimental farms in Italy.**

The same management was used for both ECS and CCS and both experimental sites during the first period (January 2018 to June 2019); this was done to standardize the starting conditions of the experiment, and to test the hypothesis that measurements from ECS and CCS are comparable when the two managements are kept equal, on the same experimental site. Figure 2 shows that, starting from December 2019, in ECS: (a) the soil remains bare for a shorter period, (b) N availability in the soil is higher due to the use of leguminous crops (pea *Pisum Sativum* L. in Ravenna and lentil *Vicia Lens* (L.) Coss. & Germ in Foggia), (c) total productivity is increased due to relay cropping (alfalfa *Medicago Sativa* L. in Ravenna and a mix of horseradish *Raphanus sativus* L. var. *oleiformis* Pers., clover *Trifolium repens* L. and *Phacelia tanacetifolia* in Foggia). Cereal residues are removed from field and sold, while other production crops, as well as cover crops, are never removed, just incorporated in the soil.

130



135 **Figure 2: Crop rotation management in (a) Ravenna and (b) Foggia experimental sites.**

2.3 Measurements

The measurements taken in both experimental sites can be divided in: data collected continuously through monitoring stations (shown in detail in Figure 1), and data collected by researchers, regularly, in the field. The former category of data can be divided into: meteorological timeseries collected using a weather station located few meters from the experimental site, soil temperature and soil moisture timeseries collected using dedicated dataloggers on all the plots, soil respiration timeseries collected on the central plots (4 and 5 in Figure 1, representative of ECS and CCS conditions) using a dedicated datalogger. More specifically, the weather stations monitored: air temperature, air humidity, net total solar radiation, precipitations, wind direction and velocity, and leaf wetness (METOS sensor, from Pessl, Austria), with data stored hourly. The soil timeseries were acquired using water content reflectometers and thermistors (20-25 cm depth) connected to a wireless datalogger (xNode, xFarm, Italy), with data stored hourly. The soil respiration measurements were taken using alternatively all the chambers (LI-COR: LI-850 gas analysers for CO₂ and N₂O, respectively, from West System and Sant'Anna School of Advanced Studies, Volpi et al., 2020), in each plot, in a way to get a measurement every 15 minutes (1 hour sampling frequency for each chamber).

The data collected by hand consisted in: dry matter production, marketable yield, biomass humidity, root index, irrigation, fertilization, pesticide application, depth of soil tillage. From 2020 onwards crops biomass was sampled right before crop harvest or, in case of a cover crop, crop destruction. Samples were weighed before and after oven drying to obtain humidity and plot's biomass production. One sample for each plot and each plant part was made from 4 randomized repetitions across the whole plot, then externally analysed to get carbon and nitrogen content in percentage of dry matter (analytical method ISO



16634-2:2016). Soil samples for SOC measurements were collected at depth 30 cm with a manual auger on October (in
Ravenna) and November (in Foggia) in 2019, while on September from 2020 onwards. One sample for each plot was formed
155 from 4 randomized areas (3 samples in each area for a total of 12 samples) and then sent to a nearby analysis centre for the
determination of SOC in g kg^{-1} dry matter using Walkley-Black method (Walkley & Black, 1934). Later, SOC values were
converted into tC ha^{-1} as in equation 1, using soil samples' depth and standard bulk density corresponding to the USDA soil
texture classification:

$$SOC = \frac{10^4 \cdot \text{depth} \cdot \text{bulk density} \cdot SOC}{10^6} \quad (1)$$

160

2.4 Soil respiration partitioning

The regression method developed by Kucera & Kirkham, (1971) assumes that root biomass and root derived soil respiration
are correlated, and that the correlation can be determined with a linear fit. This method does not modify soil or root conditions,
and it is applicable whenever enough information on soil root biomass and soil respiration is available. This method is
165 particularly interesting for crops, since a lot of information on root development is usually available, the vegetation is
comprised of a single or maximum two species, and other methods are not usually available (girdling, root excision, separation
between root and soil is very difficult). A recent application of the method is described in Tomotsune et al., (2013). The only
limit of this method is that root biomass is expected to correlate with root derived respiration (R_r), which is the respiration of
the rhizosphere, and not R_a , which is the respiration of the root only; therefore the method allows a separation of R_b and R_r
170 rather than R_a and R_h .

To partition R_s , we used the continuous, 4+ years dataset of soil respiration in ECS and CCS, and, for each management type,
we analyzed: (i) R_s measured at various temperatures in winter; (ii) R_s measured during the growing season; (iii) R_s measured
right after the harvest during non-winter months. The assumptions we used were that: (i) winter bare soil R_s was representative
of R_b ; (ii) $R_s - R_b$ is correlated with changing root biomass during the growing season, and it can be used to estimate R_r ; (iii)
175 R_r after harvest is actually $R_r - R_a$. R_h is known to be affected by soil temperature and humidity (Bauer et al., 2012; Cook &
Orchard, 2008; Neogi et al., 2014; Zhong et al., 2016), while R_a is correlated with incoming solar radiation influencing plant
photosynthesis (Fitter et al., 1999). Using these assumptions, and controlling for the effects of soil temperature and soil
moisture using multiple regression, we can estimate a function for R_a and R_h . More specifically, the correlations used have
been:

$$180 \quad R_s = R_a + R_h, \quad (2)$$

$$R_s = R_r + R_b, \quad (3)$$

$$R_r = R_a + R_{rhizosphere} + R_{priming}, \quad (4)$$



$$R_h = R_b + R_{rhizosphere} + R_{priming}, \quad (5)$$

$$R_r = k_r \text{RootBiomass} + k_{r,Rad} \text{Rad}_{net} + k_{r,T} T + k_{r,\theta} \theta, \quad (6)$$

$$185 \quad R_a = k_a \text{RootBiomass} + k_{a,Rad} \text{Rad}_{net} + k_{a,\theta} \theta, \quad (7)$$

$$R_b = k_{b,T} T + k_{b,\theta} \theta, \quad (8)$$

With T average topsoil temperature, θ average topsoil water content, Rad_{net} net solar radiation, k_r , $k_{r,Rad}$, $k_{r,T}$, $k_{r,\theta}$, k_a , $k_{a,Rad}$, $k_{a,\theta}$, $k_{b,T}$, $k_{b,\theta}$, the empirical coefficients of the multiple regression linear functions for R_r , R_a and R_b . We analyzed the data and performed all multiple regression analyses using R lm methods (Ihaka & Gentleman, 1996, supplementary material). The results of the analyses for both areas and both managements were then used to calibrate the RothC simulations (see section 2.6).

2.5 RothC version used: RothC_20N for Mediterranean climates

The Rothamsted Carbon model (RothC-26.3) simulates soil carbon dynamics with monthly timestep, by assuming that carbon entering the soil is subdivided into four different carbon pools, and that the carbon in the pools mineralizes by first order decay kinetics (Coleman & Jenkinson, 2014). The RothC model takes into account the soil only, there is no sub-model for the simulation of plant growth; this means that the user directly sets the input of organic carbon into the soil based on field measurements or independent plant models. More specifically, in RothC, the organic carbon entering the soil is first divided into two “fresh” organic matter pools: decomposable plant matter (DPM) and resistant plant matter (RPM). The carbon mineralized from any pool then goes to: the atmosphere as CO_2 , the biological carbon pool (BIO), and the humified carbon pool (HUM); the amount of carbon going to CO_2 is defined based on the clay percentage in the soil, while the subdivision between BIO and HUM is fixed (46% and 54%, respectively). The DPM, RPM, BIO and HUM pools difference is in their degradation constants: K_{dpm} , K_{rpm} , K_{bio} and K_{hum} being 10, 0.3, 0.66, 0.02 years^{-1} , respectively. It is important to understand that these pools are theoretical (a way to fit four decay function to SOC measurements) and cannot be measured in the reality; some attempts have been performed to connect them to measurable quantities, but with limited results (Skjemstad et al., 2004; Zimmermann et al., 2007).

The pools decay functions are modified by three parameters that depend on soil temperature, soil water content, and soil vegetation cover (a , b , c parameters, respectively). The a and the b parameters are based on empirical functions that link air temperature and topsoil moisture deficit (TSMD) with mineralization rates, similarly to the empirical functions usually established between soil CO_2 emissions (R_h) and other environmental variables (Sánchez-Cañete et al., 2013). TSMD is calculated by RothC with a simple bucket model: each monthly time step, the water in the soil is calculated as the budget between previously stored water, input from precipitation and irrigation, output from evapotranspiration (calculated



independently by the user); the water in the soil is constrained between a maximum wetness (field capacity) and maximum dryness (wilting point when vegetation is present, 55.5% of wilting point when the soil is bare). The soil water deficit modifier
215 decreases mineralization rates linearly from a maximum (no effect, $b = 1$), between field capacity to water matric potential of -1 bar) to a minimum (20% of maximum mineralization rates, $b = 0.2$) between -1 bar and -15 bar (wilting point). The c parameter influences the b parameters, as explained above, and affects mineralization rates if plants are present (a simulation of priming).

Due to the empirical nature of RothC, its functions have been adapted to various different conditions around the world with
220 different approaches (Paul & Polglase, 2004; Stamati et al., 2013); with a specific version of RothC for Mediterranean conditions developed by Farina et al., (2013), called RothC20_N. The modifications introduced by Farina et al., (2013) were: (i) to allow the TSMD to reach a value corresponding to capillary water when the soil is vegetated (-1000 bar); (ii) the maximum TSMD when the soil is bare is -15 bar; (iii) the minimum value of b can change from 0.2 to 0.1. Another modification introduced in the method is to directly define the TSMD limits based on pedotransfer functions, rather than using the empirical
225 parameter already defined in the model. The empirical nature of the carbon pools means that they cannot be calibrated separately, however, their combined effect on carbon dynamics can be calibrated based on two measurable quantities: SOC stocked in the field (corresponding to the sum of all the SOC pools in RothC; slow change, slow rate of measurement, large uncertainty) and CO_2 emitted due to organic matter mineralization (R_h , corresponding to the sum of all the CO_2 emitted by all pools in RothC; fast change, fast rate of measurement, low uncertainty).

230 **2.6 RothC and RothC20_N simulations**

We set up the initial conditions for the RothC and RothC20_N model, i.e. the initial quantity of carbon in the model carbon pools, with the standard approach found in the literature: using spin-up run simulations. As explained in section 2.5, RothC carbon pools cannot be directly measured in the field; as such, the model initialization makes use of SOC measurements taken at the beginning of the experiment, with the SOC quantity fractionated among the pools using the relative relevance of the
235 pools at equilibrium conditions. The spin-up run is aimed at estimating these equilibrium conditions: it is based on the assumption that we know the input conditions for the experimental site in the past, and that said conditions were stable for a time long enough to let the carbon pools reach equilibrium. The conditions are then used to run a simulation starting from empty SOC pools, until all the pools have reached equilibrium. For the two spin-up runs (one for Ravenna and the other for Foggia field sites), we assumed 500 years of continuous cultivation of three different types of crops, using as weather
240 conditions the average of conditions measured between 2004 and 2021.

After determining the initial conditions, we set up four simulations for each experimental site: ECS and CCS simulations using RothC basic model, and ECS and CCS using RothC20_N as in Farina et al. (2013). The input provided to the RothC model consisted of: monthly air temperature and precipitation from the weather station; aboveground input of organic matter, irrigation, soil vegetation cover, soil bulk density from direct field observations; evapotranspiration calculated with Penman-



245 Monteith (Monteith, 1980) and Hargreaves (Hargreaves & Samani, 1982) using the weather station data; belowground carbon input estimated using observations of root development; DPM/RPM ratio determined based on standard values given for crops. We decided to use two different methods to estimate evapotranspiration in order to assess the sensitivity of the model to the change in calculation method.

Aboveground and belowground carbon inputs are calculated starting from direct observations of yield (*yield*), above ground
250 biomass (*Agb*) and root biomass (*dry root*), all in tonnes ha⁻¹. First the dry yield is calculated as

$$\text{dry yield} = \text{yield} - [\text{yield} \cdot \text{yield } H_2O\%], \quad (9)$$

The harvest index (*HI*) and root index (*RI*) are calculated as:

$$HI = \frac{\text{dry yield}}{Agb}, RI = \frac{\text{dry root}}{Agb}; \quad (10)$$

Finally, aboveground carbon input (*AgI*, in tonnes of C ha⁻¹) is calculated as:

255
$$AgI = \left[\frac{\text{dry yield} \cdot (1-HI)}{HI} \right] \cdot \text{residues left on field} \cdot C \text{ content}, \quad (11)$$

Where *C content* is the carbon content of the considered plant part in percentual of dry matter, *residues left on field* is in percentage; belowground carbon input (*BgI*, in tonnes of C ha⁻¹), is calculated as:

$$BgI = \frac{\text{dry yield} \cdot RI}{HI}, \quad (12)$$

Where *RI* stands for root index, the ratio of root with respect to total aboveground biomass as dry matter.

260 The input provided to the RothC20_N model is the same as the basic model, plus the TSMD calculated independently using the observed retention curve of the soil material.

We calibrated all the models using the 4+ years datasets from plot 4 and 5 from each of the field sites (the two plots with respiration and soil moisture and temperature data series). The calibration procedure followed the multi-objective calibration framework presented in Cagnarini et al., (2019), and consisted in the use of the GLUE (global likelihood uncertainty analysis) analysis (Beven & Binley, 2014), according to limits of the acceptability criteria (X. Liu et al., 2009). We calibrated the
265 following 5 parameters: kinetic constants K_{dpm} , K_{rpm} , K_{bio} and K_{hum} (four parameters), the inert organic matter pool IOM (one parameter). The objectives of the calibration were the observed: SOC, soil water content (translated into TSMD), and partitioned R_h . The data on CO₂ and N₂O soil emissions are publicly available of the Agrestic website (<https://www.agrestic.eu/ravenna-co2/>). All information on the simulation inputs is available in supplementary materials.

270 Following the calibration from plot 4 and 5, we evaluated the overall quality of the calibrations by using the calibrated models to simulate the SOC stocks in all the other plots (i.e. plots 1, 2, 3, 6, 7, 8 where no observations of R_s were available) in both



sites, then the SOC estimated were averaged for each treatment (ECS, CCS) in each site (Ravenna, Foggia) and compared with the averaged SOC observed (and their standard deviation).

3 Results

275 3.1 Soil measurements

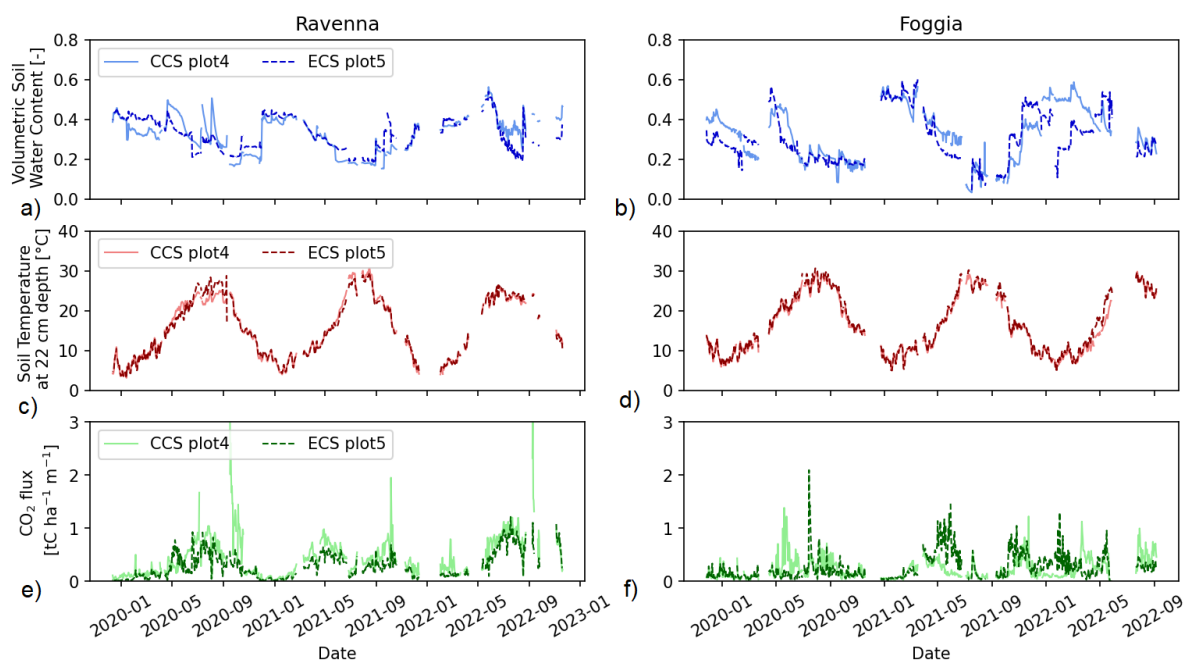


Figure 3: Measurements of soil water content, soil temperature and soil respiration, in both field sites (Ravenna and Foggia) and both treatments plots equipped with gas chambers (plot 4 CCS and plot 5 ECS).

Figure 3 shows the time series measurements taken in the field. The soil water content time series show small differences between plot 4 (CCS) and plot 5 (ECS); depending on the type of crop plantation one plot may show marginally higher or lower soil water content with respect to the other, but, on average, no clear difference is visible. During summers the soil water content is always very low in both Ravenna and Foggia sites, but the lowest values of soil water content are reached in Foggia, due to drier weather and different soil properties. The soil temperature does not change between ECS and CCS in general; the temperatures are in general 1°C higher in Foggia than in Ravenna.

285 Soil respiration is generally higher in Ravenna than in Foggia, with average respiration rates of 0.38 and 0.25 tC ha⁻¹ month⁻¹, respectively. In Ravenna, the respiration rates are larger in plot 4 (CCS, 0.45 tC ha⁻¹ month⁻¹) than in plot 5 (ECS, 0.30 tC ha⁻¹ month⁻¹); in Foggia the respiration rates are larger in plot 5 (ECS, 0.27 tC ha⁻¹ month⁻¹) than in plot 4 (CCS, 0.23 tC ha⁻¹ month⁻¹). The SOC data was available for all plots; the average values are shown in Figure 6: SOC increased more in ECS



than in CCS in both Ravenna and Foggia, but the difference is larger in Ravenna than in Foggia. The SOC trends, however, are characterized by large errors and variability among plots (see error bars in Figure 6), and it is well known that a period of around 10 years is required to observe a significant trend from field measurements (Stockmann et al., 2015). For this reason, the SOC measurements are presented and analyzed together with RothC and RothC20_N simulations in section 3.3.

Other important results of the field comparison between ECS and CCS are related to agricultural productivity, water used and soil overall emissions (considering N_2O). The dry yield was: higher in ECS for durum wheat in both field sites (+ 2% in Ravenna and + 18% in Foggia); same for other crops such as tomato (+3% in Ravenna) which produced particularly higher quality berries (+ 10% Brix degree); overall dry biomass production higher due to catch crops (around $1 \text{ t ha}^{-1} \text{ yr}^{-1}$). The water used in ECS was lower than that in CCS (-18.9 %) due to lower irrigation for tomato. The use of fertilizer was lower in ECS due to the relay crop alfalfa (-31.2 $\text{kg ha}^{-1} \text{ yr}^{-1}$) and pea (-22.4 $\text{kg ha}^{-1} \text{ yr}^{-1}$) in Ravenna, and due to lentil (-11.5 $\text{kg ha}^{-1} \text{ yr}^{-1}$) and cover crop (-18.7 $\text{kg ha}^{-1} \text{ yr}^{-1}$) in Foggia. Finally, the emissions of N_2O measured by the gas chambers were 46% lower in ECS than in CCS.

3.2 Soil respiration partitioning

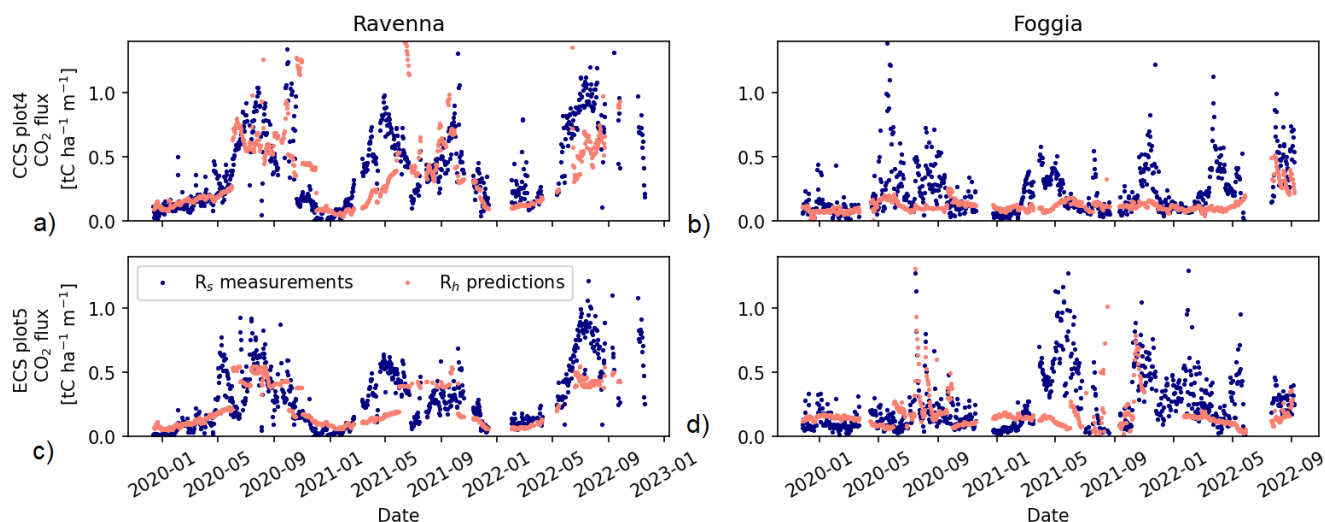


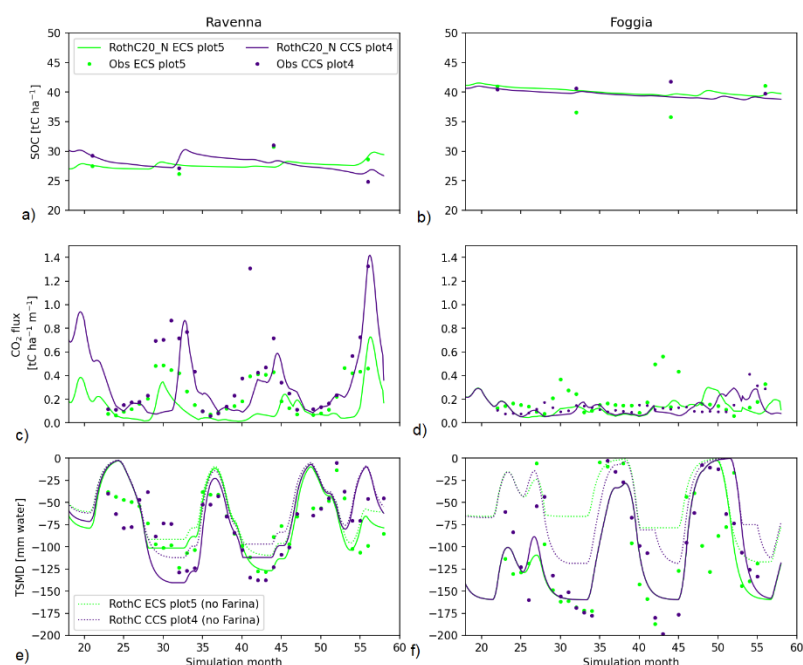
Figure 4: Soil respiration (R_s) measurements, and R_h predicted by partitioning, in both field sites (Ravenna and Foggia) and both treatments plots equipped with gas chambers (plot 4 CCS and plot 5 ECS).

The soil respiration partitioning shows very good fits for soil respiration in Ravenna in both summer and winter periods when no crop was present. The estimates of R_h are generally lower than measured R_s , as expected, apart from few periods (winter 2020 for CCS, summer 2021 for ECS, see supplementary materials). In Foggia the fitting was less satisfactory in CCS and ECS (worse in CCS) in winter, but better in summer. Figure 4 shows the results of the soil respiration partitioning; apart for



some periods in which the R_h predictions are larger than the R_s observations, especially in winter 2020 in Foggia and some
310 few days in summer in general, the predictions make sense (R_s larger when crop growing and standing, R_h large after harvest,
then decreasing). The analysis of the residuals, i.e. the difference between observed R_s and model estimates of R_h , show good
fit with: i) root depth in winter for ECS and CCS in the periods in which crop was present in the field; ii) soil water content in
summer (ECS); iii) soil water content in both summer and winter in CCS. In general, the prediction of R_h when crop was
present is larger than R_s observed when R_s was very low in Foggia (winter 2020 CCS, winters 2019-2020 in ECS), but lower
315 when R_s was large.

3.3 RothC and RothC20_N simulations



320 **Figure 5: SOC, CO₂ flux and TSMD measured and modelled using RothC20_N and RothC in the two plots where the gas chamber
measures were available (plot 4 CCS and plot 5 ECS) in both field sites (Ravenna and Foggia), after calibrating the RothC and
RothC20_N models.**

Figure 5 shows the results of the multi-objective calibration. The estimates of the soil water balance parameters from
pedotransfer functions as in Farina et al., (2013) yielded very good results for RothC20_N (Figures 5e, 5f): the very low values
reached during summer are well represented (while completely missed by RothC basic version), just like the field capacity
values during winter. Since RothC20_N provided the best fit to the water content data, from here on only this version of RothC
325 will be commented. The calibration of K_{hum} , K_{rpm} , K_{bio} and K_{dpm} were deemed site specific, that is, the parameters were fitted



using data from both treatment (ECS and CCS) for Ravenna and Foggia. However, the calibration resulted in values very similar to the original ones (less than 5% difference). The results show a general good fit with the estimated R_h , even though it is possible to see that not all peaks (sudden surges in R_h) were well simulated (especially in months 30 and 42 in Ravenna and Foggia), but the average behavior and the average fluxes matched. The calibrated RothC20_N simulations could model the general SOC trends in the data.

Figure 6 shows the averaged results for the simulations of all the plots with ECS and CCS in the two field sites; the averaged simulations for Ravenna followed very well the general trend observed in the measurements, with larger SOC values in ECS than in CCS. In Ravenna, the averaged SOC estimated by RothC20_N shows an increase in SOC for the ECS treatment (while the observation trend is not statistically significant) and a slow increase in the difference between the SOC in ECS with respect to CCS (also shown by the observations). In Foggia, the simulations show a decrease in SOC for both ECS and CCS, with ECS having slightly more SOC than CCS. However, the averaged observations show an increase in SOC in both ECS and CCS (but trend not significantly different from zero). Therefore, the averaged simulations appear to slightly underpredict the SOC with respect to the observations.

340

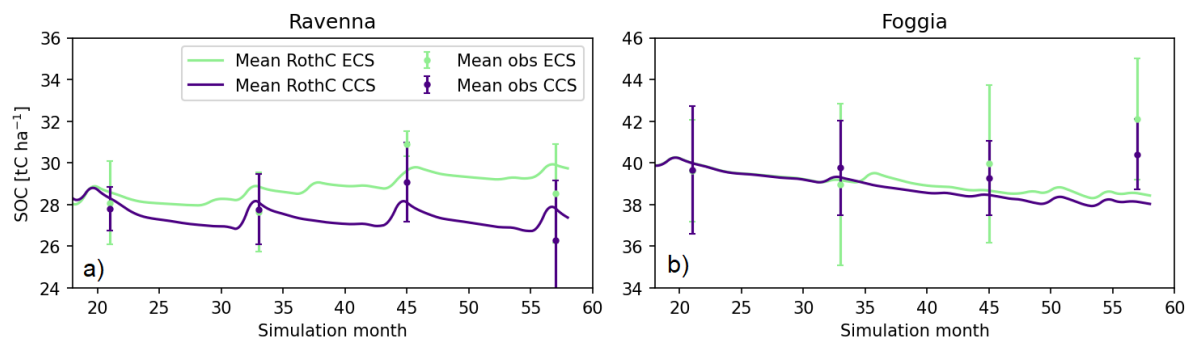


Figure 6: SOC measured in the field and modelled using RothC20_N, averaged on all ECS and CCS plots in a) Ravenna and b) Foggia field sites.

4 Discussion

Notwithstanding the difference in soil properties and climatic conditions of the two field sites, the differences in the soil conditions that affected SOC stocks (that is, soil temperature and soil water content) were very small. The main difference was in the soil water content (Figure 3a, b), that was drier in Foggia than in Ravenna, even though this difference was overall small, due to the irrigation procedure which increased soil water content whenever reaching wilting point when a crop was present. Soil water content was somehow different in ECS than in CCS, with less water used in ECS and different irrigation times. All these differences in soil water regime were simulated properly by RothC20_N. The differences in R_s measured in ECS and

350



CCS are due to the different timings of crops between the two plots 4 and 5 in both sites. R_s appears to be smaller in Foggia than in Ravenna (Figure 3e, f), probably due to the difference in carbon input between the two sites, since this difference was captured by RothC20_N as well (Figure 5c, d).

355 The partitioning method estimated R_h values very close to those predicted independently by the RothC20_N simulations, giving credibility to both methods. Some trouble was related to wild fluctuations in the data especially in the Foggia site, which could be due to the soil cracking around the chamber (observed in the field during summer) and, possibly, preferential flow of CO₂ out of the cracks. Another problem was that, when R_s was low during a cropping period, the estimates of R_h were larger than the actual measurement of R_s (see Figure 4, September 2020 in Ravenna, January 2021 in Foggia). Some mismatch in respiration peaks is visible between R_h partitioned and RothC20_N simulated, and they will be discussed further below.

360 The calibration of the RothC20_N model resulted in very good simulations of the soil water regime observed in plots 4 and 5 for both sites, especially of the dry periods with almost no rain during summer. The calibration could fit satisfactorily both SOC trends and R_h measurements without changing appreciably the calibrated parameters, with less than 5% change in the initial values. More specifically, the calibration had a small effect on K_{dpm} (12 instead of 10 yr) and K_{hum} (0.018 instead of 0.02 yr⁻¹).

365 Some mismatch is evident in the peaks of R_h partitioned and simulated by RothC20_N, especially in August-September 2020 and May-June 2021 (Simulation months 30-31 and 41-42, Figure 5c, d). In Ravenna, ECS simulation shows similar peaks as R_h partitioned, but of lower intensity, while CCS seems to miss the peaks by few months; in Foggia, ECS partitioned R_h shows peaks that are completely absent from the R_h simulated. One possibility for this mismatch is that the partitioned R_h was incorrect due to missing data from catch crop and legumes root development; however, if that was true we would see different mismatch in 2020 (when the catch crop was incorporated in the soil) and 2021 (no catch crop in Foggia), but the mismatch is the same in both years. Another possibility is related to the soil water content: in both August 2020 and May-June 2021 the two sites experienced very dry periods; even if the soil water conditions were well simulated by RothC20_N, it is possible the model missed the effect of dry soil on the microbial metabolism: in dry soil conditions, microbial metabolism (and, thus, R_s) is supposed to decrease; however, in soils experiencing regular dry periods, microbial communities may adapt to dry soil conditions (Brangarí et al., 2021). Unfortunately we cannot test this hypothesis, since we did not measure enzymatic activity in the soil.

380 The comparison between the two field managements, ECS and CCS, showed that ECS increased SOC stocks, decreased CO₂ emissions from the field, while still being profitable from the farmer point of view. In general, we could see less CO₂ emissions (-33%) and N₂O emissions, less use of fertilizers, less use of water, same or better crop yields, and increase in SOC from measurements, with a small increase trend in SOC (but with small statistical significance). Thus, the ECS method generates more or equal agricultural output (and profits) with respect to CCS, while decreasing the amount of input. The decrease in fertilizers use and irrigation practices is likely to result in a reduction in indirect CO₂ emissions as well, i.e. emissions produced



before field activity, but this should be evaluated properly in a LCA study (available upon request). If carbon accounting methods would be put in place, or in the case there was a standardized methodology to reward soil carbon sequestration alongside emission reductions, ECS would be even more interesting as a practice for the farmers, due both to reduced emissions and increased carbon stored in the soil. Moreover, Labouyrie et al., (2023), using LUCAS data from across the EU, showed that properly managed agriculture may result in larger soil microbial diversity with respect to other land uses, even natural grasslands.

Comparing the present study with literature, shows that our contribution is important toward assessing the effects of catch and cover crops in increasing SOC stocks. Starting from a comprehensive review by Poeplau & Don, (2015), various articles have claimed that catch and cover crops have the potential to increase SOC; however, a recent article by Chaplot & Smith, (2023) put those results into question. Our study seems to contribute to the debate, since it fits in the description of the “acceptable” study by Chaplot & Smith, (2023), and showing that catch and cover crops increase SOC stocks, as discussed further below.

Poeplau & Don, in their 2015 review compiling data from 139 plots at 37 different sites, show that the use of catch crop increased SOC by $0.32 \text{ t C ha}^{-1} \text{ yr}^{-1}$ within the first ~ 50 years, and then discuss the amount of carbon which could be sequestered up to soil saturation; however, how carbon saturation works is still under discussion at the time of writing this article (Georgiou et al., 2022; Lavalée & Cotrufo, 2021). Hansen et al., (2015) found that soil organic carbon (SOC) did not increase significantly after 7 years of straw incorporation in two experimental sites in Denmark. Shackelford et al., (2019), reviewing data from experiments in Mediterranean climate, shows that cover crops resulted in 13% less water, 9% more organic matter, 41% more microbial biomass, 16% higher yields when legume cover crops used. Similar results were found in an experiment in Germany (Gentsch et al., 2020). In a review on potential methods for soil carbon sequestration in agriculture, Tiefenbacher et al., (2021) showed that the use of catch crops is one of the most promising (together with other strategies applied in this study, i.e. smart irrigation and diversified crop rotation). Lessmann et al., (2022) meta-analysis of other meta-analyses, showed that increasing crop diversification as well as the use of catch crops and cereals within arable crop rotation schemes has the potential to sequester an additional 153 Mton of C yr^{-1} globally. Cerdà et al., (2022), in an experiment conducted in Valencia (Spain), showed that soil organic matter increased from 1.14 to 1.63% after 10 years of treatments using catch crops.

Modeling shows more uncertain effects of catch and cover crops on SOC stocks. Modelling of carbon sequestration potential from organic agriculture using Century (Foereid & Høgh-Jensen, 2004) predicted that cover crops could increase soil organic matter during the first 50 years of about $0.1\text{-}0.4 \text{ t C ha}^{-1} \text{ y}^{-1}$. However, Seitz et al., (2022) used RothC and C-Tool to predict potential of cover crops for carbon sequestration in Germany, accounting only for the direct effects of cover crops. They found that cover crops alone cannot turn croplands from carbon sources to sinks. However, growing them reduces bare fallow periods and SOC losses and thus is an effective climate change mitigation strategy in agriculture.

There are only few studies showing no or negative effects of cover and catch crops on SOC stocks: Hijbeek et al., 2017, using data from 20 long term experiments, showed that catch crops do not increase yields, if there is no N deficiency in the soil;



415 however, this means they may be useful to reduce fertilization, as observed here (and as observed by Couëdel et al., 2018).
Triberti et al., 2016 showed that the application of catch crops may result in a decrease in SOC stocks; however, this was
mainly due to the impact of intensified agricultural management in the same plot where the catch crop was applied. Finally, a
recent re-analysis of published studies by Chaplot & Smith, (2023) questioned the findings of previous review articles (e.g.
Poeplau & Don, 2015) on the basis of the type of experiments collected. Chaplot & Smith, (2023) claim that 31 out of the
420 collected 37 studies had a duration of 3 years or less or kept cover crops on the field too long, and thus could not discriminate
the effects of catch crops. Of the remaining 6 studies, only one study showed a positive impact. Using the methodology of
Chaplot and Smith, the study presented here qualifies as acceptable to estimate SOC trends due to catch-cover crops in the
first 30 cm soil, and we were able to show an increase in SOC, with predicted rate of $0.4 \text{ t C ha}^{-1} \text{ yr}^{-1}$.

However, the findings of this study need to be put into context, so we list here its limitations. The duration of the study, even
425 if longer than 4 years, is still below the 10 years usually needed to identify a SOC trend with certainty. The analysis of two
different areas in two different climates increases the robustness of the findings, but two sites are still not enough to represent
the whole Mediterranean conditions (even though they give indications about the variability involved). The fact that long (8+
years) SOC datasets are required to properly calibrate the model is partly solved using partitioned R_h data, but this still leaves
doubts on the relevance of the trends observed from SOC measured in the field. Another important limitation is the lack of
430 proper root biomass growth estimates for some of the catch crops used, which resulted in poor partitioning of R_s into R_h and
 R_a . Even though the partitioning method used here has some uncertainties, the use of trenching has also its own difficulties,
related to dead material increasing R_h in the trenched vs non trenched sites, lack of rhizospheric priming, lack of R_h from
exudates and other material from roots. The final limitation here is that RothC is a well-established and widely used model,
but the scientific consensus is that such models are not able to properly model the processes regulating the carbon dynamics
435 in the field, and thus is not robust against changes in the soil conditions (e.g. due to climate change or due to changes in soil
management). Even though this problem has been recognized since more than a decade (Wieder et al., 2018), there are still no
reliable and applicable successors to RothC and Century like models. Another limitation of RothC is that it simulates only the
C cycle and does not account for N limitation; this is usually not a problem in agricultural fields due to N fertilization, but we
will fix this problem in the future, including N cycle modeling calibrated using the N_2O emissions measured with the gas
440 chambers.

5 Conclusions

This study shows that the use of cover and relay crops, integrated with the use of precision agriculture techniques (field sensors
communicating with agricultural DSS models to advise the farmer on how to act and when) can result in agricultural practices
that are both economically and environmentally sustainable. In this particular case, we were able to show increases in the
445 carbon sequestered by the soil, a reduction in fertilizers and water used in the field, without any negative impact on the



production of food (or, in some cases, even increasing it). The sequestration of carbon from the atmosphere is paramount to balance anthropic emissions and help reach carbon neutrality; in the future, a carbon market or some types of carbon accounting will probably increase the desirability of carbon farming in agriculture.

The soil respiration partitioning method used here, inspired by the regression method developed by Kucera (1971), gave results very similar to those obtained independently from a RothC simulation, showing that the method is viable whenever enough data on soil respiration are collected (typically, by a battery of automated gas chambers). The method could be applied to Eddy Covariance data as well, when used in agricultural settings, with a careful determination of the measurements footprint. The partitioned R_h could be used to calibrate soil biogeochemical models in a reliable way, using shorter observation times with respect to calibrations based on SOC measurements alone.

The version of RothC modified by Farina et al., (2013) for Mediterranean conditions performed very well in its prediction of soil water content in the experimental fields, since it was able to properly reproduce the soil conditions during long periods without precipitations. This is particularly relevant in a changing climate, where more intense precipitation events and longer dry spells are expected over most of the European continent. The multiobjective calibration method used is potentially a very good method, but in our case the estimates from RothC were already very good, and as such, the calibration had only a very small effect on the model parameters (< 5%).

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Additional informations

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