

1     **Modeling topsoil carbon sequestration in two contrasting crop production**  
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3     **to set aside conversions with RothC – calibration issues and uncertainty**  
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6     **analysis**  
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16 **ABSTRACT**

17 Model simulations of soil organic carbon turnover in agricultural fields have inherent  
18 uncertainties due to input data, initial conditions, and model parameters. The RothC model  
19 was used in a Monte-Carlo based framework to assess the uniqueness of solution in carbon  
20 sequestration simulations. The model was applied to crop production to set aside conversions  
21 in Iowa (sandy clay-loam soil, humid-continental climate) and Greece (clay-loam soil,  
22 Mediterranean). The model was initialized and calibrated with particulate organic carbon data  
23 obtained by physical fractionation. The calibrated values for the Iowa grassland were 5.05 t C  
24 ha<sup>-1</sup>, 0.34 y<sup>-1</sup>, and 0.27 y<sup>-1</sup> for plant litter input and decomposition rate constants for resistant  
25 plant material (RPM) and humus, respectively, while for the Greek shrubland these were 3.79  
26 t C ha<sup>-1</sup>, 0.21 y<sup>-1</sup>, and 0.0041 y<sup>-1</sup>, correspondingly. The sensitivity analysis revealed that for  
27 both sites, the total plant litter input and the RPM rate constant showed the highest  
28 sensitivity. The Iowa soil was projected to sequester 17.5 t C ha<sup>-1</sup> and the Greek soil 54 tC ha<sup>-1</sup>  
29 over 100 years and the projected uncertainty was 65.6% and 70.8%, respectively. We  
30 propose this methodology to assess the factors affecting carbon sequestration in agricultural  
31 soils and quantify the uncertainties.

32  
33 **Keywords:** C/N sequestration, aggregation, particulate OM, RothC modeling, calibration,  
34 uncertainty

## 36 1. Introduction

37 Conversion of native vegetated lands to croplands is known to induce soil organic matter  
38 (SOM) losses due to ploughing. In medium to fine textured, structured soils, during  
39 ploughing, aggregates are partly destroyed and physically protected SOM is exposed, the bio-  
40 available fraction becomes then bio-accessible and can be microbially oxidized causing SOM  
41 decline and consequent CO<sub>2</sub> emissions (Balesdent et al., 1998). Most of the SOM loss, in  
42 structured soils, is attributed to the destruction of large macro-aggregates which consist  
43 primarily of labile SOM (Emadi et al., 2009). The free or occluded labile (light fraction with  
44 a density lower than 1.6-2 g cm<sup>-3</sup>) SOM, consisting primarily of particulate organic matter  
45 (POM), is the fraction mostly affected by ploughing (Wagai et al., 2008), while the denser  
46 mineral fraction is mostly affected in the medium- to long-term time scale (Don et al., 2009).  
47 POM, unprotected though, is also most affected due to ploughing in structureless sandy soils.  
48 On the other hand, the conversion of cultivated fields to grasslands, shrublands or forests has  
49 been shown to sequester carbon in soils (Guo and Gifford, 2002), which is primarily as  
50 attributable to POM changes in the topsoil (Potter and Derner, 2006). Soussana et al (2004)  
51 have reported that it takes twice as long to restore the carbon content in restored grasslands  
52 compared to the time it takes to lose the carbon through cultivation (i.e. ploughing). The re-  
53 formation of soil aggregates after such land use change indicates the ‘soil resilience’ potential  
54 (Lal, 1997). Particle aggregation provides structure to soils and has been related to soil  
55 fertility. An “agronomically valuable” soil is a soil where greater than 60% of the particle  
56 mass is in the range between 0.25 and 10 mm (Banwart et al., 2011). The extent of soil  
57 degradation and restoration depends on management practices as well as the factors that  
58 determine soil structure and aggregation (Angers and Carter, 1996) which includes SOM  
59 content, soil mineralogy, and clay content, soil macrofauna (e.g. earthworms), and root, root  
60 hair and (vesicular-arbuscular) mycorrhizal hyphal densities, as well as the climate under

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which the soil was formed. Understanding the factors controlling land use effects on soil particle aggregation is therefore very important in order to improve SOM modelling and soil restoration techniques (Rees et al., 2005; Angers and Carter, 1996). Physical fractionation schemes can be used to appraise the effect of land use change on particle aggregation and estimate the fractions of carbon such as the humus and particulate fractions that can be used to calibrate the turnover of carbon with mathematical models.

The turnover of SOM is usually described with multi-compartment models. Many soil carbon models have been utilized in the scientific literature and comparison of their structure and modeling results can be found in review papers (e.g. Nikolaidis and Bidoglio 2011; Battle-Aguilar et al., 2010; Manzoni and Porporato, 2009; Smith et al., 1997; Shibu et al., 2006; Fallon and Smith, 2000). These models have been used to simulate SOM turnover of discrete soil organic carbon pools using either default or calibrated decomposition rate constants mostly without accounting for the uncertainty that arises from the initial conditions, model parameters, inputs and model structure with very few exceptions (Juston et al., 2010; Paul et al., 2003). However, models can be greatly constrained by using field measured carbon pools to initialize and calibrate the model. For example physical fractionation schemes like dispersing and sieving (Krull et al., 2005) as well density fractionation (Zimmermann et al., 2007) have been used to measure modeled carbon pools in RothC carbon model. The complexity of the interrelationships among the carbon turnover model parameters and inputs require a modeling framework to assess the uniqueness of solution and the uncertainties due to model structure, initial conditions, model parameters and input data.

The objective of this study was twofold: a) develop a procedure for model parameter estimation applied to cases of cropland to fallow conversions (through initialization and calibration with field derived physical fractionation data) where the litter/manure input has not been measured and b) quantify the uncertainties in RothC modeling results.

## 86 **2. Methodology**

87 A Microsoft Excel version of the Rothamsted Carbon Model-version RothC-26.3 (Coleman  
88 and Jenkinson, 1999) was developed and used in conjunction with a statistical simulation  
89 package @RISK (PALISADES Corp.) in order to simulate the uncertainty in topsoil carbon  
90 turnover during cropland to set aside from crop production (which was cultivated in the past  
91 and was left uncultivated with native vegetation) conversion in Iowa (grassland) and Greece  
92 (shrubland). Field data derived by a physical fractionation scheme were used to initialize and  
93 calibrate the model.

### 94 **2.1. Study sites**

95 Two sites were chosen with paired plots of adjacent cropland and set aside fields. The first  
96 site (indicated as IA) was Iowa City, IA, USA (41°45'N, 91°44'W, 230 m), indicative of  
97 humid continental climate with soils with coarse texture-sandy loam. Mean annual  
98 temperature in the region is 10 °C (21.6±3.1 °C, MAY – SEP) and mean annual precipitation  
99 923 mm (60%, MAY - SEP). The second site (indicated as GR) was in the northern part of  
100 Chania Prefecture, Crete, Greece (39°25'N, 51°41'E, 10 m), where typical semi-arid,  
101 Mediterranean climate dominates and soils have finer texture-clay loam. Mean annual  
102 temperature in the region is 18 °C (12.5±3 °C, NOV – MAR) and mean annual precipitation  
103 652 mm (82%, NOV-MAR). Although, during the winter period in Iowa soil is covered by  
104 snow there is a long wet and warm cropping period from May to September, while in Greece  
105 most of the precipitation is taking place during the winter time, from November to March  
106 where the temperature is low and therefore warm cropping period is dry.

107 Soils in both sites were recent alluvial depositions. Iowan soils were very deep, poorly  
108 drained soils, formed in colluvium alluvial fans, and characterized as colo-ely (Udolls  
109 Mollisols or Phaeozems-FAO, 1998). Udolls are more or less freely drained Mollisols of

110 humid climates that naturally were tall grass prairies and are used as croplands. Cretan  
111 alluvial deposits (Quaternary formations) are shallower than those at Iowa and characterized  
112 as calcaric Regosols-known also as para-rendzinas, or Entisols in the American System  
113 (FAO, 1998). Regosols are frequently associated with Leptosols and Arenosols and are soils  
114 with very limited soil development. Regosols and Phaeozems are used as arable lands and  
115 correspond to 7.1% and 9.7% of the total world area used for arable cultivation (which is 18  
116 % of total world land cover) (The World Factbook, 2008).

117 The Iowa arable field was disk plowed to about 30 cm soil depth and used for the production  
118 of corn and soybeans. Prior to being set-aside, about 20 years ago, the Iowa uncultivated field  
119 received the same management. Mollisols in the Midwest have been cultivated for more than  
120 150 years. However, most of the organic matter decline occurred by the 1960 and has been at  
121 steady state under production practices in place since then (David et al., 2009). The Greek  
122 arable field was used for the production of green vegetables and tillage was lighter and  
123 shallower compared to Iowa. The Greek field had been set aside from crop production about  
124 35 years. For modelling purposes, the soils have been assumed to be at steady state in terms  
125 of carbon content, however this assumption is inherently uncertain.

126 Soil sampling was designed based on the objective to simulate the evolution of soil carbon  
127 from cultivation to set aside conditions. Most of the changes in aggregation and SOM storage  
128 are known to take place in the topsoil due to plant rooting depth (Potter and Derner, 2006).  
129 Typical rooting depth of Mediterranean shrublands found in Greece was 10 cm (Beier et al.,  
130 2009). Organic carbon has been found to concentrate in the top 10 cm and a sharp decline has  
131 been observed in the subsoil of both tilled ( $2.1\pm 0.5$  times) and no tilled ( $1.6\pm 0.3$  times) fields  
132 of olive groves in the alluvial plain of Koiliaris River Basin (Fig. S1 in the supporting  
133 information, data taken from the 'soiltrec' project ([www.soiltrec.eu](http://www.soiltrec.eu)) field campaign). On the  
134 other hand although the rooting depth in Iowa grassland is deeper most of the root biomass is

135 found in the topsoil. Studies have indicated that 73-87% of the total root biomass (0-125 cm)  
136 across different plants in Iowa was found in the 0-35 cm (Tufekcioglu et al., 2003), while  
137 90% of prairie root biomass (0-50 cm) was found in the 0-25 cm and 60% in the upper 5-cm  
138 (Buyanovsky et al., 1987). In order to compare the results between Greece and Iowa, surface  
139 soil was sampled from 0-10 in both croplands and set aside fields. Each sample was a  
140 composite of five representative subsamples. Bulk density was calculated using 100 cm<sup>3</sup>  
141 cores. The 10-30 cm soil depth was also sampled from the Iowa soils in order to account for  
142 the greater rooting depth and validate the changes in total SOM storage. Recent plant residues  
143 and stones were removed from the fresh soil samples by hand. The samples were air dried  
144 and stored in a cool-dry place not more than 2-3 months until further analysis. Subsoil  
145 samples were measured for SOM content and bulk density.

## 146 **2.2. Physical fractionation**

147 The methodological approach was based on a physical fractionation scheme (Fig. S2 in the  
148 supporting information) described in detail in the supporting information material. Briefly,  
149 the soils were separated into five (slake resistant) water stable aggregate (WSA) fractions  
150 according to the procedure based on Elliott (1986): i) large macro-aggregates (>2000 µm), ii)  
151 medium macro-aggregates (1000-2000 µm), iii) small macro-aggregates (250-1000 µm), iv)  
152 micro-aggregates (53-250 µm), and v) silt-clay sized micro-aggregates and minerals (<53  
153 µm). Mean weight diameter (MWD) was also calculated as an index of aggregate stability. In  
154 order to reveal possible differences in the composition and turnover rates of the macro-  
155 aggregates of different sizes and the patterns of the decomposition sequence the  
156 microaggregate isolation procedure outlined in Lichter et al., (2008) was applied to both  
157 small macro-aggregates (250-1000 µm) and composite macro-aggregates (>250 µm) samples.  
158 Sub-samples of these aggregates were separated into the following fractions: i) coarse  
159 particulate organic matter and sand (cPOM: >250 µm), ii) micro-aggregates (mM: 53-250

160  $\mu\text{m}$ ), and iii) easily dispersed silt-clay fractions (sc-M  $<53 \mu\text{m}$ ). The mM fraction as well 53-  
161  $250 \mu\text{m}$  sized aggregates (micro-aggregates) were further separated to fine particulate organic  
162 matter and sand (fPOM:  $53\text{-}250 \mu\text{m}$ ) and silt-clay fraction of the micro-aggregate (sc-mM  
163  $<53 \mu\text{m}$ ). All fractions were measured for their content in C and N.

164 Analytical methods used for the physicochemical soil characterization and further soil  
165 characteristics are presented in the supporting information material.

### 166 **2.3. Carbon turnover modeling**

167 A Microsoft Excel version of the Rothamsted Carbon Model-version RothC-26.3 (Coleman  
168 and Jenkinson, 1999) was developed. An initial excel version was provided to us by  
169 Todorovic et al (2010). The calculation of RothC 'abc' parameters (described in the  
170 following paragraph) was added in the excel version we developed. The model was crossed  
171 verified with the original exe of RothC-26.3 (Coleman and Jenkinson, 1999) with the default  
172 values of model parameters and gave identical results. No structural changes were made.

173 RothC is based on a monthly time step calculation and can simulate SOC turnover over a  
174 period ranging from a few years to a few centuries. Soil organic carbon is split into four  
175 active pools which decompose by a first-order process with its own characteristic rate and an  
176 amount of inert organic matter (IOM) resistant to decomposition. The four active  
177 compartments are Decomposable Plant Material (DPM), Resistant Plant Material (RPM),  
178 Microbial Biomass (BIO) and Humified Organic Matter (HUM). The model default  
179 decomposition rate constants ( $k$ ,  $\text{years}^{-1}$ ) for each compartment are: DPM: 10.0, RPM: 0.3,  
180 BIO: 0.66, and HUM: 0.02. The decomposition rate constants are corrected by a rate  
181 modifying factor for temperature (a), the topsoil moisture deficit rate modifying factor (b),  
182 and the soil cover factor (c). The model apportions plant litter input between DPM and RPM  
183 using the factors reported by Coleman and Jenkinson (1999); i.e. 1.44 for grassland and 0.67

184 for shrubland. Both DPM and RPM decompose to form CO<sub>2</sub>, BIO and HUM. The proportion  
185 that goes to CO<sub>2</sub> and to BIO and HUM is determined by the clay content of the soil. The BIO  
186 and HUM is then split into 46% BIO and 54% HUM. BIO and HUM both decompose to form  
187 more CO<sub>2</sub>, BIO and HUM.

188 The meteorological data used in the modeling exercise (average monthly mean temperature,  
189 precipitation, and open pan evaporation) were obtained by the Local Climate Estimator (New  
190 LocClim 1.10, 2006) and are presented in Table S1 in the supporting information material.  
191 Soil thickness was set to 10 cm. The set-aside fields were covered the whole year by grass  
192 (IA) and shrubs (GR). Field measured SOC and POM content, derived by the applied  
193 physical fractionation scheme were used to estimate initial carbon pools and calibrate the  
194 model, since the C of POM (particle sizes >50 μm) has been associated with the RPM and  
195 DPM pool of RothC (Galdo et al., 2003; Krull et al., 2005; Gottschalk et al., 2010). Initial  
196 SOC was partitioned among the different carbon pools (RPM, DPM, BIO, IOM and HUM)  
197 using the following approach. The POM content is the sum of RPM and DPM fractions  
198 apportioned by calibration. Following RothC-26.3-model recommendations, BIO was  
199 assumed to be 3% of the total SOC and IOM using the equation suggested by Falloon et al.  
200 (1998):  $IOM = 0.049SOC^{1.139}$ . For the estimation of IOM content, we used the set-aside SOC  
201 content since the inert carbon it is not usually considered to change significantly because of  
202 cultivation in a few decades. However, due to the uncertainty of this assumption in the  
203 uncertainty analysis the range of the IOM pool is from zero to the value taken by the  
204 Falloon's equation. Finally, the HUM pool was calculated by difference of the rest pools  
205 from the total SOC.

206 The developed excel version of the RothC model was used in combination with @RISK  
207 (PALISADES Corp.) in order to simulate the uncertainty in carbon turnover during set-aside  
208 conditions due to initial conditions and model parameters and inputs. First, RothC/@RISK

209 was used in a Monte-Carlo fashion in order to identify the optimal/unique solution of model  
210 parameters and input that best simulates each soil. Plant litter input and six model parameters  
211 were considered simultaneously with uniform distributions (Table 4). Since the plant litter  
212 had not been measured, the appropriate range found in the literature for the specific climate  
213 and land use was used to constrain the model. The carbon input of plant residues for the set-  
214 aside field in Iowa (grassland) was the sum of average values for the above and below-  
215 ground (0-15 cm) potential input for recently restored grasslands in south central Iowa as 5 to  
216 10 t C ha<sup>-1</sup> (Guzman et al., 2010); while within this range has been also found the maximum  
217 litter residue (7.59 t C ha<sup>-1</sup>) in prairies in central Missouri (Buyanovsky et al., 1987). Beier et  
218 al (2009) found in six shrublands across Europe that plant litter ranged from 1.0 to 5.3 t C ha<sup>-1</sup>  
219 for the 0-20 cm soil depth and above ground litter contributed 14.7 to 62.3% (0.33 to 1.43 t  
220 C ha<sup>-1</sup>). If we assume that 80% of the belowground litter was found in the 0-10 cm, which  
221 was the main rooting depth in all six sites as it is indicated in the study, the plant litter should  
222 range from 1 to 4.5 t C ha<sup>-1</sup>. Similar values for litterfall in Mediterranean shrublands (0.65-  
223 1.45 t C ha<sup>-1</sup>, assuming that carbon content of litterfall biomass is 50%, as it is indicated by  
224 the study) were reported by Fioretto et al (2003). An expanded range based on the values that  
225 have been reported in the literature was introduced for the RPM (0.1 to 0.8 y<sup>-1</sup>) and HUM  
226 (0.0001 to 0.3 y<sup>-1</sup>) decomposition rate constants. For the remaining model parameters (DPM  
227 and BIO decomposition rate constants, DPM-to-RPM ratio, and BIO%, the range was  
228 established as ±10% of their default RothC values.

229 Monte-Carlo simulations with 5000 iterations were conducted using the distributions of the  
230 plant input and the six model parameters described above. The solution with the lowest  
231 deviation (<±1%) from both SOC and POM measurements was considered as the optimum  
232 solution and the values of the parameters that generated the solution as the calibrated values.  
233 The assemble of solutions falling within the ±5% of the SOC and POM field measured values

234 was also examined in order to confirm the uniqueness of the optimum solution. This  
235 assumption correspond to 5% standard uncertainty of the mean SOC and POM field  
236 measured value and stand for  $\pm 11\%$  standard deviation of the SOC and POM field measured  
237 values, if it is assumed that five samples were taken and measured separately. The expanded  
238 range of plant input as well RPM and HUM decomposition rate constants was narrowed  
239 (Table 4), so as more iterations to pass the criterion and calculate better statistics. In  
240 particular, the standard deviations of the selected solutions of the plant input and the six  
241 model parameters were calculated and their values were compared with the initial  
242 distributions. Low standard deviations suggest the uniqueness of the optimum solution since  
243 the system is inherently constrained and does not allow solutions with extreme combinations  
244 of parameter values.

245 Once the model was calibrated, sensitivity analysis was conducted for the six model  
246 parameters (DPM, RPM, BIO, and HUM decomposition rate constants, DPM-to-RPM ratio,  
247 HUM%) and the plant litter input using ranges of  $\pm 10\%$  and  $\pm 50\%$  of the calibrated values.

248 Finally, the propagation of uncertainty in the simulated results due to initial conditions (SOC,  
249 DPM, RPM, BIO, HUM, and IOM carbon pools), plant litter input and soil clay content, as  
250 well the six model parameters used for calibration was conducted for each category  
251 separately and all together. The distribution for each parameter derived from the Monte-Carlo  
252 simulation for the iterations falling within the  $\pm 5\%$  of the SOC and POM field measured  
253 values was used in the uncertainty analysis. Field variability for initial conditions and clay  
254 content could not be estimated since soil samples were only one composite of five  
255 subsamples. To overcome this issue, the distribution was selected to be normal having as  
256 mean the calibrated value and a standard deviation of 5% of the mean. The uncertainty of the  
257 IOM pool was considered to be a uniform distribution ranging from zero to the value  
258 obtained by Falloon's equation. The HUM pool was calculated by difference.

### 259 3. Results and Discussion

#### 260 3.1. Soil Characterization

261 The basic physicochemical soil measurements are presented in table 1. The pH of the set-  
262 aside IA soil was almost neutral (6.9), while the cropland soil pH was acidic due to  
263 fertilization (6.2). The pH of both Greek soils was basic (7.7-7.8) reflecting the calcareous  
264 composition of the soils. The macro-element abundances (K, Ca, Mg, Na) were generally  
265 increased, under set-aside conditions and this was also depicted in the increase of the soluble  
266 salts and the cation exchange capacity (CEC). Potential mineralizable N, potential soluble  
267 organic N and C, as well carbohydrate C increased in the set-aside soils by a factor of 4.9,  
268 3.5, 2.9, and 2.7 for Iowa and only 1-1.5 times for Greece.

269 Set-aside from crop production in Iowa and Greek soils for 20 and 35 years, respectively, as  
270 it was indicated by the field measurements resulted in similar rates of C increase (0.777 and  
271 0.648 t C ha<sup>-1</sup> y<sup>-1</sup>) in topsoil (10 cm), while the N increase was doubled for Iowa as compared  
272 with Greece (0.048 and 0.019 t N ha<sup>-1</sup> y<sup>-1</sup>). The measured increase of SOM was accompanied  
273 by 19 and 6 % decrease of soil bulk density for IA and GR, respectively. Subsoil carbon  
274 density in set aside and cropland fields in the Iowa site (Table 1) was found to differ only by  
275 about 2%, verifying our assumption that most of the SOM gain found in the topsoil where the  
276 dense root system is found (Potter and Derner, 2006). Therefore, accounting also for the  
277 subsoil (0-30 cm) the increase for the Iowa soil was found to be similar for C (0.736 t C ha<sup>-1</sup>  
278 y<sup>-1</sup>; 0-30 cm), being however double for nitrogen (0.088 t N ha<sup>-1</sup> y<sup>-1</sup>; 0-30 cm). The rates were  
279 similar with values for arable to grasslands conversions reported for French sites (0.48±0.26 t  
280 C ha<sup>-1</sup> y<sup>-1</sup>) (Soussana et al., 2004) as well sites in UK (0.3 to 0.8 t C ha<sup>-1</sup> y<sup>-1</sup>) (Ostle et al.,  
281 2009). The C-to-N ratio was lower in croplands compared to set-aside fields, likely indicating  
282 less stabilized SOM. The C-to-N ratios of the SOM increase in set-aside fields was found to

283 be higher in Greece compared to Iowa, indicating the effect of the plant litter material quality  
284 (shrubland in Greece versus grassland in Iowa) and possibly less decomposed SOM.

285 Table 2 presents the (sand-free) WSA distribution of the four soils. The total WSA were  
286 found to be higher in set-aside fields by 68 % (IA) and 10 % (GR) compared to croplands.  
287 The increase of WSA weight and stability in set-aside soils was primarily attributed to large  
288 and medium sized macro-aggregate. The shift in composition to larger aggregates was very  
289 significant in the Iowa set-aside soil, where macro-aggregates increased by 110% comprising  
290 82% of soil composition compared to cropland where they were 39%. In Greece, the increase  
291 was smaller (46%) and macro-aggregates comprised 66% in the set-aside soil. At the same  
292 time, decline was observed in the micro-aggregate and the silt-clay sized fractions. The shift  
293 in composition to larger aggregates caused an increase in the MWD by 108% (IA) and 52.5%  
294 (GR) in Iowa and Greece, respectively (Table 2).

295 Table 2 also presents the C and N content in the various aggregate fractions. Soil C in IA  
296 cropland was 16.8 g kg<sup>-1</sup> with 60% in macro-aggregate fraction, while in IA set-aside was  
297 35.5 g kg<sup>-1</sup> with 90% in macro-aggregate fraction. Similarly, soil C in GR cropland was 29.1  
298 g/kg with 58% in macro-aggregate fraction, while in GR set-aside was 52.2 g kg<sup>-1</sup> with 71%  
299 in macro-aggregate fraction. Similar patterns were also observed for N.

300 The C and N concentration increased up to 2 times in the aggregate fractions in the Greek set-  
301 aside soil compared to cropland (Fig. 1). The C content of the easily dispersed silt-clay sized  
302 fraction (sc-M), the micro-aggregate related silt-clay fraction (sc-mM) and the free silt-clay  
303 sized aggregates in the Greek set-aside soil were found to be significantly higher compared to  
304 the respective concentrations of cropland, while the N content was similar. The increase of C  
305 and N concentration in macro-aggregates was attributed both to POM and mineral fractions.  
306 The POM contribution in the composite macro-aggregate fraction was 44 and 51% in set-  
307 aside and cropland field and 38 and 34 % in the 250-1000 µm fraction.

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308 In Iowa set-aside soil increase of C and N concentration was observed only in macro-  
309 aggregates while in finer aggregates presented lower concentration (Fig. 1). The latter has  
310 been also observed by Emadi et al (2009). The micro-aggregate isolation confirmed that all  
311 the mineral fractions in Iowa set aside field exhibited lower concentrations compared to the  
312 cropland. Don et al., 2009 also found that the conversion of cropland into grassland in a soil  
313 with very low clay content (5-7%) in contrast with a rich in clay soil (30%) did not resulted in  
314 the increase of the mineral-associated carbon fraction as it was limited by total clay surface  
315 area available for carbon stabilization. The increase of C and N concentration in macro-  
316 aggregates was attributed to POM. The C content of the composite and the 250-1000  $\mu\text{m}$   
317 aggregate fraction attributed to POM in the Iowa cropland was 19% and 23%, respectively  
318 and changed to 63% and 50% in the set-aside field.

319 The C-to-N ratio increased in aggregate fractions of set aside soils, indicating less  
320 decomposed SOM as compared to croplands. The relative increase was found to be higher in  
321 Greece compared to Iowa, indicating the effect of the plant material (shrubs) and possibly  
322 less decomposed SOM. More labile SOM indicated by higher C-to-N ratio (Elliot, 1986) was  
323 found in macro-aggregates, attributed to the increase of C-to-N ratio of the POM fractions in  
324 Iowa and both POM and mineral fractions in Greece.

325 As the size and stability of water stable aggregates decreases, their turnover rate has been  
326 found to increase (Six et al., 2000). The ratio of the fPOM-C to cPOM-C has been suggested  
327 (Six et al., 2000) as an indication of turnover rate (the higher the ratio, the lower the turnover  
328 rate). The fPOM-C to cPOM-C ratio of the composite macro-aggregates (IA: 7.9, GR: 6.4)  
329 and the small macro-aggregates (IA: 9.5, GR: 7.5) in cropland soils is lower as compared  
330 with set aside fields (composite macro-aggregates IA: 10, GR: 13.2 and small macro-  
331 aggregates IA: 23.4, GR: 10.2). Therefore the turnover rate is higher in croplands due to  
332 tillage and plant litter input quality. In addition, the estimated turnover rates in Iowa are

333 significantly lower for the small macro-aggregates compared with the composite macro-  
334 aggregates suggesting in accordance with the lower C-to-N ratio in the 250-1000  $\mu\text{m}$  fraction  
335 more decomposed and older POM. On the contrary, this pattern was not observed in Greece,  
336 where C-to-N ratio was high even in micro-aggregates, indicating possible lower  
337 decomposition rates.

### 338 **3.2. Carbon turnover modeling**

339 The clay content for the IA soil was 7% (Table 1), the initial SOC  $18.6 \text{ t C ha}^{-1}$  and the initial  
340 POM  $2.6 \text{ t C ha}^{-1}$  (Table 3). The initial carbon apportioning for the Iowa soil procedure was  
341  $1.94 \text{ t C ha}^{-1}$  for RPM,  $0.68 \text{ t C ha}^{-1}$  for DPM,  $0.56 \text{ t C ha}^{-1}$  for BIO, and  $2.63 \text{ t C ha}^{-1}$  for  
342 IOM. By difference the initial HUM pool was  $12.78 \text{ t C ha}^{-1}$ . The simulation results were  
343 compared with the results presented in Table 3 for the set-aside field (SOM =  $33 \text{ t C ha}^{-1}$ ,  
344 POM =  $20 \text{ t C ha}^{-1}$ ). The clay content for the GR soil was 30% (Table 1), the initial SOC  $34.3$   
345  $\text{t C ha}^{-1}$  and the initial POM  $14.3 \text{ t C ha}^{-1}$  (Table 3). The initial carbon apportioning for the  
346 Greek soil procedure was  $14 \text{ t C ha}^{-1}$  for RPM,  $0.3 \text{ t C ha}^{-1}$  for DPM,  $1.03 \text{ t C ha}^{-1}$  for BIO,  
347 and  $5.05 \text{ t C ha}^{-1}$  for IOM. By difference the initial HUM pool was  $13.89 \text{ t C ha}^{-1}$ . The  
348 simulation results were compared with the results presented in Table 3 for the set-aside field  
349 (SOM =  $58.5 \text{ t C ha}^{-1}$ , POM =  $21.8 \text{ t C ha}^{-1}$ ). Table 4 presents the Monte-Carlo simulation  
350 results where the plant litter input and model parameters were allowed to vary. The optimum  
351 solution was used in order to obtain the calibrated model results (Table 4). The calibrated  
352 values for Iowa were  $5.05 \text{ t C ha}^{-1}$  for plant litter input and  $0.34 \text{ y}^{-1}$  for RPM and  $0.27 \text{ y}^{-1}$  for  
353 HUM, while for Greece were  $3.79 \text{ t C ha}^{-1}$  for plant litter input and  $0.21 \text{ y}^{-1}$  for RPM and  
354  $0.0041 \text{ y}^{-1}$  for HUM.

355 The statistics of the assemble of simulations that fall within  $\pm 5\%$  of both SOC and POM  
356 measurements are also presented in Table 4. The distribution type in most of the parameters  
357 was found to be triangle and some of them uniform and beta general. The standard variation

358 was found to be less than 10% of the mean value for all parameters apart from the RPM  
359 decomposition rate constant where it was 13.9%, in the case of Iowa. Similarly, in Greece,  
360 the standard variation was found to be less than 10% of the mean value for all parameters  
361 while for the RPM and HUM decomposition rate constants was 10.6% and 58.1%,  
362 respectively. The narrow values of the resulted standard deviations confirm the uniqueness  
363 of optimum solution since they represent a well constrained system that does not allow  
364 acceptable solutions with extreme parameter value combinations.

365 For Iowa, the ‘adjusted’ model was able to capture the increase of POM and SOC content  
366 very well, which was likely attributed to POM material. Monthly decomposition rates for  
367 RPM and HUM pools for both sites are presented in Table S2 in the supporting information  
368 material. The annual decomposition rate for RPM was found to be  $0.093 \text{ y}^{-1}$  and  $0.105 \text{ y}^{-1}$  for  
369 Iowa and Greece, respectively, while for HUM was  $0.075 \text{ y}^{-1}$  and  $0.002 \text{ y}^{-1}$ , likewise. The  
370 high decay constant of the HUM pool in Iowa could be attributed to the very low clay content  
371 in accordance with Balesdent et al. (1998) and Gottschalk et al. (2010). Balesdent et al.  
372 (1998) showed that SOC in the size fraction  $<50 \mu\text{m}$  is made up of the relatively rapidly  
373 decomposing pool of silt associated C, and a relatively slowly decomposing pool of clay  
374 associated C. The measured clay turnover associated C had a decay constant of  $0.03 \text{ y}^{-1}$   
375 (Balesdent et al., 1998), while the silt associated C had a measured decay constant of  $0.12 \text{ y}^{-1}$ ,  
376 declining almost as rapidly as that in the POM fraction, especially under cultivation  
377 (Gottschalk et al., 2010). Nevertheless, the wet and warm summers in Iowa stimulated  
378 organic matter decomposition.

379 Similarly, in Greece, the ‘adjusted’ model was able to capture the increase of POM, HUM  
380 and SOC content, and the results were consistent with the fractionation measurements where  
381 the increase in carbon stock was attributed to both POM and HUM material. Lower HUM  
382 decomposition rate in Greece is attributed to dry climatic conditions and high clay content

383 which result in higher protection. Slaking of the soil surface can result in fine soil particles  
384 moving into inter-aggregate pores in the surface area, which can reduce the infiltration rate of  
385 rainfall or irrigation water and reduce hydraulic conductivity (Hillel, 1998). In addition,  
386 residue quality (lignin/N ratio, C/N ratio and N concentration) of shrublands affects the rate  
387 of decomposition and the aggregation rate (Aerts, 1997; Sainju et al., 2003).

388 Sensitivity analysis at the  $\pm 10\%$  and  $\pm 50\%$  ranges of the calibrated values for the six model  
389 parameters and the plant input was conducted. The tornado graphs are presented in Figure 2  
390 and the sensitivity coefficients (the absolute value of the ratio:  $(\Delta Y/Y)/(\Delta x/x)$ ) in Table S3 in  
391 the supporting information material. In both sites the total plant litter input and the RPM  
392 decomposition rate constant had the highest sensitivity. In Iowa, the BIO% as well as the BIO  
393 and DPM decomposition rate constants presented the lowest sensitivity with coefficients  
394 lower than 0.1 and 0.07 for the 50% and 10% case, respectively. In Greece, the coefficients  
395 were found to be in general lower than 0.04 for HUM, BIO and DPM decomposition rate  
396 constants for both the 50% and 10%. The results emphasize the importance of measurement  
397 of plant litter input when conducting carbon sequestration studies as well as the necessity of  
398 developing a methodology to model carbon sequestration in soils in the absence of such  
399 measurements.

400 A comparison of the optimum simulation for both SOC and POM and the uncertainties  
401 attributed to the initial conditions (SOC, DPM, RPM, BIO, HUM, and IOM carbon pools) the  
402 input data (plant litter input and clay content), and the six model parameters used for  
403 calibration parameters (DPM, RPM, BIO, and HUM decomposition rate constants, DPM-to-  
404 RPM ratio, HUM%), in terms of the mean of the assemble of Monte Carlo simulations plus  
405 one standard deviation are presented in Figure 3. In addition, Figure S3 presents the  
406 distribution of cumulative accumulation of total carbon after 100 years of set aside conditions  
407 due to uncertainties in initial condition, input and parameter values as well as the expected

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408 accumulation from the optimum simulation. In Iowa, the uncertainty of prediction (90% of  
409 the distribution, range between 5 and 95%) due to input data, model parameters, and initial  
410 conditions corresponded to 43.3%, 51%, and 14.3% of the total carbon sequestered in the  
411 100 years, respectively and the probability of over predicting carbon sequestration was at the  
412 same order 0.94, 0.25, and 0, respectively. Similarly, the uncertainty of POM due to input  
413 data, model parameters, and initial conditions corresponded to 25.5%, 42.4%, and 0% of the  
414 total increase in POM in the 100 years, respectively and the probability of over predicting  
415 POM was 0.94, 0.21, and 1, respectively. In Greece, the uncertainty of prediction due to input  
416 data, model parameters, and initial conditions corresponded to 42.1%, 49.5%, and 13% of the  
417 total carbon sequestered in the 100 years, respectively and the probability of over predicting  
418 carbon was 0.69, 0.24, and 0.12, respectively. Similarly, the uncertainty of POM due to input  
419 data, model parameters, and initial conditions corresponded to 90.5%, 102%, and 0% of the  
420 total increase in POM in the 100 years, respectively and the probability of over predicting  
421 POM was 0.7, 0.29, and 0. The Iowa soil was projected to sequester 17.5 t C ha<sup>-1</sup> and the  
422 Greek soil 54 t C ha<sup>-1</sup> in 100 years. Overall, the uncertainty of the model predictions for SOC  
423 and POM (65.6% and 140% of the total carbon sequestered and POM increase for Iowa and  
424 70.8% and 51.6% of the total SOC and POM increase for Greece, respectively) were quite  
425 significant, while the probability of over predicting SOC and POM (0.4 and 0.64 for Iowa  
426 and 0.31 and 0.46 for Greece) suggested that the mean of the Monte Carlo simulation  
427 distributions were close to the optimum simulation. Overall, our analysis showed that the  
428 resulting uncertainties are important; hampering our ability to accurately predict carbon  
429 sequestration under set aside conditions (even in the absence of climate change impacts).  
430 These results emphasize the necessity of obtaining accurate plant input data and other  
431 physical soil parameters as well as quantifying the variability of initial conditions in order to  
432 reduce the uncertainty of carbon sequestration projections.

#### 433 4. Conclusions

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3 434 A Monte Carlo based methodology was developed to assess the uniqueness of solution  
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5 435 carbon sequestration modeling of cultivated to set aside conditions and the uncertainties due  
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8 436 to initial conditions, model parameters and time series of the RothC carbon model.

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11 437 ○ POM-carbon data obtained by soil physical fractionation were used successfully to  
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13 438 initialize and calibrate the carbon model and provided boundary conditions to  
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15 439 effectively constrain the solution of the model.

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18 440 ○ The calibrated RPM decomposition rate constants in Iowa, similarly with the HUM  
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21 441 decomposition rate constants, was higher (13.3 %) and in Greece lower (31%) than  
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23 442 the defaults rates suggesting that commonly used RothC model functions for  
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25 443 decomposition rate correction due to temperature systematically underestimate  
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27 444 decomposition at low temperatures and overestimate decomposition at high  
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31 445 temperatures.

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34 446 ○ The calibrated HUM decomposition rate constants were significantly different than  
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36 447 the defaults rates in both Iowa (13.5 times higher) and Greece (48.8 times lower). The  
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38 448 significant difference between the two sites is attributed to significant differences in  
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41 449 clay content. Finer texture in Greece resulted in higher protection of the HUM pool  
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43 450 and presumably lower ‘apparent’ sensitivities (Davidson and Janssens, 2006, Kleber  
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46 451 and Johnson, 2010) to temperature and/or moisture effect on decomposition in  
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48 452 contrast with the coarser Iowa site where the opposite pattern was observed.

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51 453 ○ Model sensitivity analysis revealed that total plant litter input and the RPM  
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53 454 decomposition rate constant exhibited the highest sensitivity on predicted SOC in  
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56 455 both sites.

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456       ○ Uncertainty analysis suggested that total uncertainty of carbon sequestration under set  
457       aside from crop production conditions over 100 years can be as much as 70% of the  
458       total amount that was sequestered in both the Greek and Iowa soil. In both sites plant  
459       litter input was a major source of uncertainty. The uncertainty analysis results  
460       underlie the importance of obtaining accurate plant input data for set aside land uses  
461       for the various climates in the world in order to minimize carbon sequestration  
462       estimates and determine more accurate the variability of carbon transformation rate  
463       constants as a function of climatic conditions.

464       The methodology developed in this study can be used to assess the factors affecting carbon  
465       sequestration in agricultural soils, quantify the uncertainties in predictions as well as assist in  
466       the design of field experiments and measurements to minimize the uncertainties and improve  
467       carbon sequestration estimates that relate directly to soil fertility.

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### 478       **Supplementary Information**

479 Additional supporting information may be found in the online version of this article. Methods  
1  
2 480 on physical fractionation and soil physicochemical analysis, soil organic carbon content in  
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5 481 topsoil and subsoil in Koiliaris River Basin (Fig. S1), physical fractionation scheme used in  
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7 482 the study (Fig. S2), the output distributions by the Monte-Carlo simulations for the  
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10 483 calculation of uncertainties. (Fig. S3), meteorological data used for the application of the  
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12 484 RothC model (Table S1), monthly and annual decomposition rates for RPM and HUM pools  
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15 485 (Table S2), and sensitivity coefficients for RothC model parameters (Table S3).  
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## 20 487 **References**

- 23 488 Aerts, R., 1997. Climate, Leaf Litter Chemistry and Leaf Litter Decomposition in Terrestrial  
24  
25  
26 489 Ecosystems: A Triangular Relationship. *Oikos* 79, 439-449.
- 28 490 Angers D.A., Carter, M.R., 1996. Aggregation and organic matter storage in cool, humid  
29  
30  
31 491 agricultural soils. In: Carter M.R., Stewart B.A., eds. *Structure and organic matter storage in*  
32  
33 492 *agricultural soils*. Boca Raton, FL: Lewis Publ, 193-211.
- 35 493 Balesdent, J., Besnard, E., Arrouays D., Chenu, C., 1998. The dynamics of carbon in particle-  
36  
37  
38 494 size fractions of soil in a forest-cultivation sequence. *Plant and Soil* 201, 49–57.
- 40 495 Banwart, S., Bernasconi, S.M., Bloem, J., Blum, W., Brandao, M., Brantley, S., Chabaux, F.,  
41  
42  
43 496 Duffy, C., Kram, P., Lair, G., Lundin, L., Nikolaidis, N., Novak, M., Panagos, P.,  
44  
45 497 Ragnarsdottir, K.V., Reynolds, B., Rousseva, S., de Ruyter, P., van Gaans, P., van Riemsdijk,  
46  
47  
48 498 W., White, T., Zhang, B., 2011. Assessing Soil Processes and Function in Critical Zone  
49  
50 499 Observatories: hypotheses and experimental design. *Vadose Zone Journal* 10, 974-987.
- 52 500 Batlle-Aguilar, J., Brovelli, A., Porporato, A., Barry, D.A., 2010. Modelling soil carbon and  
53  
54  
55 501 nitrogen cycles during land use change: a review. *Agronomy for Sustainable Development*  
56  
57 502 31, 251-274.  
58  
59  
60  
61  
62  
63  
64  
65

1 503 Beier, C., Emmett, B.A., Tietema, A., Schmidt, I.K., Penuelas, J., Lang, E.K., Duce, P., De  
2 504 Angelis, P., Gorissen, A., Estiarte, M., de Dato, G.D., Sowerby, A., Kröel-Dulay, G., Lellei-  
3  
4 505 Kovács, E., Kull, O., Mand, P., Petersen, H., Gjelstrup, P., Spano, D., 2009. Carbon and  
5  
6  
7 506 nitrogen balances for six shrublands across Europe. *Global Biogeochemical Cycles*, 23  
8  
9 507 (GB4008), p. 13. ISSN 0886–6236.  
10  
11 508 Bouyoucos, G.J., 1936. Directions for making mechanical analysis of soils by the hydrometer  
12  
13 509 method. *Soil Science* 4, 225-228.  
14  
15  
16 510 Buyanovsky, G.A., Kucera, C.L., Wanger, G.H., 1987. Comparative analyses of carbon  
17  
18 511 dynamics in native and cultivated ecosystems. *Ecology* 68, 2023-2031.  
19  
20  
21 512 Coleman, K., Jenkinson, D.S., 1999. RothC-26.3 - A Model for the turnover of carbon in soil:  
22  
23 513 Model description and windows users guide: November 1999 issue. Lawes Agricultural Trust  
24  
25 514 Harpenden. ISBN 0 951 4456 8 5  
26  
27  
28 515 David, M.B., McIsaac, G.F., Darmody, R.G., Omonode, R.A., 2009. Long-Term Changes in  
29  
30 516 Mollisol Organic Carbon and Nitrogen. *Journal of Environmental Quality*, 38, 200–211.  
31  
32  
33 517 Davidson, E.A., Janssens, I.A. 2006. Temperature sensitivity of soil carbon decomposition  
34  
35 518 and feedbacks to climate change. *Nature* 440, 165–173.  
36  
37  
38 519 Don, A., Scholten, T., Schulze E.D., 2009. Conversion of cropland into grassland:  
39  
40 520 Implications for soil organic-carbon stocks in two soils with different texture. *Journal of Plant*  
41  
42 521 *Nutrition and Soil Science* 172, 53–62  
43  
44  
45 522 Elliott, E.T., 1986. Aggregate structure and carbon, nitrogen, and phosphorus in native and  
46  
47 523 cultivated soils. *Soil Science Society of America Journal* 50, 627-633.  
48  
49  
50 524 Emadi, M., Baghernejad, M., Memarian, H.R., 2009. Effect of land-use change on soil  
51  
52 525 fertility characteristics within water-stable aggregates of two cultivated soils in northern Iran.  
53  
54  
55 526 *Land Use Policy* 26, 452–457.  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

527 Falloon, P.D., Smith, P., 2000. Modelling refractory soil organic matter. *Biology and Fertility*  
1  
2 528 *of Soils* 30, 388-398.  
3  
4 529 Falloon, P., Smith, P., Coleman, K., Marshall, S., 1998. Estimating the size of the inert  
5  
6  
7 530 organic matter pool from total soil organic carbon content for use in the Rothamsted Carbon  
8  
9  
10 531 model. *Soil Biology and Biochemistry* 30, 1207–1211.  
11  
12 532 FAO 1998. World Reference Base for Soil Resources. (<http://www.fao.org/ag/agl/agll/wrb/>)  
13  
14 533 Fioretto, A., Papa, S., Fuggi, A. 2003. Litter-fall and litter decomposition in a low  
15  
16  
17 534 Mediterranean shrubland. *Biology and Fertility of Soils* 39, 37–44  
18  
19 535 Galdo, I.D., Six, J., Peressotti, A., Cotrufo, M.F., 2003. Assessing the impact of land-use  
20  
21  
22 536 change on soil C sequestration in agricultural soils by means of organic matter fractionation  
23  
24 537 and stable C isotopes. *Global Change Biology* 9, 1204–1213.  
25  
26  
27 538 Gottschalk, P., Bellarby, J., Chenu, C., Foereid, B., Smith, P., Wattenbach, M., Zingore, S.,  
28  
29 539 Smith, J., 2010. Simulation of soil organic carbon response at forest cultivation sequences  
30  
31 540 using <sup>13</sup>C measurements. *Organic Geochemistry* 41, 41–54.  
32  
33  
34 541 Guo, L.B., Gifford, R.M., 2002. Soil carbon stocks and land use change: a meta analysis.  
35  
36 542 *Global Change Biology* 8, 345–360.  
37  
38  
39 543 Guzman, J.G., Al-Kaisi, M.M., 2010. Soil Carbon Dynamics and Carbon Budget of Newly  
40  
41 544 Reconstructed Tall-grass Prairies in South Central Iowa. *Journal of Environmental Quality*  
42  
43 545 39, 136–146.  
44  
45  
46 546 Hillel, D. 1998. *Environmental Soil Physics*. Academic Press. San Diego, CA  
47  
48  
49 547 Juston, J., Andrén, O., Kätterer, T., Jansson, P.E., 2010. Uncertainty analyses for calibrating a  
50  
51 548 soil carbon balance model to agricultural field trial data in Sweden and Kenya. *Ecological*  
52  
53 549 *Modeling* 221, 1880-1888.  
54  
55  
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54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

550 Kleber, M., Johnson, K.J., 2010. Advances in Understanding the Molecular Structure of Soil  
551 Organic Matter: Implications for Interactions in the Environment. *Advances in Agronomy*  
552 106, 77-142.

553 Krull, E.S., Skjemstad, J.O., Burrows, W.H., Bray, S.G., Wynn, J.G., Bol, R. et al. 2005.  
554 Recent vegetation changes in central Queensland, Australia: Evidence from  $\delta^{13}C$  and  $^{14}C$   
555 analyses of soil organic matter. *Geoderma* 12, 241–259.

556 Lal R. 1997. Residue management, conservation tillage and soil restoration for mitigating  
557 greenhouse effect by CO<sub>2</sub>-enrichment. *Soil and Tillage Research*, 43, 81-107.

558 Lichter, K., Govaerts, B., Six, J., Sayre, K.D., Deckers, J., Dendooven, L., 2008. Aggregation  
559 and C and N contents of soil organic matter fractions in a permanent raised-bed planting  
560 system in the highlands of Central Mexico. *Plant and Soil*, 305, 237-252.

561 Manzoni, S., Porporato, A., 2009. Soil carbon and nitrogen mineralization: Theory and  
562 models across scales, *Soil Biology and Biochemistry* 41, 1355-1379.

563 *Methods of Soil Analysis, Part 3- Chemical Methods*, 2nd Ed. Rev. Soil Science Society of  
564 America, Black, C.A et al., 1982.

565 Nikolaidis, N.P., Bidoglio, G., 2011. Conceptual modeling of soil organic matter and  
566 structure dynamics: a synthesis. *Geoderma* (submitted revisions).

567 Ostle, N.J., Levy, P.E., Evans, C.D., Smith, P., 2009. UK land use and soil carbon  
568 sequestration-Review. *Land Use Policy* 26S, S274–S283.

569 Paul, K.I., Polglase, P.J., Richards, G.P., 2003. Predicted change in soil carbon following  
570 afforestation or reforestation, and analysis of controlling factors by linking a C accounting  
571 model (CAMFor) to models of forest growth (3PG), litter decomposition (GENDEC) and soil  
572 C turnover (RothC). *Forest Ecology and Management* 177, 485-501.

1 573 Piccolo, A., Zena, A., Conte, P., 1996. A comparison of acid hydrolyses for the determination  
2 574 of carbohydrate content in soils. *Communication in Soil Science and Plant analysis* 27, 2909-  
3  
4 575 2915.  
5  
6  
7 576 Potter, K.N., Derner, J.D., 2006. Soil carbon pools in central Texas: Prairies, restored  
8  
9 577 grasslands, and croplands. *Journal of Soil and Water Conservation* 61, 124-128.  
10  
11 578 Rees, R.M., Bingham, I.J., Baddeley, J.A., Watson, C.A., 2005. The role of plants and land  
12  
13 579 management in sequestering soil carbon in temperate arable and grassland. *Geoderma* 128,  
14  
15 580 130-154.  
16  
17  
18  
19 581 Sainju, U.M., Whitehead, W.F., Singh. B.P., 2003. Cover crops and nitrogen fertilization  
20  
21 582 effects on soil aggregation and carbon and nitrogen pools. *Canadian Journal of Soil Science*  
22  
23 583 83, 155–165.  
24  
25  
26 584 Six, J., Elliott, E.T., Paustian, K., 2000. Soil macroaggregate turnover and microaggregate  
27  
28 585 formation: a mechanism for carbon sequestration under no tillage agriculture. *Short*  
29  
30 586 *Communication in Soil Biology and Biochemistry* 32, 2099-2103.  
31  
32  
33 587 Shibu, M.E., Leffelaar, P.A., Van Keulen, H., Aggarwal, P.K., 2006. Quantitative description  
34  
35 588 of soil organic matter dynamics – A review of approaches with reference to rice-based  
36  
37 589 cropping systems. *Geoderma*, 137, 1-18.  
38  
39  
40  
41 590 Smith, P., Powlson, D.S., Smith, J.U., Elliott, E.T., 1997. Evaluation and comparison of soil  
42  
43 591 organic matter models using datasets from seven long-term experiments. *Geoderma* 81, 1-  
44  
45 592 225.  
46  
47  
48 593 Soussana, J.F., Loiseau, P., Vuichard, N., Ceschia, E., Balesdent, J., Chevallier, T., Arrouays,  
49  
50 594 D., 2004. Carbon cycling and sequestration opportunities in temperate grasslands. *Soil Use*  
51  
52 595 *and Management* 20, 219-230.  
53  
54  
55 596 The World Factbook, 2008 (<https://www.cia.gov/library/publications/the-world-factbook/>)  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

1 597 Todorovic, G.R., Stemmer, M., Tatzber, M., Katzlberger, C., Spiegel, H., Zehetner, F.,  
2 598 Gerzabek, M.H., 2010. Soil-carbon turnover under different crop management: Evaluation of  
3  
4 599 RothC-model predictions under Pannonian climate conditions. *Journal of Plant Nutrition and*  
5  
6  
7 600 *Soil Science* 173, 662–670.  
8  
9 601 Tufekcioglu, A., Raich, J.W., Isenhardt, T.M., Schultz R.C., 2003. Biomass, carbon and  
10  
11 602 nitrogen dynamics of multi-species riparian buffers within an agricultural watershed in Iowa,  
12  
13  
14 603 USA. *Agroforestry Systems* 57, 187-198.  
15  
16 604 Wagai, R., Mayer, L.M., Kitayama, K., Knicker, H., 2008. Climate and parent material  
17  
18 605 controls on organic matter storage in surface soils: three-pool, density-separation approach.  
19  
20  
21 606 *Geoderma* 147, 23-33.  
22  
23 607 Zimmermann, M., Leifeld, J., Schmidt, M.W.I., Smith, P., Fuhrer, J., 2007. Measured soil  
24  
25 608 organic matter fractions can be related to pools in the RothC model. *European Journal of Soil*  
26  
27  
28 609 *Science* 58, 658–667.  
29  
30

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## List of Figure Captions

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612

613 **Fig. 1.** a) C and b) N concentration of the sand free aggregate fractions ( $\text{g kg}^{-1}$ ). Values are  
614 shown as means ( $n=3$ ). Standard deviation was lower than 1-3% for OC and 1-4% for TKN.  
615 Mean values followed by the same lowercase letter under the same series label are not  
616 significantly different ( $p<0.05$ ). Mean values followed by the same uppercase letter under the  
617 same x-axis legend are not significantly different ( $p<0.05$ ).

618 **Fig. 2.** Tornado Graphs for 10% (black indicated) and 50% (grey indicated) sensitivity  
619 analysis. RPM=resistant plant material decomposition rate constant, DPM=decomposable  
620 plant material decomposition rate constant, HUM=humus decomposition rate constant, BIO=  
621 biomass decomposition rate constant, DPM/RPM ratio=the apportionment ratio of plant litter  
622 input DPM and RPM carbon pools, BIO%=The proportion that goes to BIO (100-BIO% is  
623 the proportion that goes to HUM).

624 **Fig. 3.** Uncertainty of total organic carbon (TOTAL) and particulate organic matter (POM)  
625 due to model parameters, input data, and initial conditions, as well as the total uncertainty,  
626 compared with the optimum solution (calibration).

627

628 **Table 1.** Chemical and physical properties of IA and GR soils.

	Iowa		Greece	
	Cropland	Set-aside	Cropland	Set-aside
Bulk Density /kg m <sup>-3</sup>	1110/1217 <sup>a</sup>	930/1024 <sup>a</sup>	1180	1110
pH	6.2	6.9	7.8	7.7
Sand /%	38	63	30.1	27.4
Clay /%	7	7	30	30
OC <sup>b</sup> /% – BS <sup>c</sup> /AF <sup>d</sup> /Subsoil	1.70/1.68/1.59	3.70/3.55/1.85	2.94/2.90	5.17/5.27
N /% - BS/AF/Subsoil	0.14/0.14/0.11	0.27/0.26/0.17	0.24/0.22	0.32/0.31
C-to-N - BS/AF	11.7/11.7	13.6/13.8	12.3/12.9	16.4/16.9
Recovery /%-AF: OC/TKN	98.5/98.5	95.9/94.3	98.6/93.5	102/99.3
Soluble salts /mmhos cm <sup>-1</sup>	0.11	0.17	0.36	0.61
CEC /meq 100g <sup>-1</sup>	6.8	11.5	24.1	36.3
P /mg kg <sup>-1</sup>	113.1	106.1	343.9	56.5
K /mg kg <sup>-1</sup>	141.4	614.7	702.9	290.8
Ca /mg kg <sup>-1</sup>	2694.1	5548.8	9947.1	16087.7
Mg /mg kg <sup>-1</sup>	133.3	474.9	571.8	1046.1
S /mg kg <sup>-1</sup>	20.2	21.7	60.8	125.2
B /mg kg <sup>-1</sup>	1.4	2.2	5.5	7.9
Cu /mg kg <sup>-1</sup>	2.8	4.1	15.6	11.3
Fe /mg kg <sup>-1</sup>	462.5	327.8	165.3	305.0
Mn /mg kg <sup>-1</sup>	26.3	248.3	258.4	133.3
Zn /mg kg <sup>-1</sup>	4.4	11.6	11.2	5.9
Na /mg kg <sup>-1</sup>	34.3	53.0	76.0	234.3
Bicarb-P /mg kg <sup>-1</sup>	42.4	69.9	106.4	62.6
PMN /mg kg <sup>-1</sup>	15.8	78.3	31.2	43.9
PSO <sub>N</sub> /mg kg <sup>-1</sup>	21.4	74.4	23.5	36.0
PSOC /mg kg <sup>-1</sup>	132.7	390.7	228.9	360.8
Carbohydrate-C /mg kg <sup>-1</sup>	45.1	123.1	42.0	42.3

<sup>a</sup> For the 10-30 cm subsoil.

<sup>b</sup>The Set-aside and cropland GR exhibited 0.98 and 0.17 % inorganic carbon content, respectively, while the rest lower than 0.03 %.

<sup>c</sup>BS: Bulk Soil.

<sup>d</sup>AF: Aggregate Fractionation.

**Table 2.** Soil water stable aggregate distribution (WSA, g sand-free aggregate 100 g<sup>-1</sup> soil), particle recovery, and mean weight diameter (MWD) as well C and N distribution among aggregates fractions (g kg<sup>-1</sup>). Values are shown as means and standard deviation is given in the brackets (n=3). Mean values followed by the same lowercase letter within the same column are not significantly different (*p*<0.05). Mean values followed by the same uppercase letter within the same row are not significantly different (*p*<0.05).

	Cropland IA			Set-aside IA			Cropland GR			Set-aside GR		
	WSA	C	N	WSA	C	N	WSA	C	N	WSA	C	N
> 2000 μm	8.2 (4.8)b, C	1.5	0.11	34.6 (3.3)a, A	12.8	0.96	16.6 (1.1)b, B	6.0	0.46	29.6 (5.8)a, A	16.1	1.03
1000-2000 μm	0.4 (0.04)c, D	0.2	0.01	15.7 (2.5)b, A	6.5	0.47	2.9 (0.5)d, C	1.4	0.09	8.1 (1.6)c, B	4.5	0.27
250-1000 μm	30.2 (4.4)a, AB	8.3	0.77	31.4 (2.4)a, A	12.7	0.87	26.5 (1.8)a, B	9.3	0.67	27.9 (2.4)a, AB	16.8	1.00
53-250 μm	9.4 (2.4)b, C	5.1	0.41	5.9 (0.4)c, C	3.0	0.22	23.7 (0.6)a, A	9.5	0.78	15.9 (2.8)b, B	12.6	0.65
< 53 μm	5.3 (1.1)b, B	1.7	0.13	2.2 (0.3)d, C	0.5	0.04	10.1 (0.7)c, A	2.9	0.24	5.6 (0.7)c, B	2.8	0.17
Recovery (%)	94.5 (1.0)			93.1 (1.6)			91.5 (1.5)			94.6 (3.6)		
WSA	53.4 (1.8) B			89.8 (0.19) A			79.6 (0.4) C			87.2 (2.0) A		
WSA (>250 μm)	38.7 (4.0) B			81.7 (0.10) A			45.8 (1.5) D			65.5 (5.3) C		
MWD (mm)	1.16 (0.51) B			2.42 (0.24) A			1.36 (0.09) B			2.07 (0.38) A		

1 **Table 3.** Distribution of C and N in the particulate organic matter (POM) and in the silt-clay  
 2 fractions (t C ha<sup>-1</sup>).

	<b>Total</b>	<b>POM- fractions</b>	<b>Silt-clay fractions</b>
<b>C</b>			
Cropland IA	18.6	2.6	16.0
Set-aside IA	33.0	20.0	13.0
Cropland GR	34.3	14.3	20.6
Set-aside GR	58.5	21.8	36.8
<b>N</b>			
Cropland IA	1.6	0.2	1.3
Set-aside IA	2.4	1.2	1.2
Cropland IA	2.6	0.8	1.9
Set-aside IA	3.5	1.7	1.8

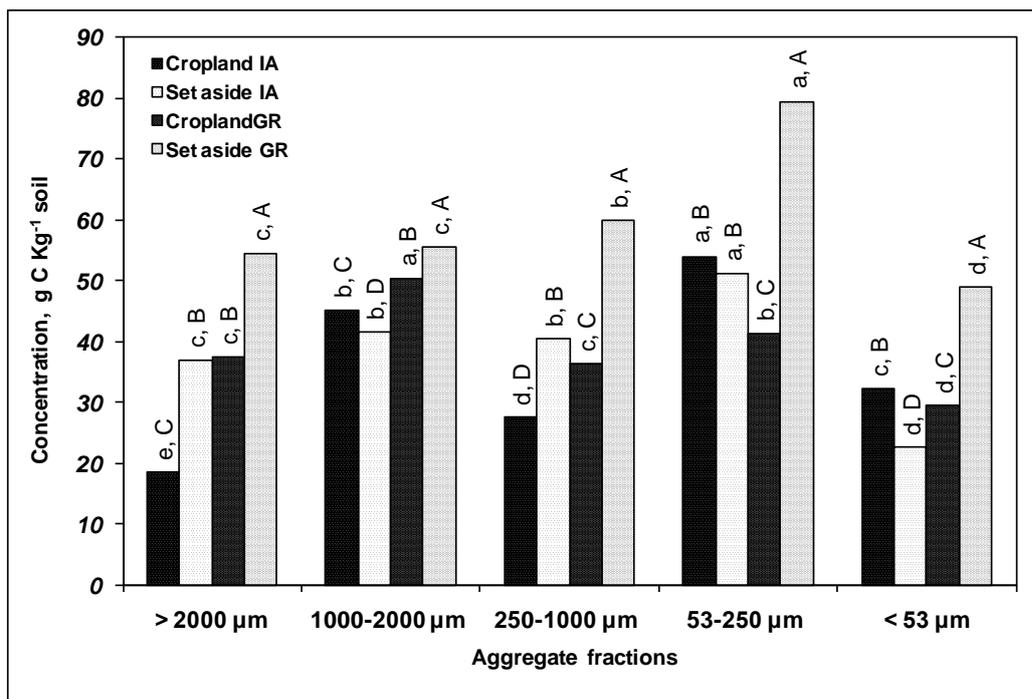
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1 **Table 4.** Parameter information about the input and output distributions for the Monte-Carlo  
 2 simulation and the optimum solutions.

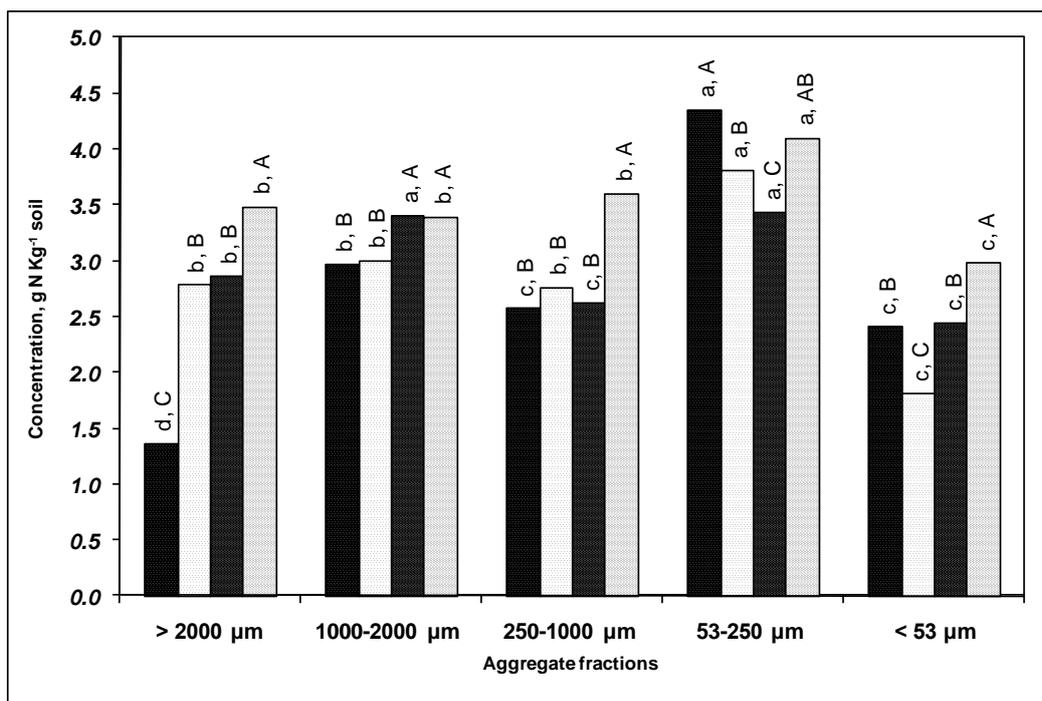
Parameter	total plant input	DPM/RPM ratio	BIO%	DPM	RPM	BIO	HUM
RothC default values	input	1.44 (grassland) 0.67 (shubland)	46	10	0.3	0.66	0.02
<b>IOWA</b>							
Input of MonteCarlo simulation							
distribution type	Uniform	Uniform	Uniform	Uniform	Uniform	Uniform	Uniform
min	5.00	1.30	41.40	9.00	0.30	0.59	0.10
max	10.00	1.58	50.60	11.00	0.80	0.73	0.30
Statistics for the iterations passed the criterion <sup>a</sup>							
distribution type	Triang	BetaGeneral	Triang	Triang	Triang	Triang	Triang
min	5.01	1.32	41.47	8.66	0.32	0.59	0.22
max	6.47	1.58	49.84	10.99	0.56	0.72	0.30
mean	5.50	1.44	47.05	10.21	0.40	0.68	0.27
std	0.35	0.09	1.97	0.55	0.06	0.03	0.02
Chi-sq	0.43	6.24	2.43	0.90	1.29	1.29	2.43
Optimum solution (<0.70%)	5.05	1.51	48.90	10.37	0.34	0.69	0.27
<b>GREECE</b>							
Input of MonteCarlo simulation							
distribution type	Uniform	Uniform	Uniform	Uniform	Uniform	Uniform	Uniform
min	2.00	0.60	41.40	9.00	0.10	0.59	0.0001
max	4.50	0.74	50.60	11.00	0.30	0.73	0.04
Statistics for the iterations passed the criterion <sup>a</sup>							
distribution type	Triang	BetaGeneral	Triang	Uniform	BetaGeneral	Uniform	Triang
min	2.95	0.60	41.41	8.99	0.14	0.59	0.00
max	4.49	0.73	50.64	10.97	0.26	0.72	0.02
mean	3.98	0.67	46.03	9.98	0.22	0.66	0.01
std	0.36	0.04	2.66	0.57	0.02	0.04	0.01
Chi-sq	7.44	9.26	10.69	3.36	3.81	6.76	2.00
Optimum solution (<0.30%)	3.79	0.67	44.95	10.45	0.21	0.60	0.0041

3 <sup>a</sup>Statistics for the iterations passed the criterion of SOC and POM falling within the  $\pm 5\%$  of  
 4 the field measured values.

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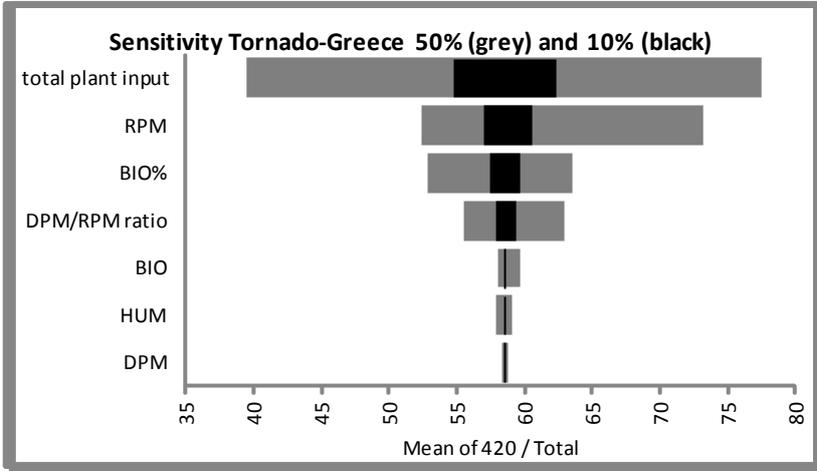
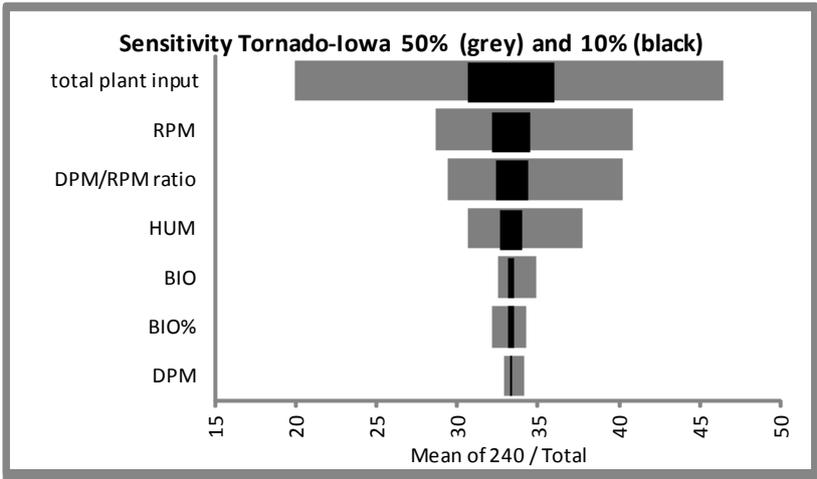


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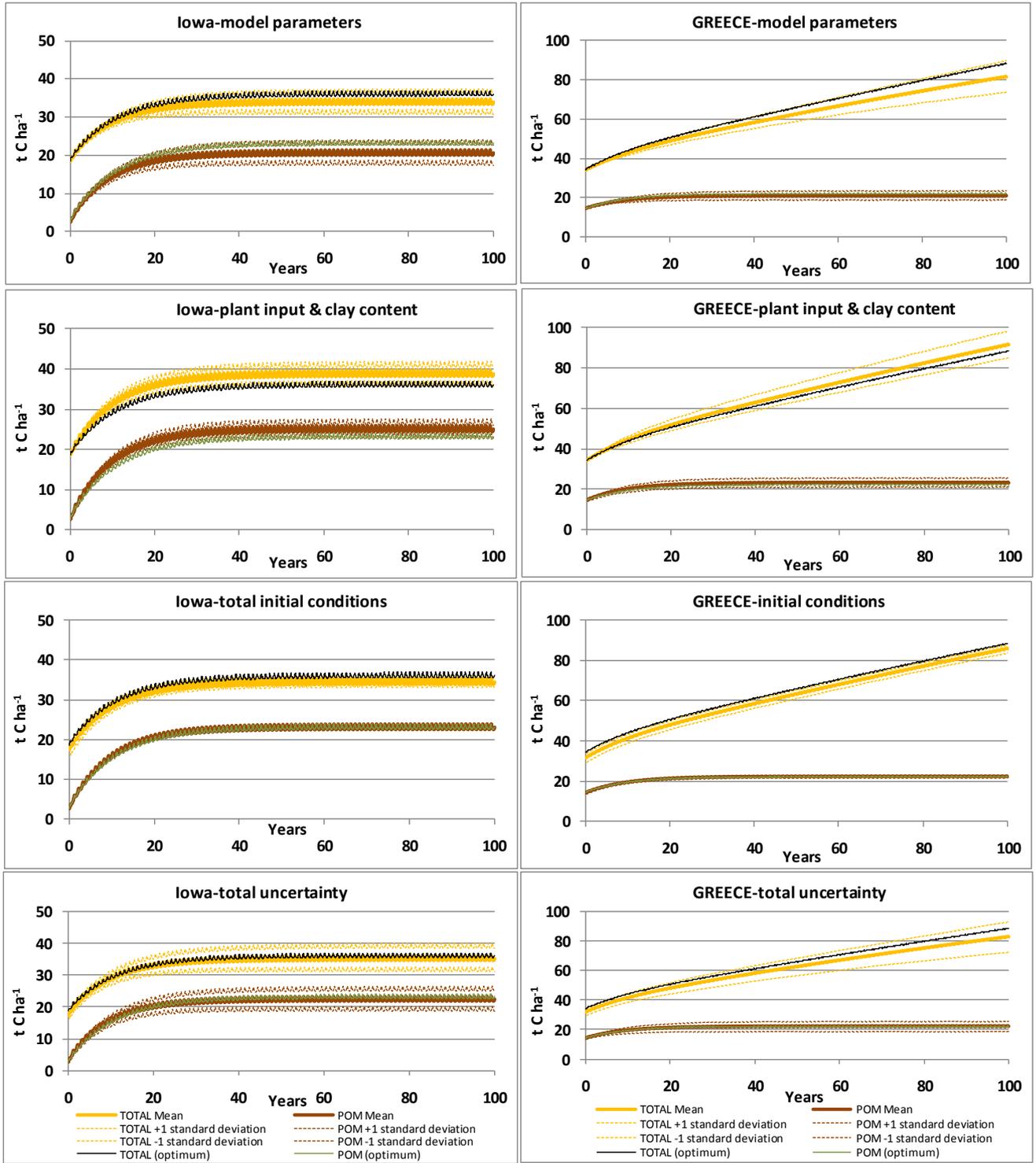
Fig. 1.



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2 Fig. 2.

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2 Fig. 3.

1 **SUPPLEMENTARY INFORMATION**

2

3 *Fotini E. Stamati, Nikolaos P. Nikolaidis, Jerald P. Schnoor*

4 **Modeling topsoil carbon sequestration in two contrasting crop production**

5 **to set aside conversions with RothC – calibration issues and uncertainty**

6 **analysis**

7 **15 pages, 3 Figures and 3 Tables.**

## 8 **S1. Methods on physical fractionation and soil physicochemical analysis**

9 The methodological approach was based on a physical fractionation scheme (Figure S2).  
10 Soils were separated in different water stable aggregate (WSA) fractions and the macro-  
11 aggregates were further separated in POM and smaller micro-aggregates. All fractions were  
12 measured for their content in C and N.

### 13 **S1.1. Aggregate fractionation procedure**

14 Bulk soil was gently sieved to pass through an 8 mm sieve and residual litter was removed.  
15 Then the soil was separated into five water stable aggregate (slake resistant) fractions  
16 according to the procedure based on Elliott (1986): i) large macro-aggregates ( $>2000\ \mu\text{m}$ ), ii)  
17 medium macro-aggregates ( $1000\text{-}2000\ \mu\text{m}$ ), iii) small macro-aggregates ( $250\text{-}1000\ \mu\text{m}$ ), iv)  
18 micro-aggregates ( $53\text{-}250\ \mu\text{m}$ ), and v) silt-clay sized micro-aggregates and minerals ( $<53$   
19  $\mu\text{m}$ ). Aggregate fractions were determined on triplicates, where air-dried soil (40 g) was  
20 quickly submerged in deionized water on top of the  $2000\ \mu\text{m}$  sieve (for 5 min), which was  
21 then moved up and down over 2 min with a stroke length of 3 cm for 50 strokes. The organic  
22 material floating on the water in the  $2000\ \mu\text{m}$  sieve was removed after the 2 min cycle  
23 because it is not considered SOM. Sieving was repeated on the  $1000\ \mu\text{m}$  (40 strokes),  $250\text{-}\mu\text{m}$   
24 ( $30$  strokes) and  $53\text{-}\mu\text{m}$  ( $10$  strokes) sieves using the soil plus water that passed through the  
25 next larger sieve. Clay from the  $53\ \mu\text{m}$  sieve was gently removed. Aggregates remaining on  
26 each sieve were oven-dried at ( $40^\circ\text{C}$ ), weighed and stored in glass jars at room temperature.  
27 Sand content was determined on aggregate fraction subsamples after dispersing soil in  
28 sodium hexametaphosphate (0.5%) for 18 h on a rotary shaker at 190 rpm. The samples were  
29 then passed the sieve corresponded to the lower limit of each aggregate size (e.g the  $1000\text{-}$   
30  $2000\ \mu\text{m}$  sized aggregated with the  $1000\ \mu\text{m}$  sieve) and the sand mass retained in the sieve  
31 was used for sand correction. Mean weight diameter (MWD) was calculated by summing the

32 weighted proportion of each aggregate fraction and was used as an index of aggregate  
33 stability.

### 34 **S1.2. Micro-aggregate isolation procedure**

35 In order to reveal possible differences in the composition and turnover rates of the macro-  
36 aggregates of different sizes and the patterns of the decomposition sequence the procedure  
37 was applied to both small macro-aggregates (250-1000  $\mu\text{m}$ ) and composite macro-aggregates  
38 ( $>250 \mu\text{m}$ ) samples. A subsample (10 g) of small macro-aggregates (250-1000  $\mu\text{m}$ ) and of  
39 composite macro-aggregates ( $>250 \mu\text{m}$ ) was further separated into the following fractions by  
40 the micro-aggregate isolation procedure outlined in Lichter et al., (2008): i) coarse particulate  
41 organic matter and sand (cPOM:  $>250 \mu\text{m}$ ), ii) micro-aggregates (mM: 53-250  $\mu\text{m}$ ), and iii)  
42 easily dispersed silt-clay fractions (sc-M  $<53 \mu\text{m}$ ). Briefly, the subsample of the aggregates  
43 was immersed in deionized water on top of a 250-  $\mu\text{m}$  mesh screen and gently shaken with  
44 50 glass beads (4 mm in diameter) with continuous and steady water flow through the device  
45 to ensure that micro-aggregates were immediately flushed onto a 53- $\mu\text{m}$  sieve and were not  
46 exposed to any further disruption by the beads (Six et al., 2000). After all macro-aggregates  
47 were broken up, the material on the 250  $\mu\text{m}$  sieve was collected (cPOM) and on the 53- $\mu\text{m}$   
48 sieve was sieved to ensure that the isolated micro-aggregates were water stable (mM). The  
49 fraction that passed through the 53  $\mu\text{m}$  sieve (sc-M) was also collected. The three fractions  
50 were oven-dried at (40°C), weighed and stored in glass jars at room temperature. The micro-  
51 aggregates within the macro-aggregates (mM) as well the 53-250  $\mu\text{m}$  sized aggregates  
52 (micro-aggregates) were similarly separated to fine particulate organic matter and sand  
53 (fPOM: 53-250  $\mu\text{m}$ ) and silt-clay fraction of the micro-aggregate (sc-mM  $<53 \mu\text{m}$ ). Using  
54 Lichter et al., (2008) as a guide, for this procedure we did not account for the separation of  
55 free POM and intra POM, as we observed that during the floatation procedure the macro-  
56 aggregates are collapsed and the separation cannot always be successful. Free POM which is

57 also reported as occluded POM is negligible in terms of mass (1% and 2% for agricultural  
58 and forest soils respectively), but has the same C-to-N ratio as the free fraction (John et al.,  
59 2005).

### 60 **S1.3. Physico-chemical analysis**

61 Soils were measured for dry bulk density (gravimetric method), pH and soluble salts  
62 (conductivity)-measured in a 1:2 soil to water ratio (Methods of Soil Analysis, 1982), and  
63 texture (Bouyoucos, 1936). Additional soil analysis conducted by A&L Analytical  
64 Laboratories, Inc., Memphis, TN, included P, K, Ca, Mg, S, B, Cu, Fe, Mn, Zn, Na and  
65 Bicarb-P. The KCl extraction (2 mol L<sup>-1</sup> KCl in a 1:5 soil-to-solution ratio) was used for the  
66 estimation of potential mineralizable N (PMN=NH<sub>3</sub>-N+NO<sub>3</sub>-N) of soils using a Hack 2010  
67 spectrophotometer for NO<sub>3</sub>-N (Cadmium Reduction Method, 8039) and NH<sub>4</sub>-N (Salicylate  
68 Method, 10023). Potential soluble organic nitrogen (PSON) was also measured by the  
69 Kjeldahl digestion technique (Nessler method, 8075). The potential soluble organic carbon  
70 (PSOC) was also estimated by a TOC analyzer (Shimadzu 5050), after the removal of  
71 inorganic carbon by air sparging for 10 min (Instruction Manual TOC-5050A, Shimadzu  
72 Corporation). The extracted pools were also measured for their content in carbohydrates  
73 colorimetrically using the phenol-sulfuric acid procedure (Piccolo et al., 1996). Bulk soil,  
74 aggregates, and fractions from the microaggregate isolation procedure were measured for  
75 their content in C by a TOC analyzer-solid sample module SSM-5000 (corrected for their  
76 inorganic C content) and N by the Kjeldahl digestion technique with a Hach digestahl  
77 digestion apparatus (Nessler method, 8075). The C and N content of the sc-mM fraction was  
78 not measured but estimated as the difference between the microaggregate (mM) and the  
79 fPOM.

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81 **References**

82 Dale, R.F., 1968. The climatology of soil moisture, evaporation, and non-moisture stress days  
83 for corn in Iowa. *Agr. Meteorol.* 5, 111-128.

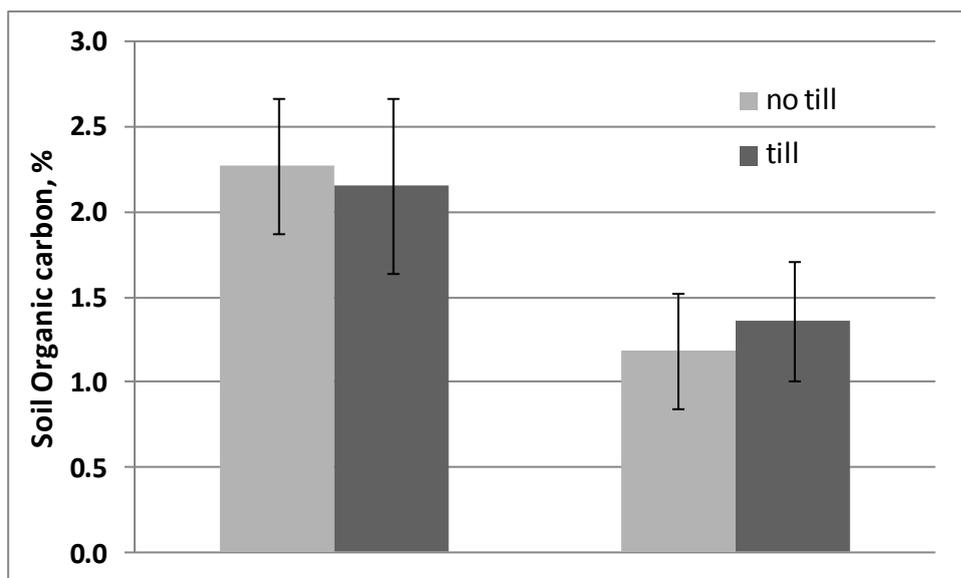
84 Elliott, E.T., 1986. Aggregate structure and carbon, nitrogen, and phosphorus in native and  
85 cultivated soils. *Soil Science Society of America Journal* 50, 627-633.

86 Instruction Manual Total Organic Carbon Analyzer Model TOC-5050A, Shimadzu  
87 Corporation, Environmental Instrumental Division.

88 John, B., Yamashita, T., Ludwig, B., and Flessa, H., 2005. Storage of organic carbon in  
89 aggregate and density fractions of silty soils under different types of land use. *Geoderma*  
90 128, 63-79.

91 Lichter, K., Govaerts, B., Six, J., Sayre, K.D., Deckers, J., Dendooven, L., 2008. Aggregation  
92 and C and N contents of soil organic matter fractions in a permanent raised-bed planting  
93 system in the highlands of Central Mexico. *Plant and Soil* 305, 237-252.

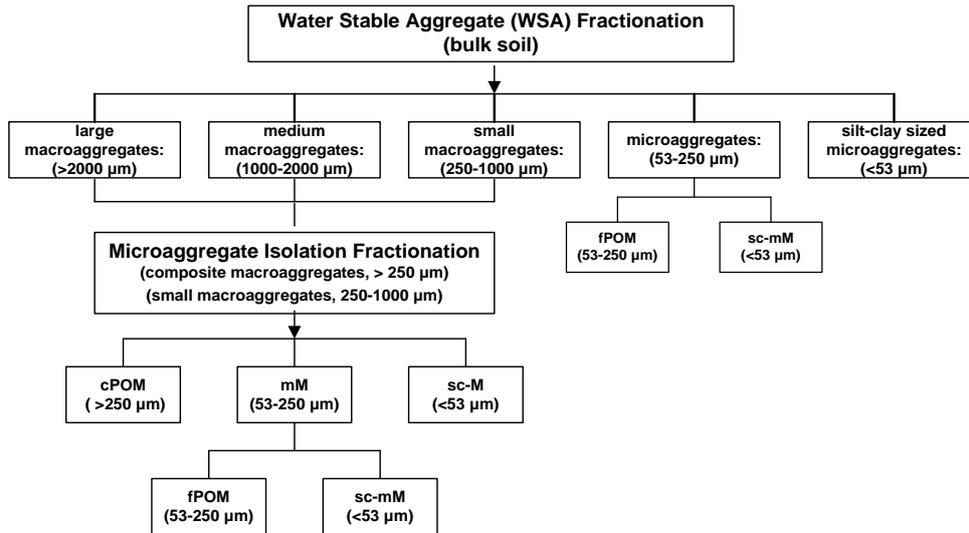
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95

96 **Fig. S1.** Soil organic carbon content in topsoil (0-15 cm) and subsoil (15-30) of tilled and no-  
 97 tilled olive groves fields (samples taken from the area between the trees) in the alluvial plain  
 98 of Koiliaris River Basin (average and standard deviation derived by three spatial samples).  
 99 Data taken from the ‘soiltrec’ project ([www.soiltrec.eu](http://www.soiltrec.eu)) field campaign.

100



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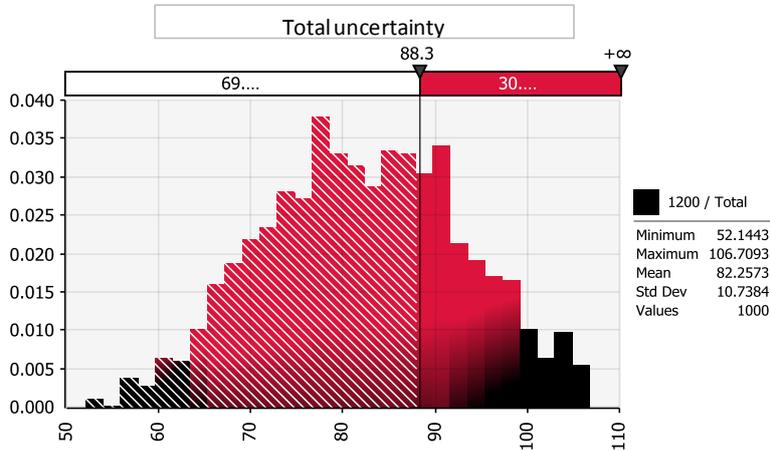
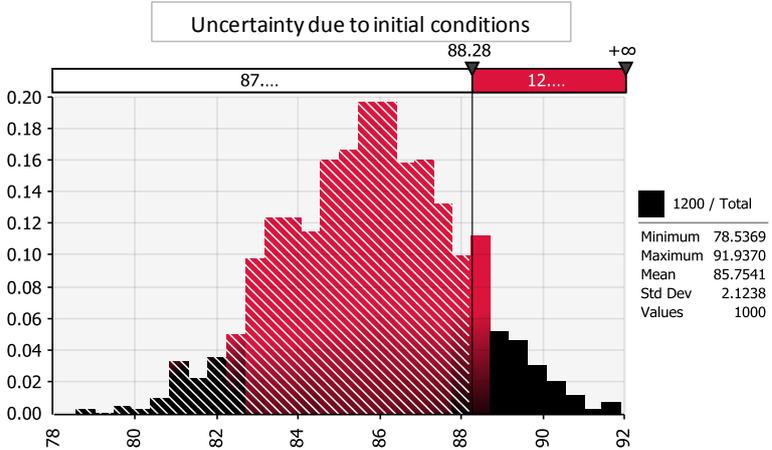
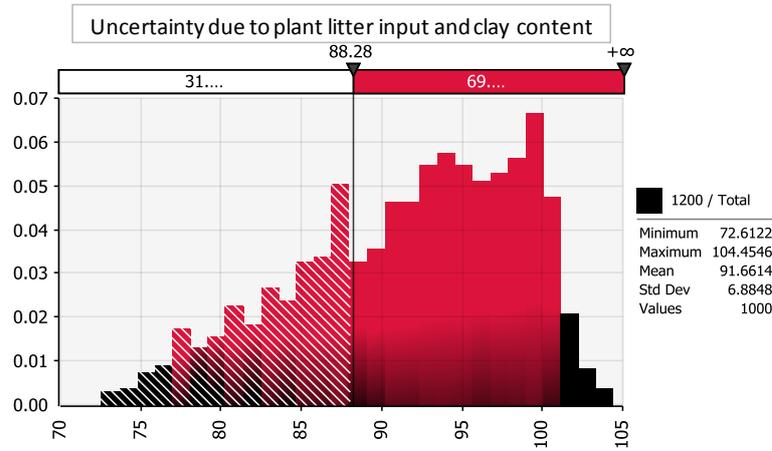
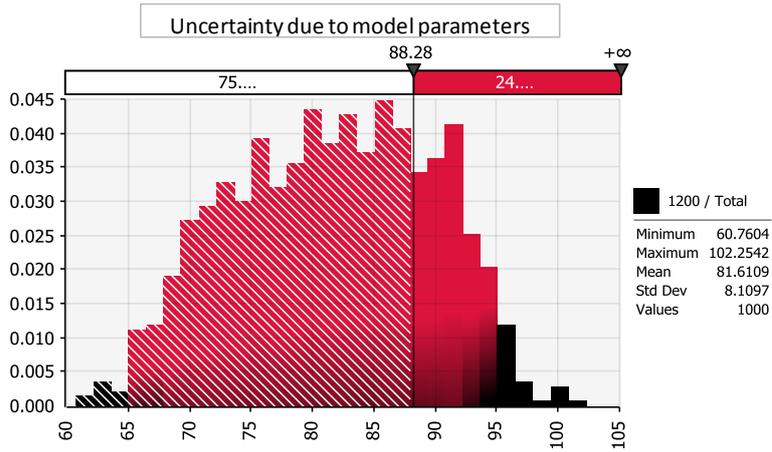
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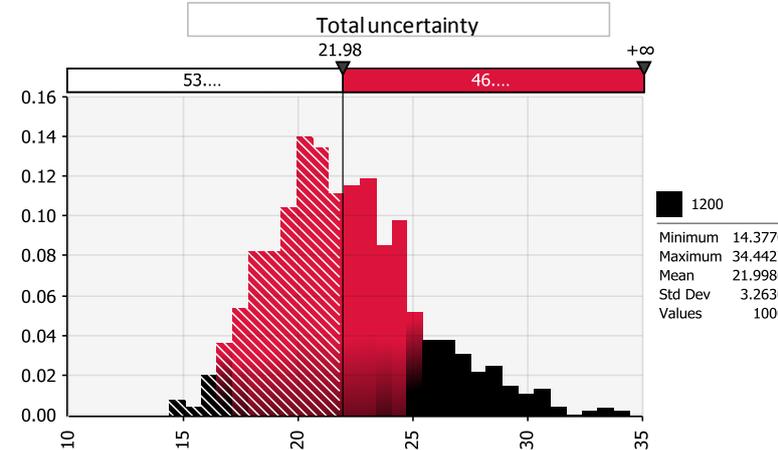
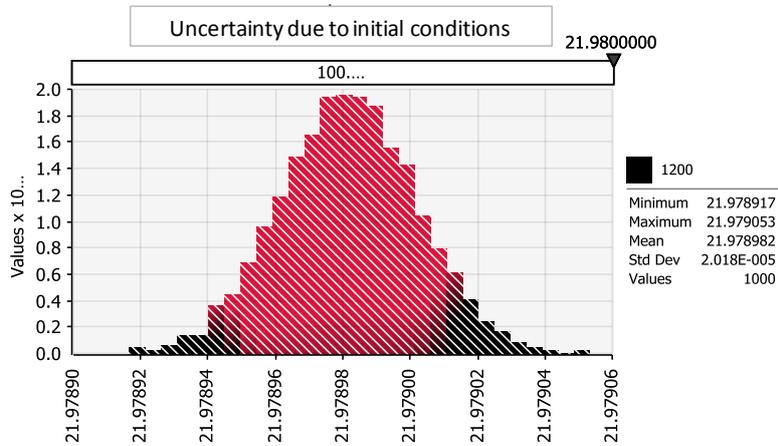
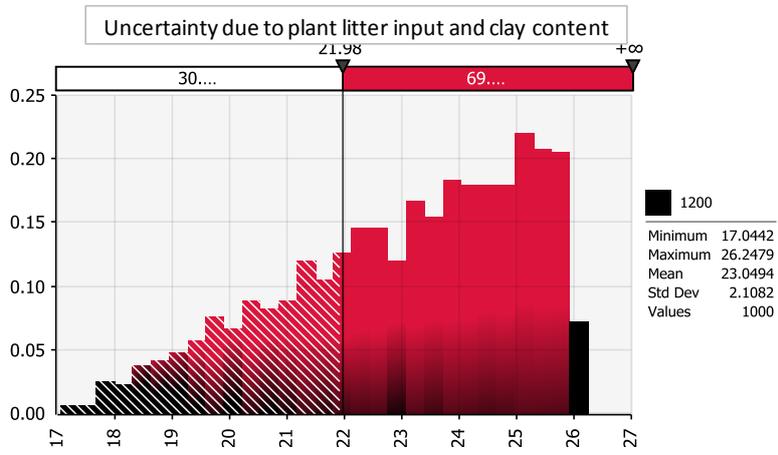
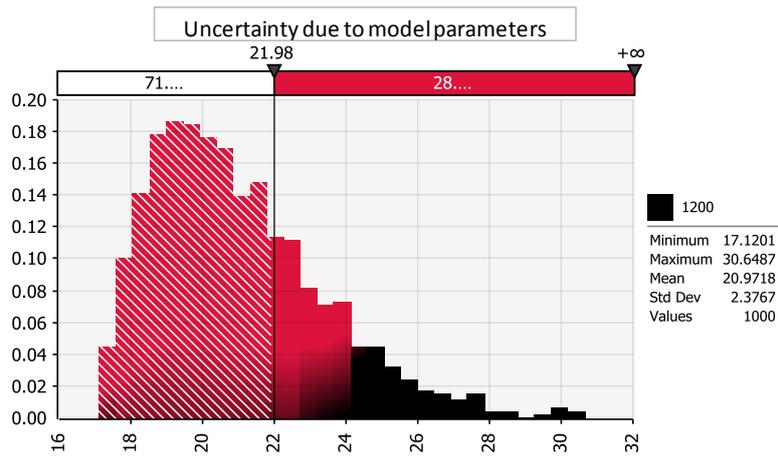
103 **Fig. S2.** Physical fractionation scheme used in the study. cPOM = coarse particulate organic

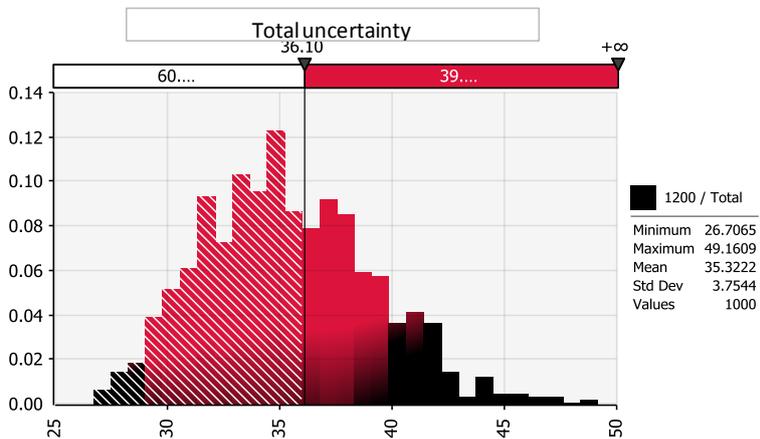
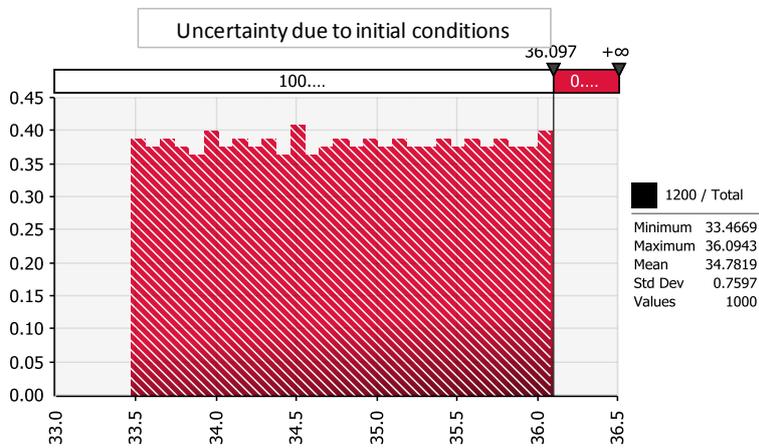
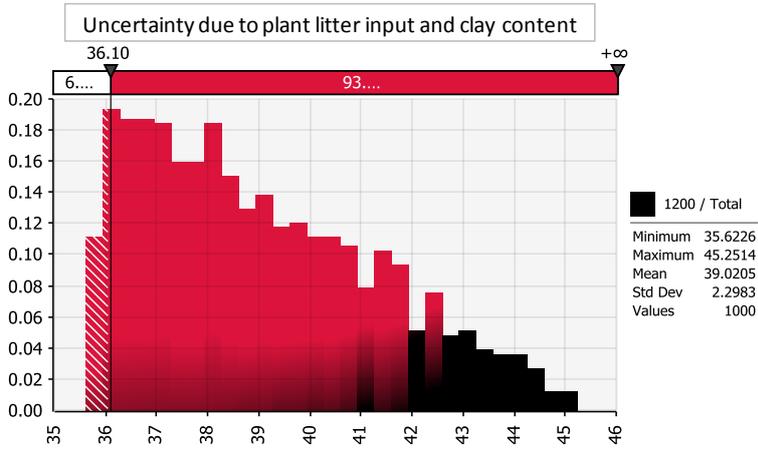
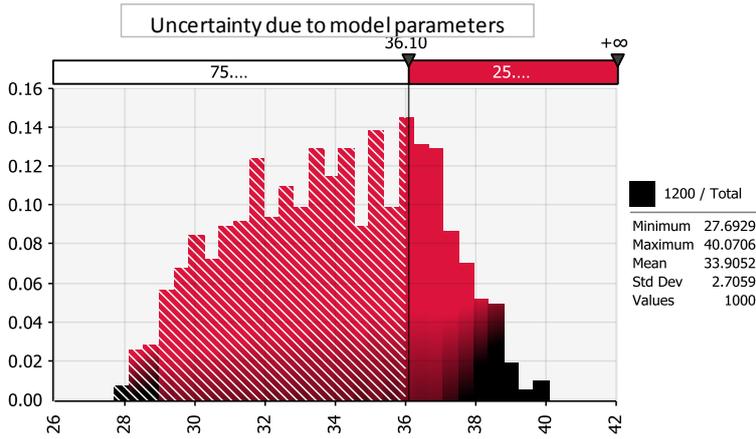
104 matter, mM = micro-aggregates within macro-aggregates, sc-M = easily dispersed silt-clay

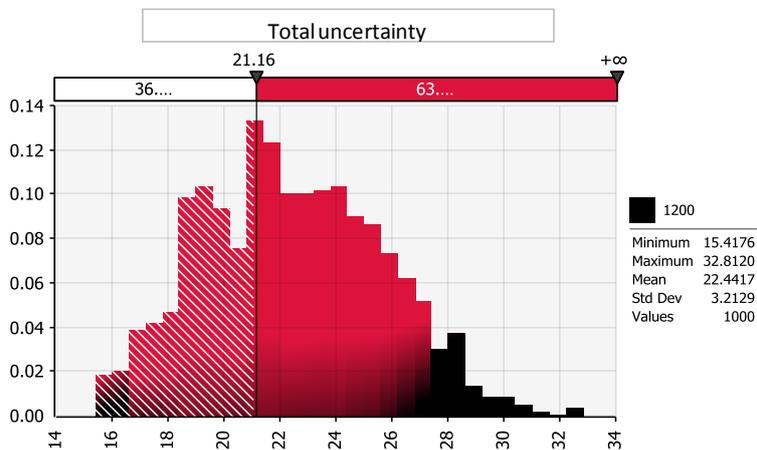
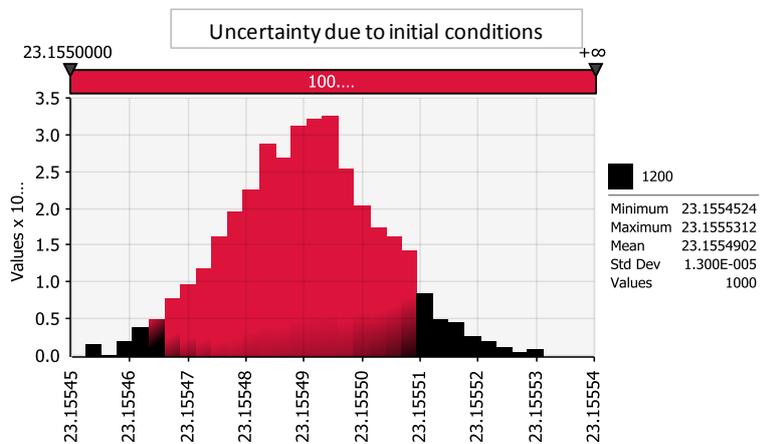
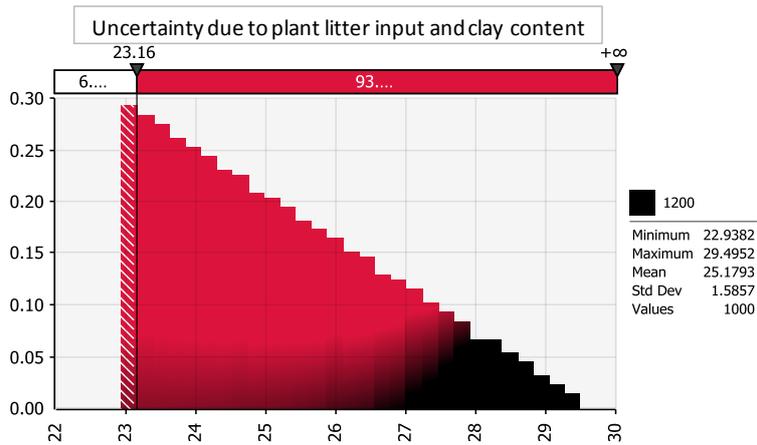
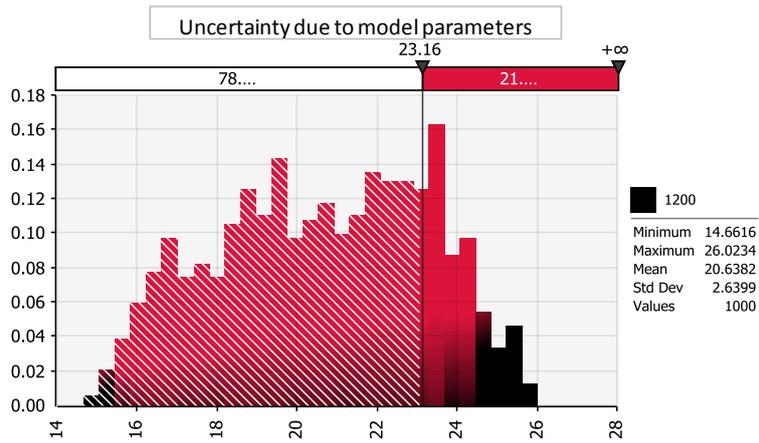
105 fraction, fPOM = fine particulate organic matter, sc-mM = silt-clay fraction of the

106 microaggregate.









111 **Fig. S2.** The output distributions by the Monte-Carlo simulations for the calculation of the  
112 total uncertainty and uncertainty due to initial conditions, input data, and model parameters  
113 for 100 year of the simulation in Greece for a) SOC and b) POM and in Iowa for c) SOC and  
114 d) POM (the value of the optimum solution is indicated).  
115

116 **Table S1.** Mean monthly meteorological data used for the application of the RothC model  
 117 in the two sites (taken from the Local Climate Estimator-New LocClim 1.10, Grieser, 2006).

	<b>IA (Iowa, USA)</b>			<b>GR (Crete, Greece)</b>		
	Temperature	Precipitation	Potential evapotranspiration	Temperature	Precipitation	Potential evapotranspiration
January	-6	25	22	11	142	51
February	-4	24	27	11	112	57
March	3	60	55	13	81	80
April	11	94	96	16	32	112
May	17	103	133	20	13	159
June	22	115	160	24	5	203
July	25	125	165	26	1	222
August	23	112	143	26	2	199
September	19	99	108	23	19	142
October	13	72	82	19	80	94
November	5	54	45	16	73	64
December	-3	40	25	13	94	55
<i>Annual</i>	10	923	1060	18	652	1437

118

119 **Table S2.** Monthly and annual decomposition rates<sup>a</sup> (y<sup>-1</sup>) for resistant plant material (RPM)  
 120 and humus (HUM) soil organic pools pools of RothC model for the Greek and Iowa sites.

	IA (Iowa, USA)		GR (Crete, Greece)	
	RPM	HUM	RPM	HUM
January	0.001	0.001	0.152	0.003
February	0.008	0.006	0.157	0.003
March	0.073	0.059	0.039	0.001
April	0.248	0.201	0.051	0.001
May	0.093	0.075	0.070	0.001
June	0.132	0.107	0.091	0.002
July	0.151	0.123	0.101	0.002
August	0.140	0.114	0.098	0.002
September	0.105	0.085	0.085	0.002
October	0.061	0.049	0.066	0.001
November	0.092	0.075	0.161	0.003
December	0.008	0.007	0.186	0.004
<i>Annual</i>	0.093	0.075	0.105	0.002

121 <sup>a</sup>Decomposition rates constant multiplied by the rate modifying factor for temperature (a), the  
 122 topsoil moisture deficit rate modifying factor (b), and the soil cover factor (c).

123

124 **Table S3.** Sensitivity coefficients (the absolute value of the ratio:  $(\Delta Y/Y)/(\Delta x/x)$ ) for  
 125 RothC model parameters for  $\pm 10\%$  and  $\pm 50\%$  change of the calibrated values for the Greek  
 126 and Iowa sites.

parameter	IA (Iowa, USA)				GR (Crete, Greece)			
	(-50%)	(+50%)	(-10%)	(+10%)	(-50%)	(+50%)	(-10%)	(+10%)
total plant input	0.802	0.802	0.802	0.802	0.649	0.649	0.649	0.649
RPM <sup>a</sup>	0.460	0.272	0.365	0.329	0.504	0.207	0.329	0.276
DPM/RPM ratio <sup>b</sup>	0.425	0.228	0.316	0.280	0.154	0.102	0.128	0.118
HUM <sup>c</sup>	0.270	0.153	0.211	0.189	0.023	0.022	0.023	0.022
BIO% <sup>d</sup>	0.064	0.066	0.065	0.065	0.175	0.193	0.182	0.186
BIO <sup>e</sup>	0.099	0.045	0.069	0.059	0.042	0.014	0.024	0.019
DPM <sup>f</sup>	0.058	0.018	0.031	0.025	0.008	0.003	0.005	0.004

127 <sup>a</sup>Resistant plant material decomposition rate constant

128 <sup>b</sup>The apportionment ratio of Plant litter input between decomposable plant material (DPM)  
 129 and RPM

130 <sup>c</sup>Humus decomposition rate constant

131 <sup>d</sup>The proportion that goes to BIO (100-BIO% is the proportion that goes to BIO)

132 <sup>e</sup>Biomass decomposition rate constant

133 <sup>f</sup>Decomposable plant material decomposition rate constant