

1 **Tillage influence on biophysical soil properties: the example of a long-term tillage**  
2 **experiment under Mediterranean rainfed conditions in South Spain**

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13

14 **Abstract**

15

16 Long-term field experiments are important to provide information about how soil  
17 carbon (C) dynamics is affected by soil tillage systems. In this study we directly  
18 diagnose the influence of the topsoil's (0-5 cm depth) C concentration on soil quality in  
19 an Entisol under Mediterranean conditions, testing a new statistical method. The  
20 objective was to estimate the impact of conservation tillage (RT) as a C management  
21 practice, versus a traditional tillage (TT) system on biophysical soil properties. We  
22 analyzed the impact of the soil C management on biological (microbial biomass C,  
23 dehydrogenase and  $\beta$ -glucosidase activities), physical soil properties (aggregates  
24 stability, conductive mean pore diameter, aggregation index) and CO<sub>2</sub> fluxes. The  
25 concentrations for total organic carbon (TOC), the active carbon (AC) normalised by  
26 the total carbon (AC TOC<sup>-1</sup>), served as a combined proxy for the soil C management  
27 related to the tillage system. Soil C management accounted for 0 to 46 % of the change  
28 of biophysical soil properties in RT versus TT. The RT led to a C increase (18.9%) of  
29 microbial activities, especially in the top 0-5 cm depth. Related to the physical soil  
30 properties, less C in TT led to a lower aggregation index, although this tendency was  
31 not observed for other physical parameters. The impact of soil C management was  
32 better correlated with soil microbial than with the physical properties. Our analysis  
33 directly quantified for the first time that the increase in the soil's carbon concentration  
34 can only explain a small fraction of the beneficial change in biophysical soil properties  
35 due to RT. In general the RT contributed to the long-term sustainability of the

36 agroecosystem by improving biological and physical soil characteristics under dryland  
37 semi-arid Mediterranean conditions.

38

39 **Keywords:** Soil Carbon, Tillage, Enzymatic Activities, Soil Aggregates, Soil Quality.

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## 42 **1. Introduction**

43 Intensive tillage frequently causes losses of total soil organic carbon (TOC) with  
44 a parallel loss of soil quality at long-term (Reicosky, 2002; Lal, 2004) and increases  
45 CO<sub>2</sub> emissions from soil to the atmosphere (Lal et al., 1989; Bauer et al., 2006;  
46 Venterea et al., 2006; Conant et al., 2007). Conservation agriculture has been promoted  
47 since approximately 1960 as a means to counteract all these constraints enhancing soil  
48 quality related to carbon (C) sequestration (Lal, 2004) that favours soil biology and  
49 physical conditions (Kandeler and Böhm, 1996; Kandeler et al., 1999; Broninck and  
50 Lal, 2005; Muñoz et al., 2007). However, so far, the positive relation between soil C  
51 sequestration and biological and physical soil properties has never been directly  
52 quantified.

53 Semi-arid Mediterranean conditions, such those of southern Spain (mild rainy winters  
54 and very hot dry summers) promote TOC decomposition by microbiological activity,  
55 which could be a limiting factor for TOC accumulation in the top soil layers.  
56 Nevertheless, those soils with low inherent levels of TOC could be the most improved  
57 with conservation tillage management (which avoid soil inversion by ploughing and  
58 maintains at least 30 percent of the soil surface covered by residues: Gajri et al., 2002),  
59 despite modest or no change in TOC within the rooting zone (Franzluebbers, 2004).

60 Small changes in TOC resulting from changes in soil management are often difficult to  
61 measure and several years are required to detect changes resulting from management  
62 practices (Roldán et al., 2005). But even with no consistent changes in TOC due to the  
63 crop residues incorporation, slight increases in labile fraction of TOC create particular  
64 conditions for the physico-chemical and biological soil dynamic. In fact, changes in  
65 small but relatively labile fractions of TOC may provide an early and useful indication  
66 of improvement in response to management practices, especially important under  
67 Mediterranean semi-arid conditions, where great increases in TOC are not expected.  
68 Active carbon (AC), water soluble carbon (WSC) or microbial biomass carbon (MBC)

69 (Melero et al., 2009a,b; López-Garrido et al., 2011) can serve to indicate these labile C  
70 fractions.

71 Not only TOC, but also soil biochemical properties (enzymatic activities) are probably  
72 the most widely known indicators of soil quality (Gil-Sotres et al., 2005). On the other  
73 hand, the overall study of tillage influence on TOC and its labile fractions and soil  
74 biochemical variables require long-term tillage studies under different soil and climatic  
75 conditions to understand the dynamics of soil organic matter under the wide diversity of  
76 environments in the world (Franzluebbbers, 2004). Interaction between different soil  
77 properties may be critical for the soil functions at long-term. In this context Deurer et al.  
78 (2008) published a new method to quantify directly the impact of soil C management on  
79 biophysical soil properties focused on two production systems: an integrated apple  
80 orchard and an organic apple orchard. This study estimated that the soil C management  
81 accounted for 0 to 81% of the degradation or enhancement of biophysical soil properties  
82 in the integrated and organic system.

83 To our best knowledge, under semi-arid Mediterranean conditions there is no study that  
84 has directly estimated how much biophysical soil properties change as a result of a  
85 change in soil C versus other factors that is caused by different tillage management  
86 practices. We hypothesize that at long-term, conservation tillage increases the soil's C  
87 concentration in the topsoil and that this in turn explains most of the positive effects on  
88 biophysical soil properties.

89 In essence, this study estimates the influence of the aspect of C sequestration of a  
90 conservation tillage technique (reduced tillage, RT) compared to a traditional tillage  
91 (TT) with soil inversion, on biophysical soil properties of the topsoil (considering  
92 different depths) of a typical Mediterranean arable land soil. For that, we have applied a  
93 recently published method (Deurer et al., 2008) to a long-term tillage experiment.  
94 Following the soil quality framework (Karlen et al., 2001, 2003; Deurer et al., 2008),  
95 we define tillage management practices to be sustainable if key soil functions did not  
96 degrade. In this study, the MBC, dehydrogenase (DHA),  $\beta$ -glucosidase ( $\beta$ -Glu) and  
97 diphenol oxidase (DphOx) activities were used as microbiological soil characteristics.  
98 For describing the soil physical properties, we chose the mean weighted diameter  
99 (MWD) of aggregates, the conductive macro-pore diameter (CMD) of water infiltration,  
100 the aggregation index (AI) and the aggregates water stability (WAS). We also measured  
101 CO<sub>2</sub> fluxes, both as a biophysical process and an indicator for the quantification of the  
102 actual C loss from the system.

## 103 **2. Materials and methods**

### 104 **2.1. Localization of the experimental area and tillage systems**

105 A long-term field trial using soil conservation management was conducted on an Entisol  
106 (Xerofluvent, Soil Survey Staff, 1999) with a sandy clay loam texture, at the  
107 experimental farm of the “Instituto de Recursos Naturales y Agrobiología de Sevilla  
108 (IRNAS-CSIC)” (37° 17' N, 6° 3' W), located 13 km southwest of the city of Seville  
109 (Spain). Some characteristics of the soil at 25 cm depth are: pH of around 7.8  
110 (calcareous) and alkaline-earth carbonates 280 g / kg. The soil has a clay content of  
111 about 24% (60% montmorillonite, 25% illite, and 15% caolinite), 18% silt and 58%  
112 sand. The climate of the zone is typically Mediterranean, with mild rainy winters (484  
113 mm mean rainfall for the time period 1971 to 2008 at the experimental site,  
114 hydrological year) and very hot and dry summers. Rainfall along the period of study  
115 was 547 mm. The mean annual daily temperature at the experimental site is around 17  
116 °C, with maximum and minimum temperatures of 33.5 °C and 5.2 °C in July and  
117 January, respectively.

118 An area of about 2500 m<sup>2</sup> was selected for establishing the experimental plots in 1991.  
119 In autumn of that year, wheat was grown. After harvesting the wheat in June 1992, the  
120 area was divided into six plots of approximately 300 m<sup>2</sup> (22 m x 14 m) each in a  
121 completely randomised experimental design (three replicates per treatment). Two tillage  
122 treatments were compared: traditional tillage, TT and conservation tillage (reduced  
123 tillage, RT). TT consisted of mouldboard ploughing (to a depth of 25-30 cm) after the  
124 straw of the preceding crop had been burned. We should note that straw burning has not  
125 occurred since 2003, when it was banned by the local government. RT was  
126 characterized by lack of mouldboard ploughing and a reduction in the number of tillage  
127 operations (retaining only chiselling at a depth of 25-30 cm) as well as by leaving the  
128 crop residues on the soil surface.

129 A wheat (*Triticum aestivum*, L.)-sunflower (*Helianthus annuus*, L.) crop rotation was  
130 established for both TT and RT. However, in 2005, a fodder pea crop (*Pisum sativum*,  
131 L.) was included in the rotation. Thus, from 2005, the annual crop rotation consisted of  
132 a basic cereal-sunflower-legumes rotation for both treatments. The sunflower and  
133 fodder pea crops were not fertilized (as is traditional in this zone), while wheat received  
134 deep fertilization with 400 kg ha<sup>-1</sup> of a complex fertilizer (15N-15P<sub>2</sub>O<sub>5</sub>-15K<sub>2</sub>O) before  
135 sowing and a top dressing with 200 kg ha<sup>-1</sup> urea (46% N). Since 2002, fertilization has  
136 been reduced to 100 kg ha<sup>-1</sup> (fertilizer complex) with no top dressing fertilizer. Weeds

137 are controlled by tillage in TT and by the application of pre-emergence herbicides in  
138 RT, at a rate of 2 l ha<sup>-1</sup> trifluraline (18%) (applied to the sunflower crop) and 4 l ha<sup>-1</sup>  
139 glyphosate (18%) (applied to the wheat and fodder pea crop).

140 In essence, when we compare the impact of TT and RT on the biophysical soil  
141 properties, then we compare the result of a different tillage management in the time  
142 from the beginning of the experiment in 1992 to our measurements in 2008 that is over  
143 16 years.

144

## 145 **2.2. Sampling and soil analysis**

### 146 *Chemical, biochemical and physical analysis*

147 Soil was collected at three depths: 0-5, 5-10 and 10-25 cm, in different soil samplings  
148 along 2008. Four samples were taken in each plot to create a composite sample per  
149 replicate and treatment. The moist field soil was sieved (2 mm) and divided into two  
150 sub-samples. One was immediately stored at 4 °C in loosely tied plastic bags to ensure  
151 sufficient aeration and prevent moisture loss prior to assaying for microbiological and  
152 enzymatic activities. The other was air-dried for chemical analysis. Biochemical  
153 analyses were carried out within two weeks.

154 Total organic carbon (TOC) was analysed by dichromate oxidation and titration with  
155 ferrous ammonium sulphate (Walkley and Black, 1934). Active carbon (AC) was  
156 determined by oxidation of 5 g of dry weight of soil with 2 ml of 0.2 M KMnO<sub>4</sub> in 1M  
157 CaCl<sub>2</sub> (pH 7.2) and non-reduced Mn<sup>7+</sup> was colorimetrically determined at 550 nm (Weil  
158 et al., 2003). Water soluble carbon (WSC) was determined in a 1/10 aqueous extract  
159 using a TOC-V-CSH/CSN analyser. Microbial biomass carbon (MBC) content was  
160 determined by the chloroform fumigation-extraction method modified by Gregorich et  
161 al. (1990). Dehydrogenase activity (DHA) was determined according to Trevors (1984)  
162 using INT (iodonitrotetrazolium chloride) as substrate. β-glucosidase (β-Glu) was  
163 measured as indicated by Eivazi and Tabatabai (1988). Diphenoloxidase (DphOx) was  
164 measured following the procedure described by Perucci et al. (2000). For each  
165 microbiological analysis, three replicates per collected sample were done. Results were  
166 based on the oven-dried weight of the soil.

167 The mean weight diameter (MWD) was determined in 1-2 mm dry aggregates following  
168 the wet sieving method of Kemper and Rosenau (1986). It was expressed in mm and  
169 corrected following the Younker and McGuiness (1956) recommendations. The  
170 aggregation index (AI) was determined following the Dobrzanski et al (1975) method,

171 using the aggregate fractions percentage and a weighting factor assigned to each one.  
172 Aggregate water stability (WAS) was determined on dry aggregates of 1-2 mm diameter  
173 following the method of Kemper and Rosenau (1986). We weighted 4 g of dry soil  
174 which was sieved (0.25 mm) in a wet sieving apparatus (Yoder Ejkelkemp). The results  
175 obtained were expressed in stable aggregates percentage. Conductive macro-pore  
176 diameter (CMD) between -20 and -60 mm tension was determined following Sauer et  
177 al. (1990) using an infiltrometer. The conductive pore diameter represents the average  
178 pore size for water infiltration near saturation.

179

### 180 *Soil CO<sub>2</sub> fluxes*

181 Soil CO<sub>2</sub> fluxes were measured by attaching a 6400-09 chamber with an area of 71.6  
182 cm<sup>2</sup> to a 6400 LICOR gas-exchange system (LI-COR, Environmental Division, Lincoln,  
183 NE, USA). The period of measurement was 2008. The system was provided with a  
184 thermocouple probe to measure soil temperature. To minimise soil surface disturbances,  
185 the chamber was mounted on PVC soil collars sharpened at the bottom and inserted into  
186 the soil to about 3.8 cm. To prevent an overestimation of soil fluxes, typically observed  
187 immediately after the collars have been installed, the latter were inserted some days  
188 before the measurements were made. Furthermore, 6 collars were placed at random  
189 locations in each treatment in order to describe statistically the spatial variability. Only  
190 one measurement was made on each collar on each observation day. The sampling time  
191 in each collar varied in accordance with the CO<sub>2</sub> concentrations inside the chamber,  
192 ranging from 3 to 8 min. All the observations were performed during daylight hours,  
193 beginning at variable times ranging from 10:30 to 13:00 h. Measurements made at this  
194 time of the day were assumed to represent the average flux of the day (Kessavalou *et*  
195 *al.*, 1998; Álvaro-Fuentes *et al.*, 2007).

196

### 197 *Statistical data analysis*

198 Statistical analyses were carried out using SPSS 11.0 for Windows, and the results were  
199 expressed as mean values. Significant differences between management systems (TT,  
200 RT) were shown based on an analysis of variance (ANOVA) and a Student's t-test at  
201  $p < 0.05$ . A correlation matrix of different properties was based on Pearson correlation  
202 coefficients ( $p < 0.05$ ). Data normality was tested prior to analysis; when necessary,  
203 variables were transformed to achieve normality. If, after transformation, the data still  
204 did not have a normal distribution, we used non-parametric tests: the Mann-Whitney U

205 test for comparison of mean values and the Kruskal-Wallis ANOVA by ranks test for  
206 variance analysis.

207

### 208 **2.3. Definition of soil carbon management**

209 The soil C management cannot be identified as one particular management practise. Soil  
210 C management is defined as a specific management practice that maintains or increase  
211 soil C (Kimble et al., 2007). Several management practices and other variables, such as  
212 soil type and climate, influence a soil's C status. We used TOC and AC normalised by  
213  $AC TOC^{-1}$ , served as a combined proxy for the soil's C status related to the tillage  
214 system. The TOC describes the size of the entire soil C pool and the AC characterizes  
215 de labile C fraction that is well correlated with microbial activities (Ghani et al., 2003).

216

### 217 **2.4. Framework to quantify the impact of soil carbon management on biophysical** 218 **soil properties**

219 This framework is based on a recently published concept (Deurer et al., 2008) and was  
220 adapted for comparing the influence of the soil C management component of different  
221 tillage treatments (TT and RT). In the following we briefly outline the concept as it  
222 applies to our study.

223 The observation time ( $\Delta t$ ), for should be long enough to represent the interaction of the  
224 local climate with the biophysical soil properties ( $f$ ). Originally (Deurer et al., 2008) a  
225 minimum measurement period of 1 year was suggested. Our study covers a period of 16  
226 years.

227

#### 228 ***Formal Setup of Variables***

229 The values of a biophysical soil property  $f$  at location  $\mathbf{x}_i$  and  $\mathbf{x}_j$  over a time interval  $\Delta t$   
230 were compared. The location  $\mathbf{x}_j$  served as a reference, which represents TT, comparable  
231 to  $\mathbf{x}_i$  which represents RT. This means, that  $\mathbf{x}_i$  and  $\mathbf{x}_j$  had the same soil type, texture and  
232 climatic conditions and the same initial conditions when the experiment started in 1992.

233

#### 234 ***Calculation of the impact of the soil carbon management component when reducing*** 235 ***tillage***

236 The impact of the C management on the biophysical soil property at  $\mathbf{x}_i$  and over the time  
237 interval  $\Delta t$ ,  $f(\mathbf{x}_i; \Delta t)$ , was calculated in five steps (Fig.1), as it was done by Deurer et al.,  
238 2008. The term 'soil C management' is defined in detail in section 2.3.

239 *Step 1:* Are the proxies of soil C management, TOC and AC TOC<sup>-1</sup>, in the managed  
 240 treatment in our case RT and the reference in our case TT statistically different (checked  
 241 by step 1)? When this applies, then proceed. We selected a measurable proxy for the  
 242 soil C management  $P$  in the soil at  $\mathbf{x}$ . For our study, we used TOC and the AC (AC  
 243 TOC<sup>-1</sup>) as a combined proxy for the soil C management at  $\mathbf{x}$  (see section 2.3). If  $P(\mathbf{x}_i;\Delta t)$   
 244 and  $P(\mathbf{x}_j;\Delta t)$  were statistically significantly different ( $p<0.05$ ), then a potential impact of  
 245 the soil C management on the biophysical soil property  $f(\mathbf{x}_i;\Delta t)$  was probable, and we  
 246 proceeded to the next step.

247 *Step 2:* Are the selected biophysical parameters between RT and TT statistically  
 248 different? When this applies, then proceed to step 3. We tested if the soil biophysical  
 249 property of the soil under RT  $f(\mathbf{x}_i;\Delta t)$  and of the soil under TT  $f(\mathbf{x}_j;\Delta t)$  were statistically  
 250 significantly different. Only if this was the case, we assumed that there was an impact of  
 251 reduced tillage on  $f(\mathbf{x}_i;\Delta t)$ , and we proceeded to the next step.

252 *Step 3:* What is the total impact of reducing tillage on the biophysical soil property in  
 253 RT? The ratio  $\Phi$  of the biophysical soil property measured at  $\mathbf{x}_i$  and  $\mathbf{x}_j$  and averaged  
 254 over  $\Delta t$  yielded the overall impact of reducing tillage on  $f(\mathbf{x}_i; \Delta t)$ :

255

$$256 \quad \Phi[f(x_{i,j}, \Delta t)] = - \left[ 1 - \frac{f(x_i; \Delta t)}{f(x_j; \Delta t)} \right] \quad (1)$$

257 The value of  $\Phi$  multiplied by 100 denoted the percentage difference (larger = positive  
 258 value and smaller = negative value) in the biophysical soil property at  $\mathbf{x}_i$  (RT) compared  
 259 with the reference  $\mathbf{x}_j$  (TT). Therefore,  $\Phi$  is a measure of the impact of the reduction in  
 260 tillage on the biophysical soil property at  $\mathbf{x}_i$ .

261 *Step 4:* What is the correlation between the proxy for soil C management and the  
 262 biophysical soil property? We performed a regression of the biophysical soil property  
 263  $f(\mathbf{x}_{i,j};\Delta t)$  (dependent variable) versus the respective C management proxy values  
 264  $P(\mathbf{x}_{i,j};\Delta t)$  (independent variable). This yielded the variance fraction ( $R^2$ ) that could be  
 265 explained by the C management proxy. We denoted it by  $R(f(\mathbf{x}_{i,j}; \Delta t);P)$ . The C  
 266 management proxy had to be a statistically significant variable in the regression.

267 *Step 5:* What is the impact of soil C management on the biophysical soil property in  
 268 RT? The correlation between the biophysical soil property and the proxy for the soil C  
 269 management,  $R(f(\mathbf{x}_{i,j};\Delta t);P)$  (step 4), was multiplied by the impact of reducing tillage on  
 270 the biophysical soil property  $\Phi$  (step 3). By this we estimated the partial impact  $I$  of the



271 soil C management as only one consequence of reducing tillage,  $P$  on the biophysical  
272 soil property at  $\mathbf{x}_i$ :

273

$$274 \quad I[f(x_{i,j}; \Delta t); P] = \Phi[f(x_{i,j}; \Delta t)]R[f(x_{i,j}; \Delta t); P] \quad (2)$$

275

276 The value of  $I$  multiplied by 100 denoted the percentage increase in the partial impact of  
277 the soil C management  $P$  on the particular biophysical soil property at  $\mathbf{x}_i$ .

278

### 279 **Reference**

280 We used the soil from the TT treatment as the reference for the managed soil in the  
281 comparison between treatments. We selected the top soil, 0.25 m depth, which is the  
282 main soil affected by the mouldboard ploughing and other tillage practices. For  
283 analysis, we separated the samples into three increments (0-5, 5-10 and 10-25 cm). This  
284 enabled us to estimate the depth of the impact of soil C management on the biophysical  
285 soil properties.

286

## 287 **3. Results**

### 288 ***Proxies for the soil carbon management***

289 In general, TOC, AC, AC TOC<sup>-1</sup> and WSC variables tended to have greater values in  
290 RT than in TT, with statistical differences ( $p < 0.05$ ) at 0-5 cm depth (Fig. 2). AC was the  
291 only parameter that showed statistical differences at 5-10 cm depth. This shows that  
292 after 16 years of reducing tillage, soil C management, indicated by TOC and AC TOC<sup>-1</sup>,  
293 had a significant impact only down to a soil depth of 5 cm.

294

### 295 ***Soil microbial functioning and CO<sub>2</sub> fluxes of the soil: comparison of TT and RT***

296 Values of MBC, DHA,  $\beta$ -Glu and DphOx enzymatic activities were higher at 0-5 cm  
297 depth in RT, compared to TT, with statistical differences for MCB, DHA and  $\beta$ -Glu  
298 (Table 1). DphOx activity, also showed greater values in RT, although differences were  
299 not significant. At 5-10 cm depth, the tendency was the same, but without statistical  
300 differences between treatments. At 10-25 cm depth, results were practically the same in  
301 RT and TT. In general, the CO<sub>2</sub> flux was higher in TT ( $0.40 \pm 0.03 \text{ g m}^{-2} \text{ h}^{-1}$ ) compared  
302 to RT ( $0.31 \pm 0.02 \text{ g m}^{-2} \text{ h}^{-1}$ ), with statistical differences between treatments (Table 2).

303 We conclude that four (MBC, DHA,  $\beta$ -Glu, CO<sub>2</sub> fluxes) of the five indicators for soil  
304 biological activities increased in parallel with an increase in the proxies for soil C  
305 management in the top 5 cm of the soil.

306

### 307 *Physical functioning of the soil: comparison of TT and RT*

308 We did not find clear results for the different methods used for assessing a possible  
309 change of the soils aggregate structure and stability in the different treatments. On the  
310 one hand, greater values of WAS were recorded under TT, in the three depths, although  
311 statistical differences were only found at 5-10 cm depth (Table 3). On the other hand,  
312 RT tended to enhance the MWD and AI variables in all depths (with statistical  
313 differences for AI at 0-5 cm depth). The tillage treatments made no difference for the  
314 water infiltration characteristics near saturation. The CMD near water saturation was  
315 measured only at the soil surface. We conclude that there is no clear difference of soil  
316 physical properties between the tillage treatments, and consequently also no clear  
317 influence of soil C management on the set of soil physical properties that we selected.

318

### 319 *Correlation of biophysical soil properties with proxies for soil carbon management*

320 The proxies for soil C management explained a fraction of the variability of the  
321 biophysical soil properties between treatments. We use these fractions to quantify how  
322 much soil C management is responsible for any change in biophysical soil properties  
323 (see equation 2 in Step 4). For example, the proxies explained 46% of the variation of  
324 DHA, which means that the TOC and AC TOC<sup>-1</sup> explained 46% of the variation of  
325 DHA in both tillage systems (TT and RT) (Fig. 3A). For the  $\beta$ -glucosidase activity, the  
326 TOC and AC TOC<sup>-1</sup> explained 37% ( $R^2 = 0.37$ ) of the variation (Fig. 3B). For the  
327 MBC, 25% could be explained, and only AC TOC<sup>-1</sup> was a significant variable in the  
328 regression (Fig. 4). In our multiple step-wise regressions the TOC was a significant  
329 variable for two of the biological soil properties studied (DHA and  $\beta$ -Glu) and AC  
330 TOC<sup>-1</sup> was a significant variable in all cases.

331 In general, the soil physical properties that we considered were not (MWD, CMD and  
332 WAS) or only poorly (AI) correlated with the proxies for C management. For example,  
333 the AI tended to be slightly correlated with AC TOC<sup>-1</sup>, which explained 8.4 % of the  
334 variation of this soil property.

335

336 *Total and partial impact of the soil carbon management on the microbial and*  
337 *physical functioning of the soil*

338 The microbial properties decreased between 0 and 75% in the TT system compared to  
339 RT (Table 4). We could attribute 0 to 46% of the change in microbial properties to soil  
340 C management (Table 4). Therefore, the impact that can directly be attributed to the  
341 change of the soil C management on the microbial properties was always less than half  
342 of the total impact of the treatments. For example, the DHA decreased in total by 46%  
343 in TT versus RT, but we attributed only a reduction of 17% to the soil C management.

344 An impact of the soil C management on the physical soil properties was only significant  
345 for AI, which decreased between 0 and 5% in the TT system compared to RT, but only  
346 between 0 and 0.4% can be attributed to soil C management.

347 This disproves our hypothesis that soil C management is able to explain most of the  
348 impact of reduced tillage on biophysical soil properties.

349

350 **4. Discussion**

351 *Soil carbon management*

352 The soil quality framework was not helpful in the decision as to which soil depths  
353 should be selected for an assessment of the influence of tillage management (Letey et  
354 al., 2003). Initially, we chose the depth of the ploughing layer (0.25 m), and only after  
355 using the new framework with clear decision criteria we selected the 0.05 m depth,  
356 because the greatest differences between treatments were found only to this depth (Fig.  
357 2). In this study, a tendency for improvement derived from the conservation tillage was  
358 also reported at 5-10 cm depth. From an agro-ecological point of view it is an important  
359 fact, as the superficial layer (0.05 m) is the interface between soil and atmosphere and is  
360 considered a very important part of the soil for various ecosystem services, and also  
361 where the most important biological processes take place (Madejón et al., 2007; López-  
362 Garrido et al., 2011). Although conservation tillage will progressively introduce  
363 improvements in the soil profile, it is desirable to have a robust statistical tool to  
364 establish at which depth these benefits are currently consolidated.

365 In our case, a Xerofluent calcareous soil cropped under rainfed agriculture for 16  
366 years, RT has managed to reduce losses of organic C that is typically caused by TT with  
367 soil inversion. These higher values, not only for TOC, but also for AC and WSC in RT  
368 compared to TT (Fig. 2) can be associated to the crop residues left at surface under  
369 conservation tillage treatments (RT in our case) and with the lower decomposition

370 process (Reeves, 1997; Salinas-García et al., 2002), resulting in lower emissions of CO<sub>2</sub>  
371 to the atmosphere (Álvaro-Fuentes et al., 2008; López-Garrido et al., 2009), a fact also  
372 reported here (Table 2). One of these parameters, AC has been considered to be more  
373 sensible to soil management (tillage) than even TOC (Weil et al., 2003), and it has been  
374 evaluated as a possible soil quality index under rainfed, semi-arid Mediterranean  
375 conditions (Melero et al., 2009a,b).

376

377 Greater values for TOC, AC and WSC at 0.05 m depth under RT compared to TT have  
378 been reported for other scenarios (De la Horra et al., 2003; Madejón et al., 2007; Melero  
379 et al., 2009a,b). In general, differences were detectable during the first two to five years  
380 of the experiment, but significant increases often occurred only five to ten years after  
381 the conservation tillage system was established (Franzluebbers and Arshad, 1996). For  
382 this reason, long-term experiments, such as that studied here, are very important to  
383 evaluate differences in soil C in a reliable way. Soil organic matter increase is a very  
384 important objective in Mediterranean areas, where most agricultural soils have low  
385 organic matter values (typically around 1-2%, Madejón et al., 2009).

386 However, as pointed out before, under Mediterranean conditions TOC accumulation  
387 near the soil surface is not common, due to the high temperature which promotes  
388 microbial activity and organic matter mineralisation. Although moderate increase of  
389 TOC at the soil surface has been postulated to trigger an increase in some biological  
390 properties (Madejón et al., 2007; Melero et al., 2009a,b; López-Garrido et al., 2011)  
391 there is little literature (Deurer et al., 2008) that directly assessed to which degree a  
392 change in soil C stocks (TOC) are responsible for an improvement of soil biophysical  
393 properties versus other effects of agronomic management such as the use of fertilizers  
394 or pesticides.

395

### 396 ***Soil microbial functioning and CO<sub>2</sub> fluxes***

397 Soil microbial properties, such as microbial biomass C and soil enzyme activities, have  
398 been used in other studies to predict soil biological status and the effect of farm  
399 managements in soil quality (Eivazi et al., 2003). Soil enzyme activities have also been  
400 used as discriminatory indicators for a wide range of soil management practices  
401 (Kandeler and Böhm, 1996; Kanderler et al., 1999; Eivazi et al., 2003; De la Horra et  
402 al., 2003; Roldán et al., 2005; Melero et al., 2009a,b) and evaluated as productivity,  
403 contamination and nutrient recycling index (Nannipieri et al., 1990; Pérez-De-Mora et

404 al., 2006). It means that enzymatic activity changes are interpreted as changes in soil  
405 quality (Dick, 1994; Madejón et al., 2007), being early indicators to different soil  
406 management practices (Albiach et al., 1999; Benítez et al., 2006; Melero et al., 2006).  
407 Therefore, it is important to quantify at long-term the influence that small C increases  
408 could have on these variables that are considered to be good indicators of soil quality. It  
409 would be desirable for this knowledge to be extended to other semi-arid Mediterranean  
410 areas, for which large increases in C in the soil surface derived from conservation tillage  
411 practices cannot be expected.

412 Small increases of organic C fractions under conservation tillage have influence on the  
413 DHA, associated to respiratory microorganisms processes (Nannipieri, 1994), thus  
414 being one of the most important indicators of soil quality (García et al., 1997). This  
415 corroborates previous studies on this subject (Madejón et al., 2007; Melero et al.,  
416 2007a,b; Madejón et al., 2009). The C increase could explain 46% of the variability of  
417 the DHA (Fig. 3A), a high value indicating the sensibility of this enzymatic activity  
418 with respect to the C management component of tillage treatment.

419 Organic C also influences  $\beta$ -Glu, explaining 37% of its variability (Fig. 3B).  $\beta$ -Glu  
420 belongs to the enzymes group that catalyzes hydrolysis of various glycosides, providing  
421 substrate for soil microorganisms (De la Horra et al., 2003; Madejón et al., 2003). This  
422 could be related to the MBC increase under RT, which could explain 25% of its  
423 variability (Fig. 4). Both values of  $\beta$ -Glu and MBC could be considered normal for  
424 our soil-climatic conditions (Angers et al., 1993; García et al., 1994; Smith et al., 1995;  
425 Bandick y Dick, 1999; Moore et al., 2000; Pascual et al., 2000) and significantly greater  
426 at soil surface in RT in relation to TT.

427 The CO<sub>2</sub> fluxes were not correlated with the soil C contents. Apart from the immediate  
428 physical losses of CO<sub>2</sub> following tillage operations that are especially high with deep  
429 tillage and soil inversion (Prior et al., 1997; Reicosky et al., 1997; Ellert y Janzen, 1999;  
430 Álvarez et al., 2001; Álvaro-Fuentes et al., 2007), soil respiration is a complex  
431 ecosystem process. For example, the release of CO<sub>2</sub> occurs not only via microbial  
432 decomposition of TOC, and litter, but also via plant root and faunal respiration (Luo and  
433 Zhou, 2006). Root respiration can account for a half of the total soil respiration, which  
434 usually varies from 10 to 90% (Hanson et al., 2000). On the other hand, soil respiration  
435 usually responds to the most limiting factor in the soil; when both temperature and  
436 moisture are not extreme, both factors influence interactively, accounting for most of its  
437 | variability at the field scale. For croplands, literature (Luo and Zhou, 2006) reports a

438 mean value of about  $550 \pm 80 \text{ g C m}^{-2} \text{ yr}^{-1}$ , with intermediate values ranging from the  
439 low value of tundra ( $60 \pm 6 \text{ g C m}^{-2} \text{ yr}^{-1}$ ) and tropical moist forests ( $1260 \pm 60 \text{ g C m}^{-2}$   
440  $\text{yr}^{-1}$ ). In our soils this value is around  $800 \text{ g C m}^{-2} \text{ yr}^{-1}$  between results reported by Luo  
441 and Zhou (2006) for croplands and forest. As a general rule, root contributions for sites  
442 dominated by forest vegetation account, on average, for 48.6% of soil respiration. The  
443 values of root contributions in the non-forest ecosystems are widely scattered  
444 throughout the entire range (10 - 90%), with an overall average of 36.7%. Also, root  
445 contributions exhibit a strong seasonality, increasing dramatically during the active  
446 growing season, when roots receive a consistent supply of carbohydrates from canopy  
447 photosynthesis (Luo and Zhou, 2006).

448

#### 449 *Soil physical functioning*

450 In general, soil C management was better correlated with biochemical than with  
451 physical properties, a fact so far only observed by one study in an orchard system and a  
452 humid climate (Deurer et al., 2008). However, the smaller increase of TOC at surface  
453 under RT could occasion a slight but non significant increase in the AI and MWD  
454 (Table 3). Nevertheless, the two variables show seasonal variability, being somewhat  
455 higher in spring than in summer, when plants are scarce (Yang and Wander, 1998;  
456 Álvaro-Fuentes et al., 2007, 2008). Plant development, root growth specially, can  
457 promote soil aggregation, due to the release of organic compounds, aggregating soil  
458 particles and promoting microbial activity (Angers y Caron, 1998; Six et al., 2004).

459 The opposite tendency was observed for WAS (Table 3), in discordance with other  
460 authors which observed that less C usually leads to lower aggregate stability  
461 (Franzluebbers and Arshad, 1996; Le Bissonais and Arrouays, 1997; Deurer et al.,  
462 2008). In our case, it is possible the decrease of WAS could be due to somewhat soil  
463 hydrophobicity in the TT treatment at the sampling date, derived from the straw burning  
464 followed in the TT treatment from 1992 to 2003. Fire is considered an important factor  
465 that could have influence in soil aggregation, affecting its hydrophobicity (MacDonald  
466 and Huffman, 2004; Poulénard et al., 2001). Some authors have been found important  
467 hydrophobicity in soils after 22 months from the straw burning (Huffman et al., 2001).

468

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471

## 472 **5. Conclusions**

473 The statistical framework to quantify the impact of soil C management on biophysical  
474 soil properties was successfully applied to a tillage treatment. Important conclusions  
475 from this study are:

476

477 - In this study a change of soil quality related biophysical soil properties as a  
478 function of soil C management (reduced versus traditional tillage) occurred after  
479 16 years mainly at 0-5 cm depth. This shows how much time is needed for any  
480 management change to have an impact under these environmental conditions.

481

482 - The soil C management proxies increase by 15.9 % for TOC and 37.4 % for AC  
483  $\text{TOC}^{-1}$  at 0-5 cm depth over 16 years in the reduced compared to the traditional  
484 tillage caused an improvement in microbial activity between 28.4 to 42.9 % at  
485 this depth. This agrees with other studies, that soil carbon management mainly  
486 affects soil biological activities.

487

488 - The improvement of the soil C management proxies in the reduced compared to  
489 the traditional tillage over 16 years had very little impact on soil physical  
490 properties. For example the aggregation index decreased due to a change in the  
491 carbon management only by 0.4%. This agrees with other authors that soil  
492 physical properties are less representatives of changes by soil C management.

493

494 - The soil C management component of a tillage management explained less than  
495 half of the total impact of reducing tillage on various biophysical soil properties.  
496 More research is needed to identify the other factors associated with tillage  
497 management that cause a change in soil quality.

498

499 Overall and over 16 years conservation management (reduced tillage) improved soil  
500 quality, enhancing organic C content and particularly the biological status of the soil,  
501 but was limited mainly to in the upper layer (0.05 m).

502

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509

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**Table 1:** Mean values  $\pm$  standard error of biochemical properties for traditional (TT) and reduced tillage (RT) at 0-5, 5-10 and 10-25 cm depth. The results were derived from a 16 year tillage experiment.

Treatment	Depth	MBC	DHA	$\beta$ -Glu	DphOx
TT	0-5	620 $\pm$ 76.4	1.69 $\pm$ 0.16	126 $\pm$ 13.1	1.51 $\pm$ 0.06
	5-10	627 $\pm$ 88.6	1.04 $\pm$ 0.18	97.4 $\pm$ 11.3	1.66 $\pm$ 0.08
	10-25	637 $\pm$ 119	0.88 $\pm$ 0.22	98.9 $\pm$ 12.9	1.68 $\pm$ 0.12
RT	0-5	885 $\pm$ 84.8*	2.96 $\pm$ 0.49*	185 $\pm$ 13.4*	2.11 $\pm$ 0.28
	5-10	732 $\pm$ 119	1.06 $\pm$ 0.15	133 $\pm$ 25.3	1.92 $\pm$ 0.26
	10-25	624 $\pm$ 121	0.33 $\pm$ 0.16	59.7 $\pm$ 9.04	1.70 $\pm$ 0.06

MBC: microbial biomass carbon ( $\text{mg kg}^{-1}$ ); DHA: dehydrogenase activity ( $\text{mg TPF dwt kg}^{-1} \text{ h}^{-1}$ ); DphOx: diphenol oxidase ( $\text{mg cathecol } 10 \text{ min}^{-1} \text{ g}^{-1} \text{ dwt}$ );  $\beta$ -Glu:  $\beta$ -glucosidase activity ( $\text{mg p-nitrophenol kg}^{-1} \text{ dwt h}^{-1}$ ). Differences between treatments are indicated by (\*) ( $p < 0.05$ ).

**Table 2:** Mean values  $\pm$  standard error of measurements of CO<sub>2</sub> fluxes (g m<sup>2</sup> h<sup>-1</sup>) for traditional (TT) and reduced tillage (RT) at ten different times in the period of study (2008). The results were derived from a 16 year tillage experiment. Differences between treatments are indicated by (\*) ( $p < 0.05$ ).

Measurement	TT	RT
1	0.31 $\pm$ 0.03	0.30 $\pm$ 0.00
2	0.56 $\pm$ 0.03	0.42 $\pm$ 0.02*
3	0.32 $\pm$ 0.04	0.21 $\pm$ 0.01*
4	0.26 $\pm$ 0.02	0.21 $\pm$ 0.02
5	0.27 $\pm$ 0.02	0.21 $\pm$ 0.01*
6	0.22 $\pm$ 0.02	0.34 $\pm$ 0.03*
7	0.48 $\pm$ 0.11	0.30 $\pm$ 0.04
8	0.70 $\pm$ 0.11	0.50 $\pm$ 0.08
9	0.38 $\pm$ 0.02	0.32 $\pm$ 0.01*
10	0.50 $\pm$ 0.04	0.31 $\pm$ 0.02*
<i>Average</i>	<i>0.40 <math>\pm</math> 0.03</i>	<i>0.31 <math>\pm</math> 0.02</i>



**Table 3:** Mean values  $\pm$  standard error of physical properties for traditional (TT) and reduced tillage (RT) at 0-5, 5-10 and 10-25 cm depth. The results were derived from a 16 year tillage experiment.

Treatment	Depth	WAS	MWD	AI	CMD
TT	0-5	49.1 $\pm$ 2.82	2.45 $\pm$ 0.12	505 $\pm$ 7.73	0.33 $\pm$ 0.08
	5-10	49.4 $\pm$ 1.82*	2.93 $\pm$ 0.07	521 $\pm$ 20.8	
	10-25	49.8 $\pm$ 2.04	2.86 $\pm$ 0.10	524 $\pm$ 27.3	
RT	0-5	42.4 $\pm$ 2.77	2.73 $\pm$ 0.17	532 $\pm$ 8.54*	0.28 $\pm$ 0.03
	5-10	39.1 $\pm$ 1.96	3.03 $\pm$ 0.12	533 $\pm$ 19.7	
	10-25	43.0 $\pm$ 3.21	2.99 $\pm$ 0.19	532 $\pm$ 16.2	

WAS: water aggregate stability (%), MWD: mean weigh diameter (mm), AI: aggregation index, CMD: conductive macro-pore diameter between -20 and -60 mm tension (mm). Differences between treatments are indicated by (\*) ( $p < 0.05$ ).

**Table 4:** Impact of all management practices (¥), and of only the soil organic C management (I) on soil microbial parameters and aggregation index. The values are given only when the differences were statistically significant. The value of R refers to a linear regression with total organic C (TOC) or (given in brackets) to a multiple linear regression with TOC and AC TOC<sup>-1</sup>.

Properties	¥	R	I
MBC	0.75	(0.46)	0.34
DHA	0.46	(0.37)	0.17
β -Glu	0.43	0.25	0.11
AI	0.05	0.08	0.004

## **FIGURE CAPTIONS**

**Fig. 1:** Schematic overview of the framework to quantify the impact of soil C management on biophysical soil properties. Adapted from Deurer et al., 2008.

**Fig. 2:** Mean values ( $N=7$ )  $\pm$  standard errors of total organic carbon (TOC), active carbon (AC), AC  $\text{TOC}^{-1}$  and water soluble carbon (WSC) in the top 0.25 m of the soils under TT and RT. Values with asterisk were significantly different ( $p<0.05$ ). The results were derived from a 16 year lasting tillage experiment.

**Fig. 3:** Prediction of measured enzyme activities by the proxies for the soil C management. The predictive power of the proxies for the soil C management was quantified by multiple regression. **A)** Dehydrogenase activity (iodo-nitrotetrazolium formazan [INTF] with  $N = 21$  for each treatment). The  $R^2$  is 0.46. Total organic carbon (TOC) and active carbon fractions (AC  $\text{TOC}^{-1}$ ) were significant variables. **B)**  $\beta$ -Glucosidase activity ( $\beta$ -Glu) with  $N = 21$  for each treatment. The  $R^2$  is 0.37. Both, TOC and AC  $\text{TOC}^{-1}$  were significant variables.

**Fig. 4:** Prediction of measured microbial biomass carbon (MBC) by the proxies for the soil C management. The predictive power of the proxies for the soil C management was quantified by multiple regression. We used  $N = 21$  samples for each treatment. The  $R^2$  is 0.25. Only the factor AC  $\text{TOC}^{-1}$  was a significant variable.

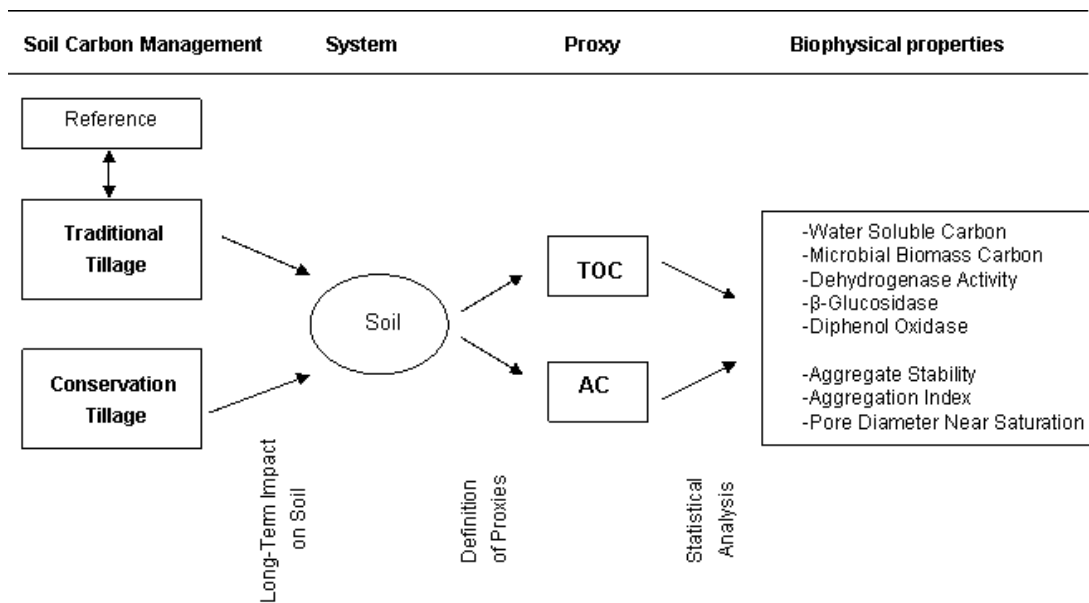


Fig. 1.

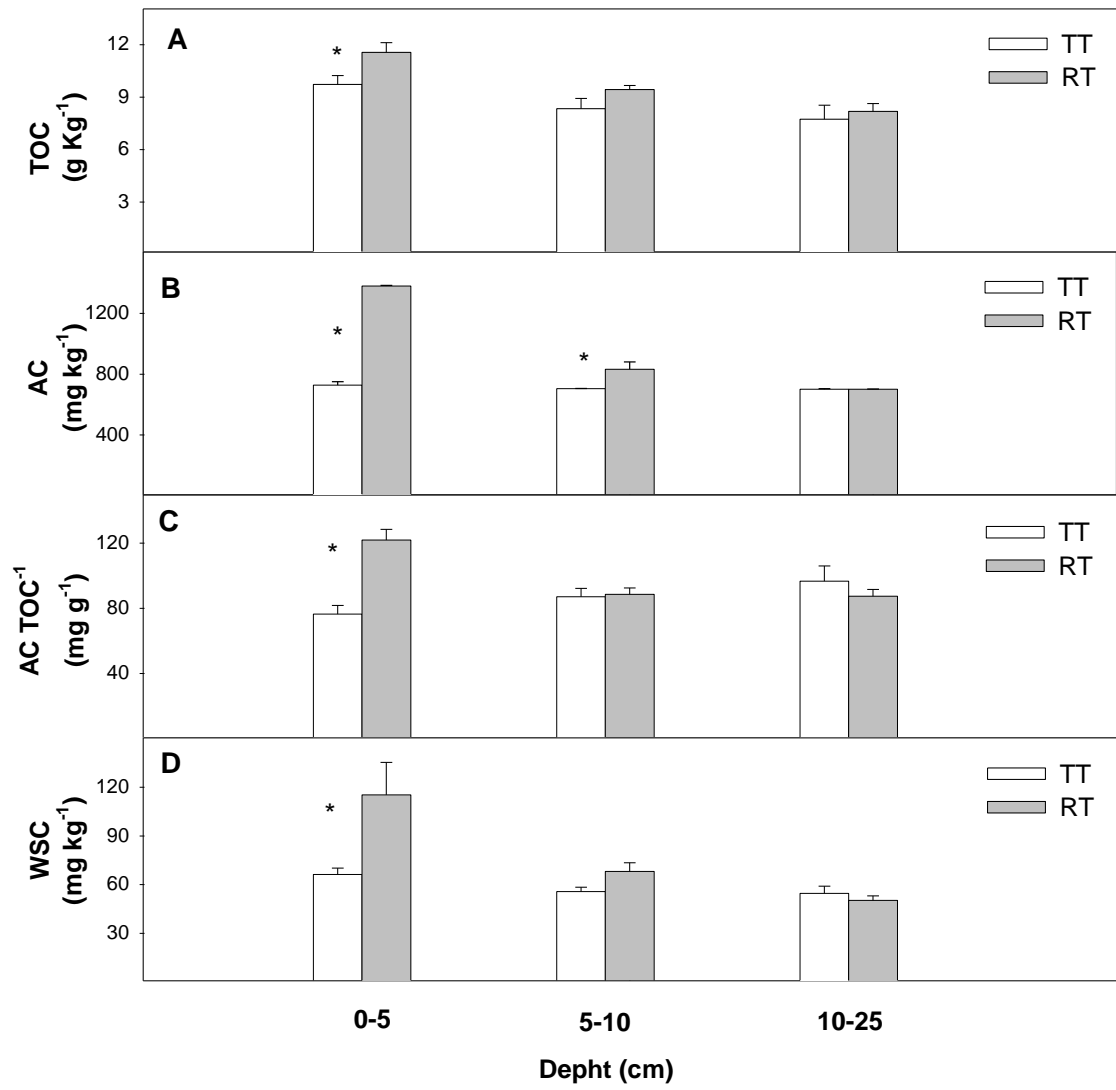


Fig. 2.

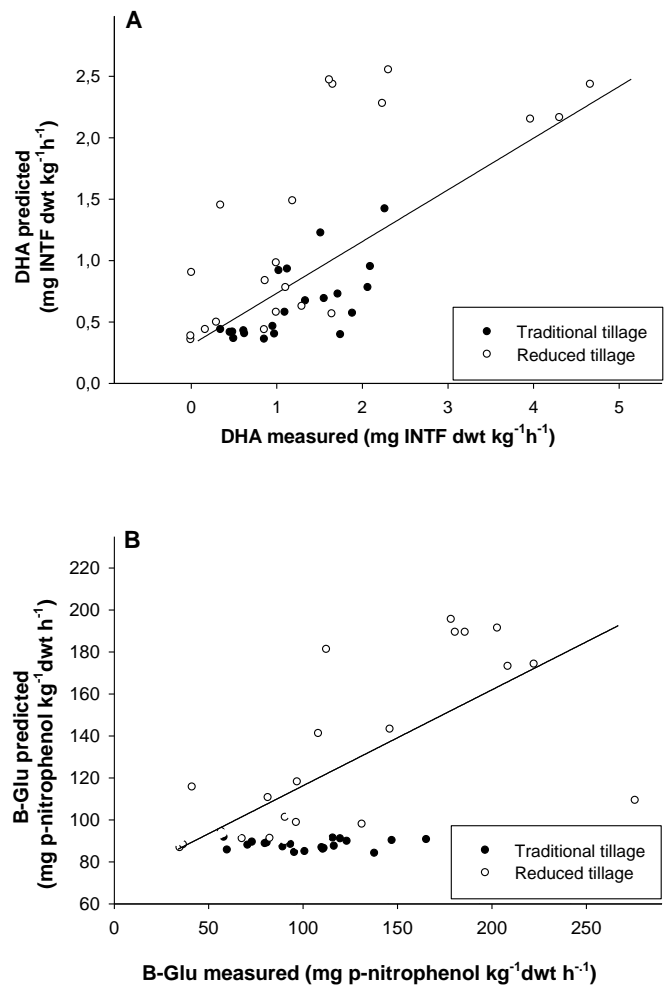


Fig. 3.

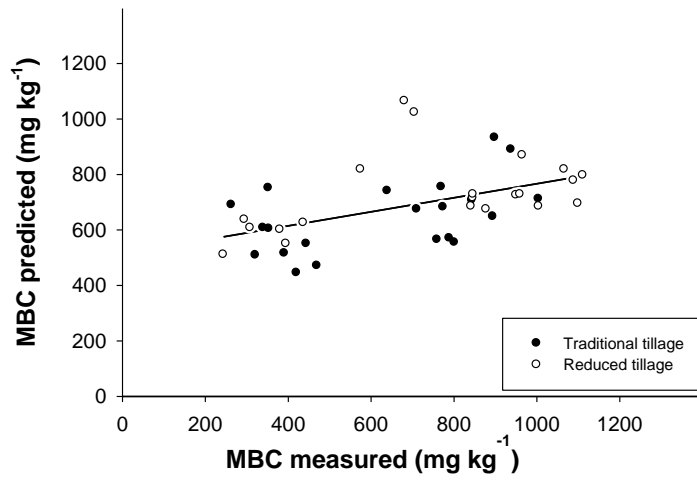


Fig. 4.