1 **Effective carbon sequestration in tilled agricultural soils by *in situ***

2 **photocatalyzed polymerization of soil organic matter**

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20 **Current practices adopted in agriculture to mitigate climate change induce little and**

21 **temporary soil organic matter stabilization, while they negatively affect crop yields.**

22 **Facing an increasing intensification of agriculture to match an exploding population**

23 **growth, new chemical technologies are being sought to control CO_2 emissions from soil.**

24 **Here we report that an effective organic carbon sequestration in cropped soils is**

25 **obtained by an innovative technology based on an *in situ* photo-oxidative coupling**

26 **among organic matter molecules catalysed by a biomimetic synthetic iron-porphyrin. A**

27 **three-years long field study shows that the catalyst-assisted *in situ* photo-polymerization**

28 **of organic matter enables significant sequestration of soil organic carbon under a**

29 **conventional tillage management without reducing crop yields and soil biological**

30 **quality. This "green" catalytic technology is a promising soil management practice that**

31 **concomitantly provides the *in situ* stabilization and maintenance of carbon in arable**

32 **soils, the protection of soil qualities and the sustainable mitigation of climate change,**

33 **even within an intensified agricultural productivity.**

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35 Agricultural lands under food and bio-energy crops, managed grass and permanent crops

36 including agro-forestry, account for about 40-50% of the Earth's land surface[1]. Land-use

37 changes, technological advancement and varietal improvement enabled world grain harvests

38 to double from 1.2 to 2.5 billion t y^{-1} between 1970 and 2010, thus ensuring food to

39 population growth[2]. This occurred at the cost of an annual total greenhouse gases (GHGs)

40 emission from cropland, that in 2010 is estimated to be 5.2 to 5.8 Gt CO_2-eq y^{-1} and

41 comprises 10-12% of global anthropogenic emissions[3] However, only agricultural non-CO_2

42 sources are reported as anthropogenic GHG emissions in IPCC calculations[3], since emitted

43 CO_2 is assumed to be neutral, being associated to annual cycles of carbon fixation and

44 oxidation through photosynthesis[3]. It is thus overlooked that much of carbon lost from

45 cropland upon conventional tillage practices is due to the heterotrophic respiration of the most

46 stable organic carbon pool contained in soil aggregates and hardly reestablished by a short-

47 term photosynthetic cycle[4-7].

48 The increasing need for cropland for food production and renewable forms of energy

49 to support a global population of 10 billion by 2050[8], put soils at larger risk of SOC depletion,

50 and, consequently, erosion and desertification[9]. Measures to mitigate GHGs emission from

51 agricultural soils by reducing SOC oxidation are now limited to management practices such

52 as the use of leys and green manures in crop rotation, addition of organic fertilizers, crop

53 residue incorporation, no-till (NT)/reduced tillage (RT) management, agroforestry, and return

54 to natural vegetation[3]. These practices do not only fail in substantially reducing GHGs

55 emissions from soil or permanently stabilizing soil organic matter[10-12], but are also predicted

56 to hardly match more than a maximum of 25% of the GHGs reductions required by the Kyoto

57 Protocol within 2050[13]. For example, the RT practice for SOC sequestration is ineffective on

58 poorly drained soils[14], or when crop residues are removed from soil[15]. Despite the widespread

59 adoption of NT/RT farming, its global rate of SOC sequestration is rather small (< 0.5 t C ha^{-1}

60 y^{-1})[16], highly variable among soils and land uses ($> 50\%$ error)[17, 18], and effective only over a

61 long-term[19]. Moreover, NT/RT practices do not persistently sequester SOC in

62 microaggregates, since C is concentrated in upper soil layers[20], and as tillage is resumed

63 (possibly by lack of sufficient incentives to farmers), the temporary fixed carbon may be

64 rapidly lost again from soil[21]. Finally, the potential contribution of no-till to the sustainable

65 intensification of agriculture to feed increasing population has been recently questioned[22]. It is

66 thus necessary to find alternatives to current soil management practices for carbon

67 sequestration in agriculture in order to enable soils to support the expected intensification of

68 food production, while still contributing to mitigate GHG emissions.

69   The humified organic matter in soil (70-80% of SOM)[23] represents the most persistent

70 SOC pool with mean residence time of several hundreds of years[24], and, thus, the principal

71 long-term C sink in the biosphere. It is expected that any advanced comprehension of the

72 chemical nature and reactivity of soil humus should help to devise novel technologies to

73 mitigate CO_2 emissions from soil[25]. Humic matter is a complex mixture of heterogeneous

74 aliphatic and aromatic molecules of plant and microbial origin[26, 27], which accumulate on soil

75 particles[28] by progressively separating from the soil solution for thermodynamic reasons[29, 30],

76 and, thus giving rise to chemical and physical protection of SOC[28, 31]. Recent scientific

77 evidence changed the paradigm on the chemical nature of humus by describing humic

78 molecules as heterogeneous but relatively small in mass (≤ 1000 Da)[32], rather than the

79 previously assumed macropolymers[33]. Humic molecules were shown to be associated in

80 supramolecular structures, which are prevalently stabilized by weak non-covalent bonds and

81 may be easily disrupted by interactions with organic acids[34, 35]. This new view even led some

82   scientists to the extreme assumption that the persistence of SOM should be due less to the

83   structure of humic components and their arrangement with clay particles than to the prevailing

84   environmental and biological conditions in soil[36].

85   We still believe that the chemical structure and reactivity of organic molecules in the

86   soil matrix are crucial to develop effective measures for the control of SOC persistence. For

87   example, it is known that oxidative enzymes induce a free-radical driven coupling reaction

88   among small humic-like aromatic molecules[37], and this catalyzed formation of intermolecular

89   covalent bonds may be usefully exploited to enhance OC fixation in soil[38]. Synthetic

90   biomimetic metal-porphyrin catalysts that mimic the activity of oxidative enzymes, were

91   found to produce large and chemically-stable humic molecules during oxidative (H_2O_2)

92   treatments of dissolved organic matter[39, 40]. Even the simple solar irradiation under both

93   homogeneous and heterogeneous metal-porphyrin catalysts enabled the photo-oxidative

94   formation of intermolecular C-C and C-O-C bonds among humic phenolic molecules [41, 42].

95   We thus conceived that a photo-oxidative polymerization of SOM can be activated

96   directly *in situ* by spreading a biomimetic catalyst on soil. The catalytic formation of multiple

97   covalent bonds among humic molecules and the consequent increase of chemical energy in

98   SOM would result in a reduction of its bioaccessibility to microbial mineralization and in a

99   concomitant OC accumulation in soil. Furthermore, larger and branched humic molecules

100   would promote the association of soil particles into greater soil aggregates, thus improving

101   soil physical quality[43-45]. These hypotheses were proved by treating whole soils with

102   biomimetic catalysts in either laboratory or mesocosm experiments, whereby the enhanced

103   chemical stabilization of SOC both significantly reduced microbial respiration[46, 47] and

104   improved soil structural stability even after several wetting and drying cycles[47].

105   The field scale effectiveness in SOC fixation by a water-soluble iron-porphyrin

106   treatment (FeP) is reported here for different soils under conventional soil management for

107   wheat production. In the silty-loam soil of Torino (Table SI-2 in supplementary information),

108   the FeP added to soil significantly enhanced the OC content of the bulk soil after the first year

109   in respect to that of both control and original soil (Fig. 1). The significant increase of OC

110   fixation in the bulk soil was confirmed after the second year, whereas this increasing trend

111   lost significance after the third year (Table SI-2). However, a significant SOC stabilization by

112   the FeP treatment after both the second and third year of management was shown by the sum

113   of OC content in the water-stable aggregates (WSA), which were separated by wet sieving

114   from the bulk soil. In fact, despite the inherent loss of the soluble OC during wet-sieving

115   separation[48], the FeP-treated field soil maintained up to 97% of SOC in WSA at the end of the

116   third year of cultivation, as compared to only 93% of SOC retention in WSA of the control

117   soil (Fig.1, Table SI-2). The evidence of SOC fixation in the Fe-amended soil was also

118   supported by the significant shift of carbon content in WSA towards larger size-aggregates

119   after the second and third experimental year, whereby the relative SOC enhancement in larger

120   particles (0.50-0.25 mm) corresponded to a concomitant decrease of OC content in the <0.25

121   mm microaggregates (Fig.1, Table SI-2). This indicates that the occurred coupling among

122   humic phenolic molecules and consequent enhancement of SOM hydrophobicity, determined

123   the association of microaggregates and formation of larger soil particle-sizes[43, 49].

124   The FeP treatment on the silty clay loam soil of Piacenza (Table SI-3) produced an

125   increase of OC content in the bulk soil that was significantly larger than control only after the

126   second experimental year, whereas a significant OC fixation as sum of WSA was evident for

127   every year (Fig. 2, Table SI-3). In fact, the mechanical particles separation in water depleted

128   only about 2 % of SOC for the FeP-treated soil, whereas the OC loss from control was

129   significantly greater than this value for each experimental year (Fig. 2, Table SI-3). The

130   stabilization of SOM by the photo-catalyzed reaction after the second year was confirmed by

131   the significant enhancement of OC content in each separated aggregate-size (Table SI-3).

132   Moreover, the overall improvement of soil aggregation due to the FeP treatment was shown

133   by the significant relative increase in OC content in the largest size-aggregate (4.75–1.00 mm)

134   at each experimental year

135   The effect of the catalyst addition to the clay-loam soil of the Napoli site was still

136   slightly different from the other two soils. A significant SOC increase (>10%) in the bulk soil

137   of FeP-treated plots was found only after the third experimental year (Fig. 3, Table SI-4).

138   Nevertheless, a different carbon distribution between FeP-treated and control soils was noted

139   already after the first experimental year in the separated size-aggregates. In fact, the

140   significant reduction in OC content induced by the catalyst treatment in the 1.00–0.50 mm

141   aggregate fraction (Fig. 3) is in line with the observed relative percent increase of OC content

142   for the largest (4.75–1.00 mm) size-aggregate (Table SI-4), thus indicating a progressive

143   incorporation of carbon-rich particles into soil macroaggregates. The occurred OC fixation by

144   the FeP treatment after the second and third year of cultivation was shown by the significantly

145   larger OC content in the sum of WSA, in respect to control (Fig.3, Table SI-4). This effect

146   was accompanied by a significant OC stabilization in the 0.50–0.25 and <0.25 mm size-

147   aggregates after the second year, and a definite increase of OC content and relative percentage

148   in the largest aggregates (4.75–1.00 mm) in the third year.

149   These results showed that the soil treatment with the biomimetic water soluble iron-

150   porphyrin in field conditions determined a significant sequestration of organic carbon in

151   respect to untreated soils in all three experimental plots of this study. Already after one year

152   of wheat cultivation following the FeP amendment, the catalyzed photo-polymerization of

153   SOM in Torino and Piacenza bulk soils provided an OC fixation of 3.15 and 1.56 Mg ha^{-1}

154   larger than control, respectively, while it was not effective for the Napoli soil (Table 1).

155   However, the FeP treatment produced a significant net increase of OC fixation after the

156   second and third experimental year for all three field plots with values as low as 2.75 Mg ha^{-1}

157 and as large as 5.46 Mg ha^{-1} for Torino and Piacenza bulk soils, respectively. The magnitude

158 of carbon sequestration obtained by the photo-oxidative catalyzed coupling of humic

159 molecules was also evident from the sum of WSA, whose OC content larger than control

160 varied from 1.96 Mg ha^{-1} in Torino field soil after the first year of cultivation, to 7.41 Mg ha^{-1}

161 in Piacenza plot soil for the second experimental year (Table 1). The differences in fixed

162 carbon among soils and years are likely to depend on the specific soil properties or rainfall

163 events which may vary the activity of the biomimetic catalyst, depending on either its

164 adsorption equilibrium on surfaces of soil particles or its transport down the soil profile.

165 Despite the variability in OC fixation due to heterogeneity of soil matrix and inherent

166 randomness of representative sampling, the carbon sequestered in bulk soil by the photo-

167 activity of the iron-porphyrin catalyst for three consecutive years reached an average of 3.54,

168 3.90, and 2.24 Mg ha^{-1} y^{-1} for the Torino, Piacenza and Napoli experimental sites, respectively

169 (Table 1). These significant amounts of fixed SOC were achieved under conventional tillage

170 (CT) management, whereas the current highly experimented NT or RT practices are far from

171 reaching such a large OC increase in cropped bulk soils even after several years, whether

172 under boreal North European[50] or Mediterranean[51] agroecosystems. This considerable SOM

173 incorporation even under CT management was not due to a reduced exposure of SOC to

174 microbial respiration, as in NT and RT management[12], but rather to an enhanced chemical and

175 biochemical stabilization of SOM achieved by the *in situ* catalyzed photo-oxidative coupling

176 among soil organic molecules. The phenolic molecules undergone the catalyzed coupling

177 reaction in soil are likely to originate from either humic moieties already existing in soil, or

178 lignin monomers released from heterotrophically respired crop residues, or exudates of plant

179 roots, or metabolites of soil microbial activity[47].

180 The SOC accumulation in the cropped field plots of this study is to be attributed to the

181 sole abiotic coupling reaction induced by the synthetic FeP catalyst, without any involvement

182   of biological processes. The invariance of soil biological activity upon FeP treatment was

183   shown by the phospholipids fatty acids (PLFA) (Fig. 4) and the activities of soil β-

184   glucosidase, phosphatase, urease, and invertase enzymes (Tab. SI-5), which were measured

185   on the same cultivated soils and over the three experimental years[52]. The PCA multivariate

186   elaboration of PLFA results failed to reveal significant differences between untreated and

187   FeP-treated plots in terms of structure and activity of living soil microorganisms (Fig. 4). In

188   particular, results from Torino and Piacenza plot soils grouped separately according to

189   sampling year, while in the Napoli soil the grouping became evident only at the third year. No

190   significant clustering was instead found as a result of the catalyst treatment, being control and

191   treated samples grouped together in all three sites at each year (Fig. 4). The PCA description

192   of results were confirmed by applying the distance-based redundancy analysis (db-RDA)

193   model, in order to verify the significant effect of either sampling year or FeP treatment, as a

194   function of PLFA patterns found for the three experimental sites. The model showed a

195   significance ($P<0.05$) for sampling year in each site, with percent of explained variance

196   ranging from 26.7% in Napoli to 40.2% in Piacenza and 61.2% in Torino. Conversely, the

197   model did not reveal any significant difference between the control and catalyst-treated soil,

198   since the FeP effect showed a very low percent of explained variance (<4%) and was never

199   significant (Table SI-6). These results confirm that the considerable SOC accumulation

200   observed in FeP plots (Table 1) was not linked to changes in structure and activity of

201   microbial communities, and it must be most likely related to the abiotic catalyst-assisted

202   photopolymerization of SOM. Furthermore, the biochemical index of soil quality alteration[53]

203   based on the enzymes activities measured in plot soils of this study, excluded that the catalyst

204   treatments had any effects on the biological quality of soils (Table SI-5). It is also important

205   to underline that the significant SOC sequestered by the *in situ* catalytic treatment of these

206   field plots did not imply any reduction in crop yield and yield attributes[54]. Plant N uptake was

207　not affected either, thus confirming that soil mineralization was not modified by the

208　treatment[54]. This indicates that such an *in situ* sequestration technology is not only effective

209　towards SOC sequestration, but it also conserves the full plant nutrition capacity of soil and

210　its potential crop productivity. Conversely, the NT and RT practices aimed to increase carbon

211　sequestration by reducing the bio-oxidation of SOM, limit the mineralization of plant

212　nutrients in soils and result in lower crop yields[22, 55, 56].

213　　　　The conceptual approach of soil organic matter fixation has long considered

214　decreasing the rate of its biological decomposition by reducing tillage intensity and frequency

215　and adding organic materials to soil[57], despite their temporary effectiveness due to limited

216　SOM stabilization[58].　But this view is evolving and many now try to develop chemical

217　technologies to both reduce CO_2 emissions from soil and increase SOM, such as replacement

218　of agricultural lime with silicates[59] or promotion of carbonation with additions of Ca-and Mg-

219　rich salts to soils[60, 61] or use of polyphenols to complex SOM or inhibit enzymes that

220　decompose it[62]. Here we demonstrate that an *in situ* photo-oxidative coupling reaction among

221　humic molecules catalyzed in soil by an eco-friendly water-soluble metal-porphyrin is highly

222　effective in organic carbon sequestration even under conventional soil management. This

223　catalyst-assisted abiotic enhancement of covalent bonds in soil molecular components favours

224　the processes of chemical and physical protection of OC in soil and reduces the extent of

225　microbial mineralization of SOM, without either altering the soil biological quality or

226　questioning the maximum crop yields required to feed an increasing population.　This

227　innovative catalytic technology of SOC fixation may become an easy soil management

228　practice to control SOM dynamics and sustain the intensification of agriculture, while

229　contributing to mitigate climate change.

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Methods

The biomimetic catalyst used here was a water-soluble iron–porphyrin (FeP) synthesized in the laboratory as meso-tetra (2,6-dichloro-3-sulfonatophenyl)porphyrinate of iron(III) chloride, [Fe-(TDCPPS)Cl], as previously described[39, 47].

Three different sites along an Italian North-South climate gradient were selected for the field experiments to represent important conditions of intensive agricultural production. The sites are located within the Experimental Stations of the University of Torino, the Catholic University of Sacro Cuore at Piacenza, and the University of Napoli Federico II. At all sites, volumetric (undisturbed soil cores) and bulk soil mixed samples were collected from surface horizons (0-30 cm). Core samples were used to determine soil bulk density, whereas mixed samples were stored at field moisture at 4°C until further use (see Supplementary Table SI-1 for soil classification, textural composition and other properties of selected soils).

The field experiments were conducted for three consecutive years (2007-2009) on triplicates of 1 x 1 m plots inserted in larger plots cultivated with wheat (*Triticum Durum*) and managed with a 35 cm depth ploughing, followed by surface harrowing. Treatments were: 1. control soils managed with conventional tillage and mineral fertilization with urea at the rate of 130 kg ha^{-1} of N; 2. soils managed as control but added with the water-soluble iron-porphyrin at a rate 10 kg ha^{-1}. For each replicate of the FeP-treated plot, 1g of catalyst was dissolved in 10 L of water and added to soil plots by a sprinkling irrigation each year before sowing. At the end of the cropping cycle, about 1 kg of soil was sampled from the first 30 cm of control and FeP-treated soils, air dried and stored for subsequent analyses.

Water-stable aggregates were isolated from bulk soils as reported earlier[63] by applying a wet sieving method[64]. An air–dried sub–sample (30 g) was placed on the top sieve of a set of three nested sieves (1.0, 0.50, and 0.25 mm). The sample was gently rewetted and then submerged into 2 cm of distilled water for 30 min. After this time, the sieves were manually oscillated

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257   (up and down 4 cm) for 30 times during 1 min. Recovered aggregate fractions were oven–

258   dried at 60˚C, weighed, and stored at room conditions.

259   Total amount of OC in bulk soils and in water-stable aggregates (WSA) were determined on

260   finely ground samples by an Elemental Analyzer EA 1108 (Fisons Instrument). The amount

261   of OC (%) in each aggregate fraction was normalized to the weight of each fraction: OC

262   content in fraction (g kg^{-1}) × mass of recovered fraction (g kg^{-1}) / total OC recovered (g kg^{-1}).

263   Phosphatase (E.C. 3.1.2.1), B-glucosidase (E.C. 3.2.1.21, urease (E.C. 3.5.1.5) and invertase

264   (E.C. 3.2.1.26) soil enzymatic activities were determined by colorimetry using, respectively,

265   *p*-nitrophenyl-β-D-glucoside[65], *p*-nitrophenyl-phosphate[66], urea[67] and saccharose[66] as

266   substrates. PLFAs were quantified with an Agilent 5973N GC-MS, equipped with a 30 m x

267   0.25 mm ID cross-linked methyl silicone (0.25 mm film thickness) HP-5-MS capillary

268   column after extraction of the soil lipid phase with a mixture of methanol, dichloromethane

269   and sodium bromide, transesterification of fatty acids by saponification and column

270   separation of phospholipids[68].

271   Results were statistically elaborated with ANOVA and Tukey's alternate test. PLFA patterns

272   were explored by Principal Component Analysis (PCA) and distance-based Redundancy

273   Analysis (db-RDA).

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Supplementary Information is available in the online version of the paper.

Author Contributions A.P. and R.S. conceived the project. A.P., R.S., G.C., L.Z., M.T., E.P. handled experimental filed plots. All authors contributed to the literature search. R.S. C.G., L.Z, E.P. elaborated data for publication. V.C., A.N., E.P. conducted analyses. A.P., R.S., E.P. wrote the manuscript draft and all authors contributed to interpretation of the results and writing of the final paper.
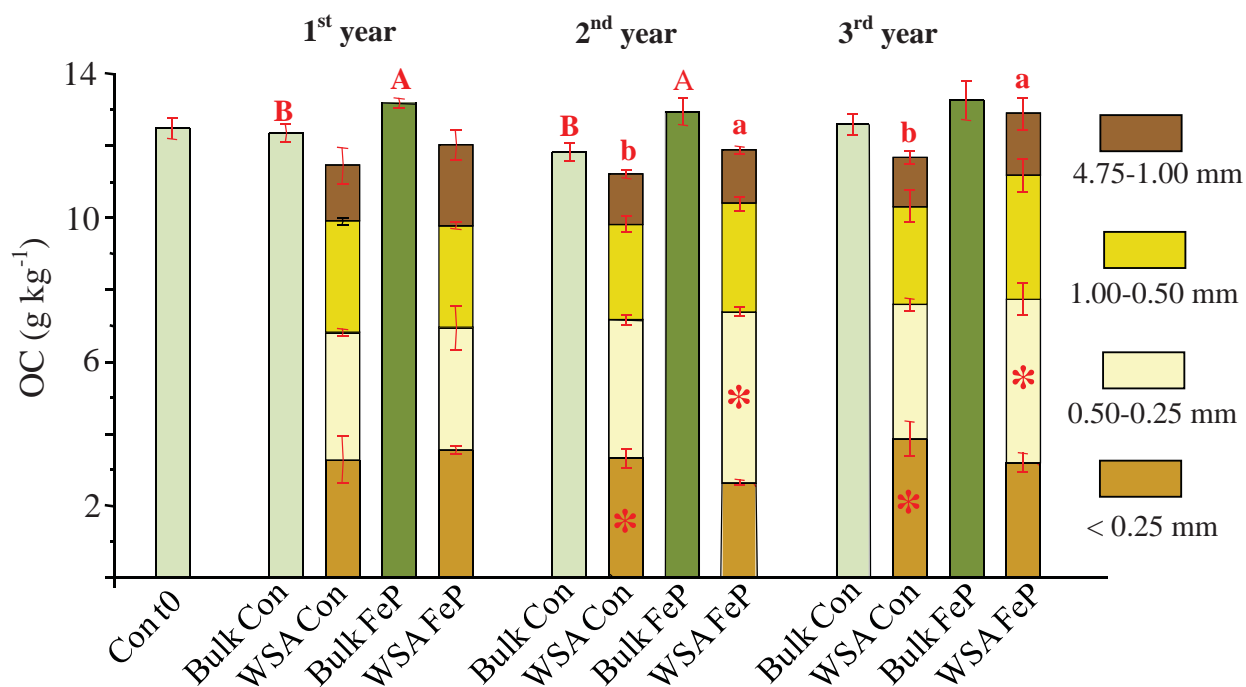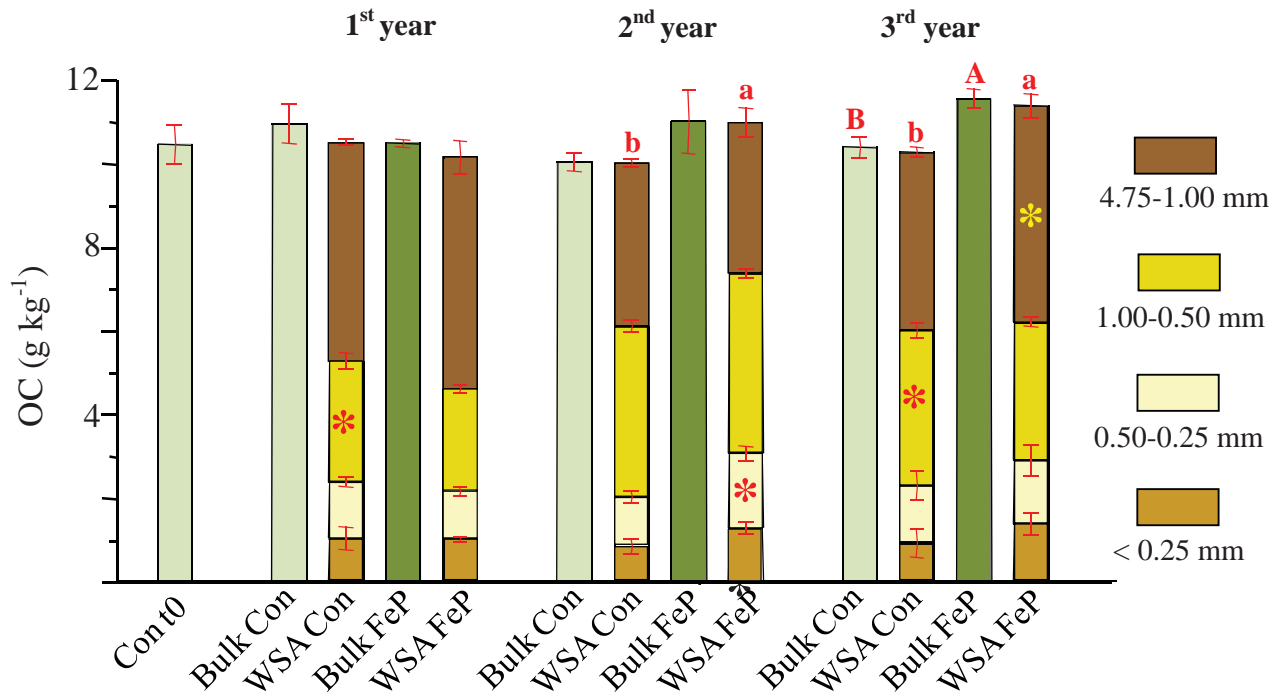
Figure 1. TOC content (g.kg⁻¹) in bulk soils and sum of separated classes of water-stable aggregates (WSA) for original soil (ContT0) and control and FeP-treated soils from the Torino experimental field plots. The OC content in size-aggregates was calculated by multiplying the OC value in fraction by the ratio of the weight of each fraction over that of the sum of fractions. Error bars indicate standard error (n=3). The asterisk denotes significant differences among size-aggregates at the P=0.05 level. Capital and small letters indicate significant differences at the P=0.05 level for bulk samples and sum of fractions, respectively. Analyses were conducted separately for each year
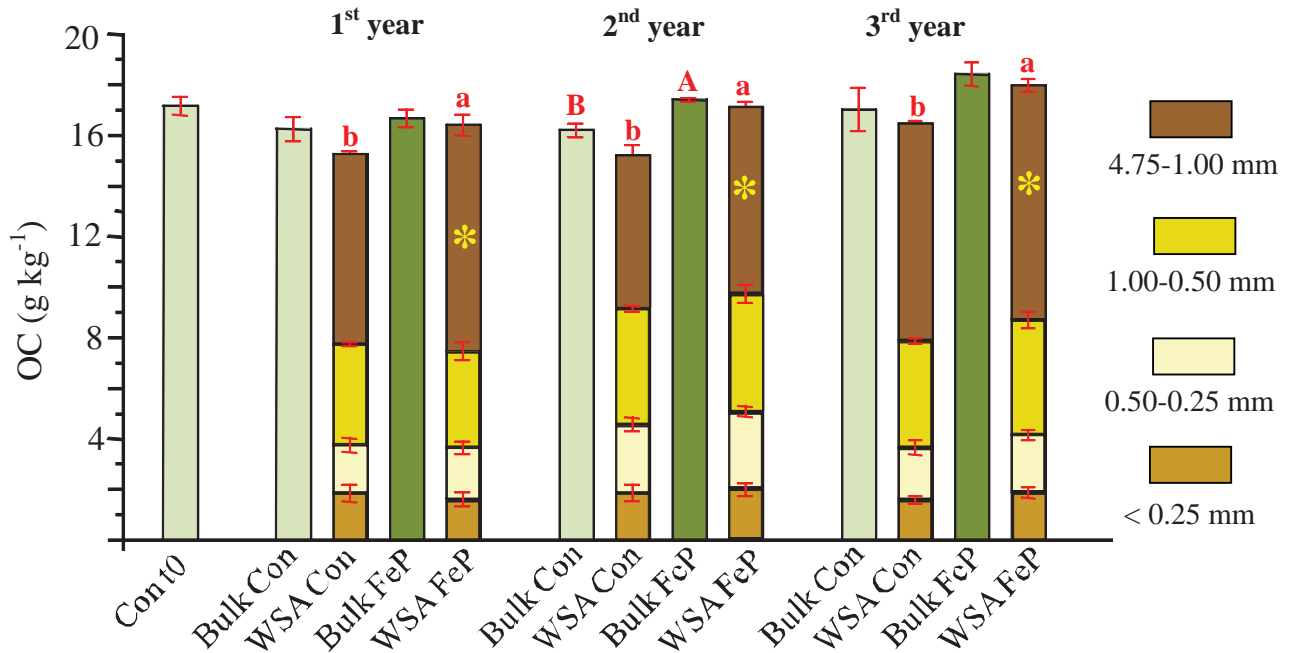
Figure 2. TOC content (g.kg^{-1}) in bulk soils and sum of separated classes of water-stable aggregates (WSA) for for original soil (ContT0) and control and FeP-treated soils from the Piacernza experimental field plots. The OC content in size-aggregates was calculated by multiplying the OC value in fraction by the ratio of the weight of each fraction over that of the sum of fractions. Error bars indicate standard error (n=3). The asterisk denotes significant differences among size-aggregates at the P=0.05 level. Capital and small letters indicate significant differences at the P=0.05 level for bulk samples and sum of fractions, respectively. Analyses were conducted separately for each year.

Figure 3. TOC content (g.kg^{-1}) in bulk soils and sum of separated classes of water-stable aggregates (WSA) for original soil (ContT0) and control and FeP-treated soils from the Napoli experimental field plots. The OC content in size-aggregates was calculated by multiplying the OC value in fraction by the ratio of the weight of each fraction over that of the sum of fractions. Error bars indicate standard error (n=3). The asterisk denotes significant differences among size-aggregates at the P=0.05 level. Capital and small letters indicate significant differences at the P=0.05 level for bulk samples and sum of fractions, respectively. Analyses were conducted separately for each year.
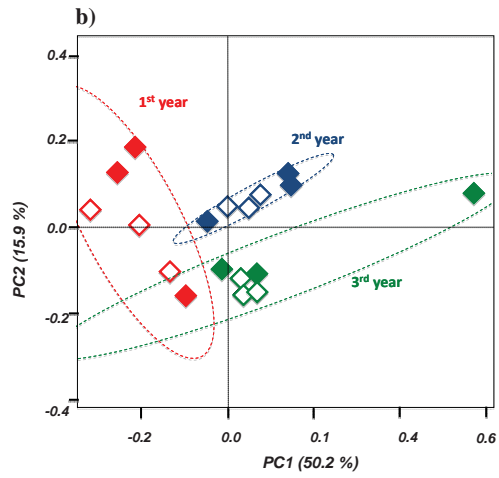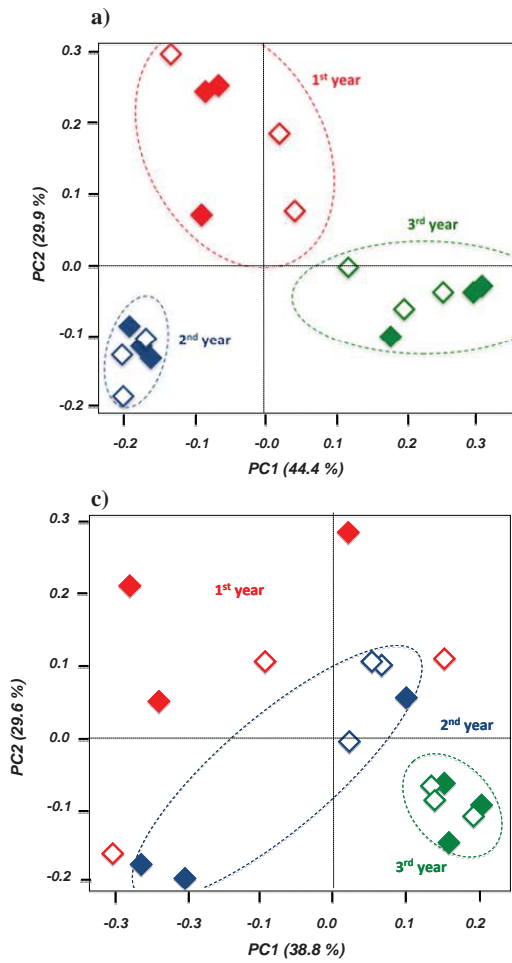
Figure 4. Principal Component Analysis of total PLFA patterns found in the plot soils of a) Torino, b) Piacenza, and, c) Napoli. Results for control and FeP treatments are indicated with empty and solid diamonds, respectively, while the three sampling years are highlighted in different colors with ordination ellipses.

Table 1. Sequestered carbon (Mg ha^{-1} y^{-1}) in both bulk soil and sum of separated WSA for three field plots (Torino, Piacenza, Napoli) under wheat cropping, due to treatment with water-soluble iron-porphyrin for three consecutive years. Values were obtained by multiplying the difference in average OC content (g kg^{-1}) between FeP-treated and untreated soil samples (Figs. 1-3; Tabs. SI2-4) by soil depth interested to conventional tillage (0.3 m), soil bulk density (Mg m^{-3} in Tab. SI-1), and hectare surface (10000 m^{2})

Bulk soil				Sum of WSA			
1st year	2nd year	3rd year	**Average**	1st year	2nd year	3rd year	**Average**
Torino							
3.15	4.72	2.75	**3.54**	1.96	2.75	4.71	**3.14**
Piacenza							
1.56	4.68	5.46	**3.90**	4.29	7.41	5.85	**5.85**
Napoli							
-2.10	4.20	4.62	**2.24**	-1.26	4.20	4.62	**2.52**