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Abstract

Facing an exploding population growth with consequent increase of agriculture intensification, new chemical technologies are being sought to limit organic matter losses and reduce land degradation. Here, we report that an effective organic carbon sequestration in different cropped soils of Italy is obtained by an in situ photo-oxidative coupling among soil humic molecules, when catalyzed under solar irradiation by a water-soluble biomimetic iron-porphyrin catalyst amended to field soils. A 3-year long field study showed that the catalyst-assisted in situ photochemical polymerization of humic matter enabled a yearly sequestration of soil organic carbon that ranged from 2.2 to 3.9 t ha−1 y−1, despite the periodical soil disturbance due to a conventional tillage management. This significant stabilization of organic matter was observed not only in bulk soils but also in water-stable aggregates, whose loss of organic carbon during separation was limited in catalyst-treated soils. Although crop yields were the same in treated and control soils, measurements of phospholipids fatty acids and soil enzyme activities indicated that the catalyzed in situ photo-oxidative coupling of humic molecules did not alter the structure and activity of microbial communities and the biological functions of soils. This innovative and ecologically safe catalytic technology may be developed as a useful soil management practice to stabilize organic matter in situ in arable soils, while concomitantly ensuring soil functions and sustainability of crop production.

1 INTRODUCTION

Agricultural lands under food and bioenergy crops, managed grass, and permanent crops, including agro-forestry, account for about 40–50% of the Earth's land surface (FAOSTAT, 2016). Land-use changes, technological advancement, and varietal improvement enabled world grain harvests to double from 1.2 to 2.5 billion t y−1 between 1970 and 2010, thus ensuring food to population growth (FAOSTAT, 2016). This intensive land exploitation occurred at the cost of an annual total greenhouse gases (GHGs) emission from cropland, that in 2010 is estimated to be 5.2 to 5.8 Gt CO2-eq y−1 and composes 10–12% of global anthropogenic emissions (Smith et al., 2014). However, only agricultural non-CO2 sources are reported as anthropogenic GHG emissions in Intergovernmental Panel on Climate Change calculations. It is thus overlooked that much of carbon lost from cropland upon conventional tillage practices is due to the heterotrophic respiration of the most stable organic carbon pool contained in soil aggregates and hardly reestablished by a short-term photosynthetic cycle (Bradford et al., 2016; Fontaine et al., 2007; Janzen, 2004; Keiluweit et al., 2015).

The increasing pressure for food production and renewable forms of energy to support a predicted global population of 10 billion by 2050 put cropland soils at larger risk of soil organic carbon (SOC) depletion, and, consequently, degradation, erosion, and desertification (Ostle, Levy, Evans, & Smith, 2009). Measures to mitigate carbon losses from agricultural land by reducing SOC oxidation are now limited to management practices such as the use of leys and green manures in crop rotation, addition of organic fertilizers or biochar, crop residue incorporation, no-till (NT)/reduced tillage (RT) management, agroforestry, and return to natural vegetation (Smith et al., 2014). These practices do not only fail in substantially reducing GHGs emissions from soil or permanently stabilizing soil organic matter (Kirkby, Richardson, Wade, Passioura, et al., 2014; Schlesinger & Lichter, 2001; Smith et al., 2014), but are also predicted to hardly match more than a maximum of 25% of the GHGs reductions required by the Kyoto Protocol within 2050 (Read & May, 2001). For example, the RT practice for SOC sequestration is ineffective on poorly drained soils (Franzlubbers, 2010), or when crop residues are removed from soil (Lal, 2011). Despite the widespread adoption of NT/RT farming, its global rate of SOC sequestration is rather small (<0.5 t C ha−1 y−1; Minasny et al., 2017; West & Post, 2002), highly variable among soils and land uses (>50% error; Smith et al., 2007), and effective only over a long-term (Six et al., 2004). Moreover, NT/RT practices do not persistently sequester SOC, because C is concentrated in upper soil layers (Alvarez, 2005), and, as tillage is resumed (possibly by lack of sufficient incentives to farmers), the temporary fixed carbon may be rapidly lost again from soil (Manley, van Kooten, Moeltner, & Johnson, 2005). Finally, the potential contribution of NT to the sustainable intensification of agriculture to feed increasing population has been recently questioned (Pittelkow et al., 2015). It is thus necessary to develop alternative management practices to enable soils to support the expected intensification of food production, while reducing the concomitant risk of land degradation due to soil carbon losses under conventional tillage.

The humified organic matter in soil (70–80% of SOM) represents the most persistent SOC pool with mean residence time of several hundreds of years (Stevenson, 1994), and, thus, the principal long-term C sink in the biosphere (Bradford et al., 2016). It is expected that any advanced comprehension of the chemical nature and reactivity of soil humus should help to devise novel technologies to mitigate carbon losses from soil (Paustian et al., 1997), and reduce the still large uncertainties in SOC prediction models (Bradford et al., 2016). Humic matter is a complex mixture of heterogeneous aliphatic and aromatic molecules of plant and microbial origin (Nebbioso & Piccolo, 2011), which accumulate on soil particles (Kleber & Johnson, 2010) by progressively separating from the soil solution for thermodynamic reasons (Tanford, 1991), and thus, giving rise to chemical and physical protection of SOC (Kleber & Johnson, 2010; Piccolo, 2002). Recent scientific evidence changed the paradigm on the chemical nature of humus by describing humic molecules as heterogeneous, but relatively small in mass (≤1,000 Da; Piccolo & Spiteller, 2003), rather than the previously assumed macropolymers (Piccolo, Conte, & Cozzolino, 2001) de novo synthesized in soil. Soil

humic molecules were shown to be associated in supramolecular structures, which are prevalently stabilized by weak non-covalent bonds and may be easily disrupted by interactions with organic acids (Piccolo, 2002; Piccolo, Conte, Trivellone, Van Lagen, & Buurman, 2002; Smejkalova & Piccolo, 2008). This new view even led some scientists to the extreme assumption that the persistence of SOM should be due less to the structure of humic components and their arrangement with clay particles than to the prevailing environmental and biological conditions in soil (Piccolo, 2016; Schmidt et al., 2011).

The new awareness of the chemical structure and conformational arrangement of organic molecules in the soil matrix (Drosos et al., 2017; Piccolo, 2016) conveys the possibility of controlling the dynamics of humus by chemical technologies in order to enhance SOC persistence (Piccolo, Spaccini, Nebbioso, & Mazzei, 2011). For example, it is known that oxidative enzymes induce a free-radical driven coupling reaction among small humic-like aromatic molecules (Bollag, 1992), and this catalyzed formation of intermolecular covalent bonds may be technologically exploited to increase the molecular mass of humic molecules (Piccolo et al., 2000). As a more convenient alternative to fragile enzymes, synthetic biomimetic metal-porphyrin catalysts that mimic the activity of oxidative enzymes, were found to produce larger and chemically stable humic molecules during oxidative (H2O2) treatments of soluble humus (Piccolo, Conte, & Tagliatesta, 2005; Smejkalova & Piccolo, 2006; Smejkalova, Piccolo, & Spiteller, 2006). Being more suitable for soil applications than H2O2, dioxygen molecules under solar irradiation and in the presence of both homogeneous and heterogeneous metal-porphyrin catalysts provide the highly oxidizing singlet oxygen that induces the photo-oxidative formation, via a free radical mechanism, of intermolecular C–C and C–O–C bonds among humic phenolic molecules (Nuzzo & Piccolo, 2013a, 2013b; Smejkalova & Piccolo, 2005).

We thus conceived that an oxidative polymerization of SOM can be conducted directly in situ in soil by the activity of a biomimetic photocatalyst under field solar irradiation. The catalytic formation of multiple covalent bonds among humic molecules and the consequent increase of chemical energy in SOM would result in a reduction of its bioaccessibility to microbial mineralization and in a concomitant OC accumulation in soil. Furthermore, larger and branched humic molecules would promote the association of soil particles into greater soil aggregates, thus improving soil physical quality (Baldock & Skjemstad, 2000; Tisdall & Oades, 1982). These hypotheses were proved earlier by treating whole soils with the biomimetic photocatalyst in either laboratory or mesocosm experiments under natural light, whereby the enhanced chemical stabilization of SOC significantly reduced microbial respiration, increased plant roots system (Gelsomino, Tortorella, Cianci, et al., 2010; Nuzzo, Madonna, Mazzei, Spaccini, & Piccolo, 2016; Piccolo et al., 2011), and improved soil structural stability even after several wetting and drying cycles (Piccolo et al., 2011).

The aim of this study was thus to apply the in situ photocatalyzed polymerization technology of carbon sequestration directly on different agricultural soils during a 3-year field experiments and follow the soil organic carbon content, microbial community distribution, and enzymes activities.

2 MATERIALS AND METHODS

Catalyst. 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 (Piccolo et al., 2005, 2011).

2.1 Soils and experimental field sites

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 Table S1 for soil classification, textural composition, and other properties of selected site soils).

2.2 Field treatments

The field experiments were conducted for three consecutive years on four replicates of 1 × 1 m plots inserted in larger (7 × 7 m) plots cultivated with wheat (Triticum durum) and managed with a 35 cm depth ploughing, followed by surface harrowing. Both ploughing and harrowing on the catalyst-treated 1 × 1 m plots were conducted manually in order to avoid soil losses and/or mixing over the plot borders. Treatments were (a) control soils managed with conventional tillage and mineral fertilization with urea at the rate of 130 kg ha−1of N; (b) 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, 1 g of catalyst was dissolved in 10 L of water and added to soil plots by a sprinkling irrigation each year before sowing (see Table S2 for time schedule of agronomic operations at the three sites). At the end of the cropping cycle, about 1 kg of soil was sampled from the first 30 cm (resulting from the mixing of five subsamples from each plot) of both control and FeP-treated plots, air dried, and stored for subsequent analyses.

2.3 Aggregate-size distribution

Water-stable aggregates (WSA) were isolated from bulk soils as reported earlier (Spaccini & Piccolo, 2013) by applying a wet sieving method (Kemper & Rosenau, 1986). 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 (up and down 4 cm) for 30 times during 1 min. The recovered aggregate fractions, which represented the 4.75–1.00, 1.00–0.50, 0.50–0.25, <0.25 mm size ranges (Tables S3–S5), were oven-dried at 60 °C, weighed, and stored at room conditions.

2.4 OC determination

Total amount of OC in bulk soils and in WSA were determined on finely ground samples (<0.5 mm) by an Elemental Analyzer EA 1108 (Fisons Instrument). The amount of OC (%) in each aggregate fraction was normalized to the weight of each fraction: OC content in each fraction (g kg−1) × mass of each aggregate fraction (g kg−1) /total OC content in all fractions (g kg−1).

2.5 Activities of soil enzymes

Phosphatase (E.C. 3.1.2.1), β-glucosidase (E.C. 3.2.1.21), urease (E.C. 3.5.1.5), and invertase (E.C. 3.2.1.26) enzymatic activities in soil were determined by colorimetry using, respectively, p-nitrophenyl-β-D-glucoside (Eivazi & Tabatabai, 1990), p-nitrophenyl-phosphate and saccharose (Sannino & Gianfreda, 2001), and urea (Kandeler & Gerber, 1988) as substrates.

2.6 Phospholipids fatty acids (PLFA)

PLFA were quantified with an Agilent 5,973 N GC–MS, equipped with a 30 m × 0.25 mm ID cross-linked methyl silicone (0.25 mm film thickness) HP-5-MS capillary column, after extraction of the soil lipid phase with a mixture of methanol, dichloromethane, and sodium bromide, transesterification of fatty acids by saponification and column separation of phospholipids (Ibekwe & Kennedy, 1998).

2.7 Statistical analysis

Measured variables were tested by a two-way analysis of variance using year, FeP addition, and their interactions as factors. The comparisons among the means were made using Tukey's test ($p < .05$). All

statistical analyses were performed using Statgraphics Centurion for Windows software (StatGraphics Centurion, version XV). PLFA patterns were explored by Principal Component Analysis (PCA) and distance-based Redundancy Analysis.

3 RESULTS AND DISCUSSION

The field scale effectiveness in SOC fixation by a water-soluble FeP treatment is reported here for three soils of different properties (Table S1) under conventional soil management for wheat production. In the silty-loam soil of Torino (Table S3), the FeP added to soil significantly enhanced the OC content of the bulk soil after the first year in respect to that of both control and original soil (Figure 1). The significant increase of OC fixation in the bulk soil was confirmed after the second year, whereas the still increasing trend lost significance after the third year (Table S3). However, a significant SOC stabilization by the FeP treatment after both the second and third year of management was shown by the sum of OC content in the WSA, which were separated by wet sieving from the bulk soil. In fact, despite the inherent loss of the soluble OC during wet-sieving separation (Piccolo et al., 2011), the FeP-treated soil maintained up to 97% of SOC in WSA at the end of the third year of cultivation, as compared to only 93% of SOC retention in WSA of the control soil (Figure 1; Table S3). The evidence of SOC fixation in the Fe-amended soil was also supported by the significant shift of carbon content in WSA towards larger size-aggregates after the second and third experimental year, whereby the relative SOC enhancement in larger particles (0.50–0.25 mm) corresponded to a concomitant decrease of OC content in the <0.25 mm microaggregates (Figure 1; Table S3). This indicates that the occurred photo-oxidative coupling among humic phenolic molecules and consequent enhancement of SOM hydrophobicity induced the association of microaggregates and the formation of larger soil particle-sizes (Kaiser, Kleber, & Berhe, 2015; Nuzzo & Piccolo, 2013b; Spaccini, Piccolo, Conte, Haberauer, & Gerzabek, 2002).
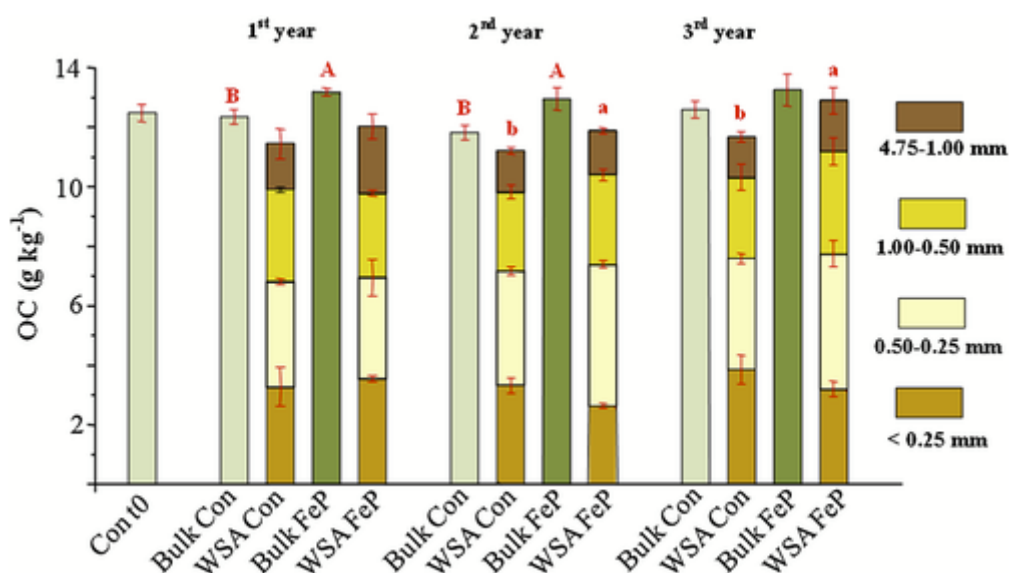

Figure 1

TOC content (g kg−1) in bulk soils and sum of separated classes of water-stable aggregates (WSA) for original soil (ContT0) and control (BulkCon, WSACon) and iron-porphyrin (FeP)-treated (BulkFeP, WSAFeP) 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 = 4). Capital and small letters indicate significant differences between means by the Tukey's test (p < .05) for bulk samples and sum of fractions, respectively. Analyses were conducted separately for each year by the one-way ANOVA

The FeP treatment on the silty clay loam soil of Piacenza (Table S4) produced an increase of OC content in the bulk soil that was significantly larger than control only after the second experimental year, whereas a significant OC fixation as sum of WSA was evident for every year (Figure 2; Table S4). In fact, the particles mechanical separation in water depleted only about 2% of SOC for the FeP-treated soil, whereas the OC loss from control was significantly greater than this value for each experimental year (Figure 2; Table S4). The stabilization of SOM by the photocatalyzed reaction after the second year was confirmed by the significant enhancement of OC content in each separated aggregate-size (Table S4). Moreover, the overall improvement of soil aggregation due to the FeP treatment was shown by the significant relative increase in OC content in the largest size-aggregate (4.75–1.00 mm) at each experimental year.
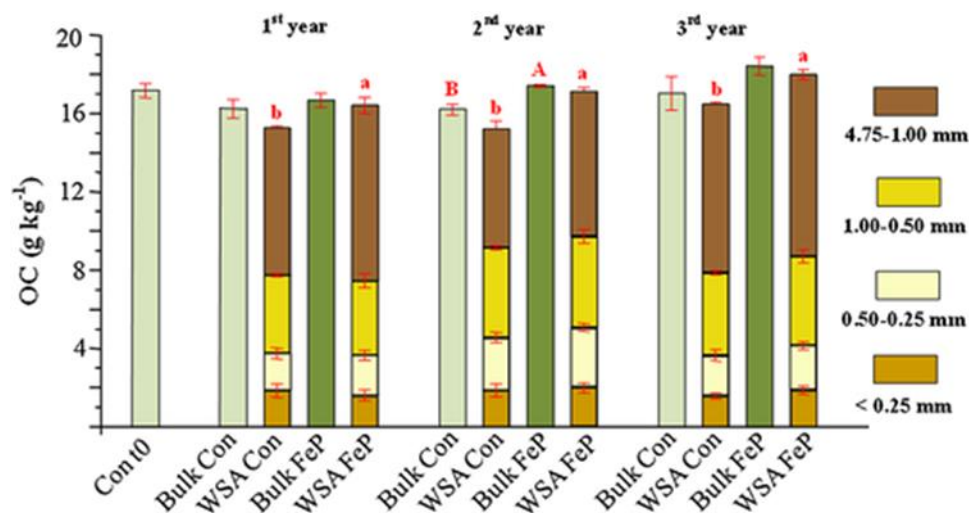

Figure 2

TOC content (g kg−1) in bulk soils and sum of separated classes of water-stable aggregates (WSA) for original soil (ContT0) and control (BulkCon, WSACon) and iron-porphyrin (FeP)-treated (BulkFeP, WSAFeP) soils from the Piacenza 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 = 4). Capital and small letters indicate significant differences between means by the Tukey's test (p < .05) for bulk samples and sum of fractions, respectively. Analyses were conducted separately for each year by the one-way ANOVA

The effect of the catalyst addition to the clay-loam soil of the Napoli site was similar to that of the other two soils. However, a significant SOC increase (>10%) in the bulk soil and sum of fractions of FeP-treated plots was found only after the second experimental year and maintained in the third year (Figure 3; Table S5). Nevertheless, a different carbon distribution between FeP-treated and control soils was noted already after the first experimental year in the separated size-aggregates. In fact, the significant reduction in OC content induced by the catalyst treatment in the 1.00–0.50 mm aggregate fraction is in line with the observed relative percent increase of OC content for the largest (4.75–1.00 mm) size- aggregate (Table S5),

thus indicating a progressive incorporation of carbon-rich particles into soil macroaggregates. The occurred OC fixation by the FeP treatment after the second and third year of cultivation was shown by the significantly larger OC content in the sum of WSA, in respect to control (Figure 3; Table S5). This effect was accompanied by a significant OC stabilization in the 0.50–0.25 and <0.25 mm size-aggregates after the second year, and a definite increase of OC content and relative percentage in the largest aggregates (4.75–1.00 mm) in the third year.
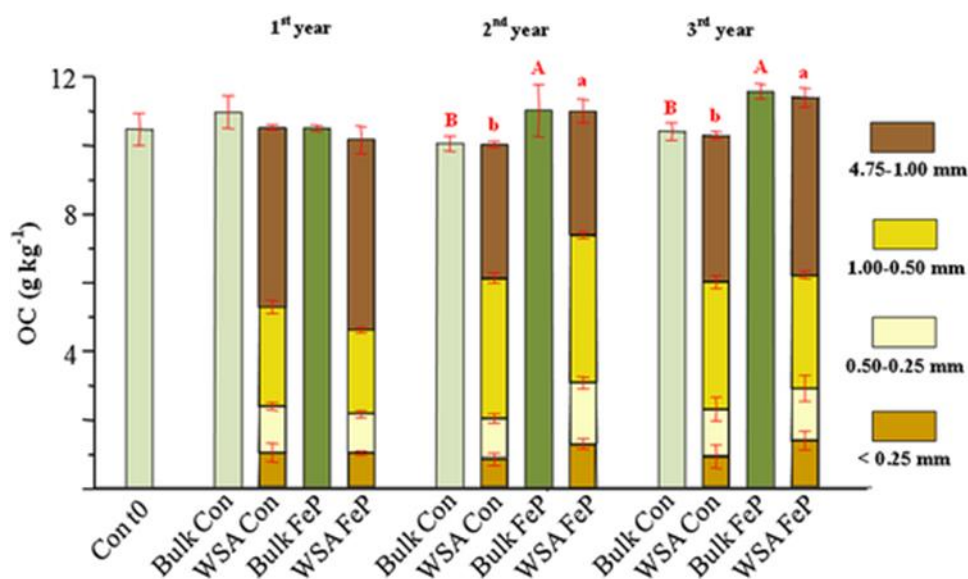

Figure 3

TOC content (g kg−1) in bulk soils and sum of separated classes of water-stable aggregates (WSA) for original soil (ContT0) and control (BulkCon, WSACon) and iron-porphyrin (FeP)-treated (BulkFeP, WSAFeP) 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 = 4). Capital and small letters indicate significant differences between means by the Tukey's test (p < .05) for bulk samples and sum of fractions, respectively. Analyses were conducted separately for each year by the one-way ANOVA

The differences in fixed carbon among soils are likely to depend on the specific soil properties, and local evapotranspiration rates and rainfall events. In fact, adsorption/desorption equilibria of the biomimetic catalyst adsorbed on the surface of soil aggregates determine its photocatalytic activity in the soil solution at the soil-atmosphere interphase, where maximum is the photochemical induction of natural light (Nuzzo et al., 2016). Similarly, the periodical water fluxes due to rainfall and soil evapotranspiration, favor the desorption from soil particles of soluble humic molecules, which become the substrates of photo-oxidative couplings occurring in the soil solution (Keiluweit et al., 2015; Narasimhan, 2009; Piccolo et al., 2011).

In order to account for these unpredictable variabilities in the complex field soil systems, the measured TOC were elaborated by a two-ways analysis of variance for three sites using not only treatment but also year, and their interactions as effect factors (Table 2). Despite the complexity of the soil system, enhanced by the differences in soil management and climatic conditions at different sites, the effect of FeP addition on soil carbon content and treatment year was generally highly significant in both bulk soils and corresponding water-stable separates. Only exception was the year factor for the Napoli site, although the

interaction between year and treatment factors in the same site has maintained a p < .01 significance for both bulk and WSA samples.

These results showed that the soil treatment with the biomimetic water soluble iron-porphyrin in field conditions determined a significant sequestration of organic carbon in respect to untreated soils in all three experimental plots of this study. Already after 1 year of wheat cultivation following the FeP amendment, the photocatalyzed polymerization of SOM in Torino and Piacenza bulk soils provided an OC fixation of 3.15 and 1.56 Mg ha−1 larger than control, respectively, although it was not effective for the Napoli soil (Table 1). This may have been possibly due to the heavy rainfall that followed the first year catalyst amendment to the Napoli soil, and have caused a substantial leaching of the water-soluble catalyst down the profile, thereby reducing its concentration at the soil surface where the photocatalysis is effective. However, a significant net increase of OC fixation was induced by the FeP treatment after the second and third experimental year for all three field plots, with values as small as 2.75 Mg ha−1 and as large as 5.46 Mg ha−1 for Torino and Piacenza bulk soils, respectively. The magnitude of carbon sequestration obtained by the photo-oxidative catalyzed coupling of humic molecules was also evident from the sum of WSA, whose OC content larger than control varied from 1.96 Mg ha−1 in Torino soil after the first year of cultivation to 7.41 Mg ha−1 in Piacenza soil for the second experimental year (Table 1).

Table 1. Sequestered carbon (Mg ha−1 y−1) in both bulk soil and sum of separated water-stable aggregates (WSA) for three field sites (Torino, Piacenza, Napoli) under wheat cropping, due to treatment with water-soluble iron-porphyrin (FeP) for three consecutive years

	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

Note. Values were obtained by multiplying the difference in average OC content (g kg−1) between FeP-treated and untreated soil samples (Figures. 1-3; Tables S3–S5) by soil depth interested to conventional tillage (0.3 m), soil bulk density (Mg m−3 in Table S1), and hectare surface (10,000 m2).

* significant difference from control at P < 0.05 based on Tukey's test

Despite the variability in OC fixation due to heterogeneity of soil matrices, and site conditions, and inherent randomness of representative samplings, the carbon sequestered in bulk soil by the photo-activity of the iron-porphyrin catalyst for three consecutive years reached an average of 3.54, 3.90, and 2.24 Mg ha−1 y−1 for the Torino, Piacenza, and Napoli experimental sites, respectively (Table 1). These significant yearly amounts of fixed SOC were achieved under conventional tillage management, whereas the current highly experimented NT or RT practices are far from reaching such a large OC increase in cropped bulk soils even after several years, whether under boreal North European (Sheehy, Regina, Alakukku, & Six, 2015) or Mediterranean (Garcia-Franco, Albaladejo, Almagro, & Martínez-Mena, 2015) agroecosystems.

This considerable SOM incorporation even under conventional tillage management was not due to a reduced exposure of SOC to microbial respiration, as in NT and RT management (Franzlubbers, 2010), but rather to an enhanced chemical and biochemical stabilization of SOM achieved by the in situ catalyzed photo-oxidative coupling among soil humus molecules. The phenolic or other molecules susceptible to undergo the catalyzed coupling reaction in soil (Nuzzo et al., 2016; Piccolo et al., 2011) are likely to originate from the large mass of carbon moieties circulating in the soil solution during and after the crop growing season. These may be either soluble humic molecules in equilibrium with those adsorbed on soil particles, or lignin monomers released from heterotrophically respired crop residues, or exudates of plant

roots, or their transformed metabolites by the intensified soil microbial metabolism (Bradford et al., 2016; Keiluweit et al., 2015; Kell, 2012). All that soluble carbon in the soil solution may follow the soil evapotranspiration/precipitation hydrological cycles (Narasimhan, 2009) and periodically become the substrate of the polymerization reaction at the soil-atmosphere interface, under the activity of the photocatalyst, that is concomitantly desorbed in the soil solution from the surface of soil particles. This photo-oxidatively coupled carbon in catalyst-treated soils can thus be removed from solution and protected from microbial mineralization, due to a thermodynamic shift, referred to as the "hydrophobic effect" (Tanford, 1991), towards adsorption on aggregates surfaces. Conversely, in the untreated control soils, most of the carbon present in the soil solution is rapidly consumed by microbial metabolism and released as CO_2 to the atmosphere, thereby reducing significantly the percent of SOC measured in control samples at the end of the growing season. In fact, our results indicate that a significant part of organic matter molecules is fixed in the soil each year due to the FeP treatment, and is, therefore, subtracted to the OC losses that occur in control soils.

The SOC accumulation in the cropped field plots of this study is to be attributed to the abiotic coupling reaction induced by the synthetic FeP catalyst, without any influence on the soil biological processes. The invariance of soil biological activity upon FeP treatment was shown by the PLFA (Figure 4) and the activities of soil β-glucosidase, phosphatase, urease, and invertase enzymes (Table S6), which were measured on the same cultivated soils and over the three experimental years (Puglisi & Trevisan, 2012). The PCA multivariate elaboration of PLFA results failed to reveal significant differences between untreated and FeP-treated plots in terms of structure of living soil microorganisms (Figure 4). In particular, results from Torino and Piacenza sites grouped separately according to sampling time for all 3 years of experimentation, whereas in the Napoli site, the grouping became evident only at the third year. No significant clustering was instead found as a result of catalyst treatment, being control and treated samples grouped together in all three sites at each sampling time (Figure 4). The PCA description of results were confirmed by applying the distance-based redundancy analysis model (Legendre & Anderson, 1999), in order to verify the significant effect of either sampling time or FeP treatment, as a function of PLFA patterns found for the three experimental sites. The model showed a significance ($p < .05$) for sampling time in each site, with percent of explained variance ranging from 26.7% in Napoli to 40.2% in Piacenza and 61.2% in Torino. Conversely, the model did not reveal any significant difference between the control and catalyst-treated soil, because the FeP effect showed a very low percent of explained variance (<4%) and was never significant (Table S7).
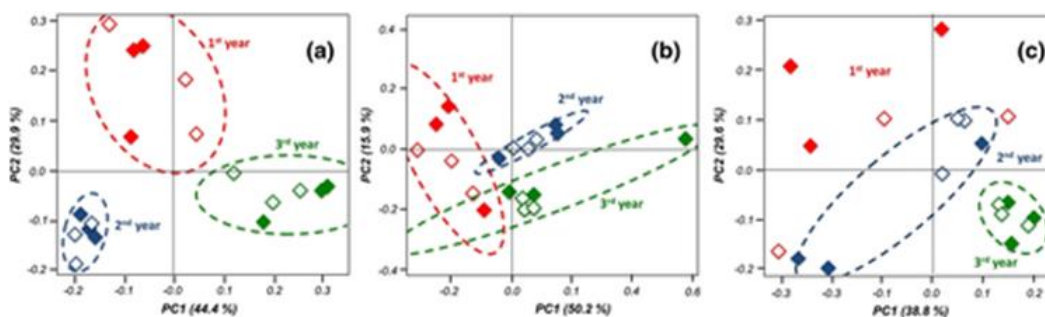

Figure 4

Principal component analysis of total phospholipids fatty acids patterns found in the plot soils of (a) Torino, (b) Piacenza, and, (c) Napoli. Results for control and iron-porphyrin treatments are indicated with empty and solid diamonds, respectively, whereas the three sampling years are highlighted in different colors with ordination ellipses

These results confirm that the considerable SOC accumulation observed in FeP-treated plots (Table 2) was not linked to changes in structure and activity of microbial communities, and it must be most likely related to the abiotic catalyst-assisted photo-polymerization of SOM. Furthermore, the biochemical index of soil quality alteration (Puglisi & Trevisan, 2012) based on enzymes activities measured in the plot soils of this study excluded that the catalyst treatments had any effects on the biological quality of soils (Table S6).

Table 2. Significant difference (P) on measured TOC content by a two-way ANOVA (year and water-soluble FeP treatment) for both bulk soils and corresponding water-stable aggregates (WSA) sizes (mm) for three field sites (Torino, Piacenza, Napoli) under wheat cropping

		Bulk soil	WSA sizes			
			4.75–1.00	1.00–0.50	0.50–0.25	<0.25
Torino	Year (Y)	0.0115	<0.0001	<0.0001	<0.0001	<0.0001
	Treatment (T)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	YxT	0.3684	<0.0001	<0.0001	<0.0001	<0.0001
Piacenza	Year (Y)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	Treatment (T)	<0.0001	<0.0001	0.0643	<0.0001	0.0038
	YxT	0.0919	<0.0001	<0.0001	0.012	<0.0001
Napoli	Year (Y)	0.2445	<0.0001	<0.0001	<0.0011	0.0031
	Treatment (T)	0.0008	<0.0001	0.0072	<0.0001	<0.0001
	YxT	0.0035	<0.0001	<0.0001	<0.0001	<0.0001

It is also important to underline that the significant SOC sequestered by the in situ photo-catalytic treatment of these field plots did not imply any reduction in crop yield (Table S8; Grignani et al., 2012). This indicates that such an in situ sequestration technology is not only effective towards SOC sequestration, but it also conserves the full plant nutrition capacity of soil and its potential crop productivity. Conversely, the NT and RT practices, aimed to increase carbon sequestration by reducing the bio-oxidation of SOM, are reported to limit the mineralization of plant nutrients in soils and result in lower crop yields (Pittelkow, 2015; Salem, Valero Ubierna, Muñoz-García, Gil Rodríguez, & Silva, 2015; Zavattaro et al., 2015).

The conceptual approach of soil organic matter fixation has long considered decreasing the rate of its biological decomposition by reducing tillage and adding organic materials to soil (Powlson et al., 2012), despite its poor effectiveness (Conant, Easter, Paustian, Swan, & Williams, 2007). But this view is evolving and many now try to develop chemical technologies to both reduce CO2 emissions from soil and increase SOM, such as replacement of agricultural lime with silicates (Renforth, Washbourne, & Taylder, 2011) or promotion of carbonation with additions of Ca- and Mg-rich salts to soils (Manning & Renforth, 2013; ten Berge et al., 2012) or use of polyphenols to complex SOM or inhibit enzymes that decompose it (Sinasbaugh, 2010).

Here, we showed that the innovative in situ photo-oxidative coupling reaction among humic molecules catalyzed by an eco-friendly water-soluble metal-porphyrin was highly effective in fixing organic carbon in soil. Even under conventional soil management, the catalytic technique increased, in treated plot soils, the carbon content from 2.24 to 3.90 Mg ha^{-1} y^{-1} more than control soil. This significant carbon stabilization is attributed to the catalyst-assisted abiotic enhancement of covalent bonds in soil molecular components. In fact, the larger molecular mass of humic molecules coupled under the catalytic photo-oxidation favours, in turn, the processes of chemical and physical protection of OC in soil and reduces the extent of OM losses

from soil. However, more work would be needed with field samples to confirm that the mechanism of catalyzed photopolymerization occurs as it is explained here and was proved in previous homogenous and heterogeneous experiments in the laboratory or mesocosms. Moreover, the objectively considerable amount of carbon sequestered yearly in this study by the applied photocatalytic technology was even achieved without either altering the soil biological quality or reducing the crop yields. Fixation of SOC by amending soils with a photocatalyst, such as metal-porphyrins, may be promising to establish an easy soil management practice that not only controls SOM dynamics and contributes to accumulate carbon stocks in agricultural soils, but also sustains the intensification of agriculture required to feed an increasing population.

REFERENCES

Alvarez, R. (2005). A review of nitrogen fertilizer and conservation tillage effects on soil organic carbon storage. Soil Use Management, 21, 38– 52. https://doi.org/10.1079/SUM2005291

Baldock, J. A., & Skjemstad, J. O. (2000). Role of the soil matrix and minerals in protecting natural organic materials against biological attack. Organic Geochemistry, 31, 697– 710. https://doi.org/10.1016/S0146-6380(00)00049-8

ten Berge, H. F. M., van derMeer, H. G., Steenhuizen, J. W., Goedhart, P. W., Knops, P., & Verhagen, J. (2012). Olivine weathering in soil, and its effects on growth and nutrient uptake in ryegrass (Lolium Perenne L.): A pot experiment. PLoS One, 7, 1– 8. https://doi.org/10.1371/journal.pone.0042098

Bollag, J.-M. (1992). Decontaminating soil with enzymes: An in situ method using phenolic and anilinic compounds. Environmental Science Technology, 26, 1876– 1881. https://doi.org/10.1021/es00034a002

Bradford, M. A., Wieder, W. R., Bonan, G. B., Fierer, N., Raymond, P. A., & Crowther, T. W. (2016). Managing uncertainty in soil carbon feedbacks to climate change. Nature Climate Change, 6, 751– 758. https://doi.org/10.1038/nclimate3071

Conant, R. T., Easter, M., Paustian, K., Swan, A., & Williams, S. (2007). Impacts of periodic tillage on soil C stocks: A synthesis. Soil Tillage Research, 95, 1– 10. https://doi.org/10.1016/j.still.2006.12.006

Drosos, M., Nebbioso, A., Mazzei, P., Vinci, G., Spaccini, R., & Piccolo, A. (2017). A molecular zoom into soil Humeome by a direct sequential chemical fractionation of soil. The Science of the Total Environment, 15, 807– 816. https://doi.org/10.1016/j.scitotenv.2017.02.059

Eivazi, F., & Tabatabai, M. A. (1990). Factors affecting glucosidase and galactosidase activities in soils. Soil Biology Biochemistry, 22, 891– 897. https://doi.org/10.1016/0038-0717(90)90126-K

FAOSTAT (2016). FAO statistics division. Food and agriculture organization of the united nations. Italy: Rome www.faostat.fao.org

Fontaine, S., Barot, S., Barré, P., Bdioui, N., Mary, B., & Rumpel, C. (2007). Stability of organic carbon in deep soil layers controlled by fresh carbon supply. Nature, 450, 277– 280. https://doi.org/10.1038/nature06275

Franzlubbers, A. J. (2010). Achieving soil organic carbon sequestration with conservation agricultural systems in the southeastern US. Soil Science Society American Journal, 74, 347– 357. https://doi.org/10.2136/sssaj2009.0079

Garcia-Franco, N., Albaladejo, J., Almagro, M., & Martínez-Mena, M. (2015). Beneficial effects of reduced tillage and green manure on soil aggregation and stabilization of organic carbon in a Mediterranean agroecosystem. Soil Tillage Research, 153, 66– 75. https://doi.org/10.1016/j.still.2015.05.010

Gelsomino, A., Tortorella, D., Cianci, V., et al. (2010). Effects of a biomimetic iron-porphyrin on soil respiration and maize root morphology as by a microcosm experiment. Journal Plant Nutrition Soil Science, 173, 399– 406. https://doi.org/10.1002/jpln.200900125

Grignani, C., Alluvione, F., Bertora, C., Zavattaro, L., Fagnano, M., Fiorentino, N., … Amato, M. (2012). Field plots and crop yields under innovative methods of carbon sequestration in soil. In A. Piccolo (Ed.), Carbon sequestration in agricultural soils (pp. 39– 60). Heidelberg, Germany: Springer.

Ibekwe, A., & Kennedy, A. (1998). Phospholipids fatty acids profiles and carbon utilization patterns for analysis of microbial community structure under field and greenhouse conditions. FEMS Microbiological Ecology, 26, 151– 163. https://doi.org/10.1016/S0168-6496(98)00032-4

Janzen, H. H. (2004). Carbon cycling in earth systems-a soil science perspective. Agriculture. Ecosystem, Environment, 104, 399– 417. https://doi.org/10.1016/j.agee.2004.01.040

Kaiser, M., Kleber, M., & Berhe, A. A. (2015). How air-drying and rewetting modify soil organic matter characteristics: An assessment to improve data interpretation and inference. Soil Biology Biochemistry, 80, 324– 340. https://doi.org/10.1016/j.soilbio.2014.10.018

Kandeler, E., & Gerber, H. (1988). Short-term assay of soil urease activity using colorimetric determination of ammonium. Biology Fertility Soil, 6, 68– 72. https://doi.org/10.1007/BF00257924

Keiluweit, M., Bougoure, J. J., Nico, P. S., Pett-Ridge, J., Weber, P. K., & Kleber, M.. (2015). Mineral protection of soil carbon counteracted by root exudates. Nature Climate Change, 5, 588– 595. https://doi.org/10.1038/nclimate2580

Kell, D. B. (2012). Large scale sequestration of atmospheric carbon via plant roots in natural and agricultural ecosystems: Why and how. Philosophical Transactions of the Royal Society B, 367, 1589– 1597. https://doi.org/10.1098/rstb.2011.0244

Kemper DW & Rosenau RC. 1986. In methods of soil analysis, part I, Klute a (ed). ASA and SSSA; Madison, WI, pp. 425-442

Kirkby, C. A., Richardson, A. E., Wade, L. J., Passioura, J. B., Battenb, G. D., Blanchardb, C., & Kirkegaarda, J. A. (2014). Nutrient availability limits carbon sequestration in arable soils. Soil Biology Biochemistry., 68, 402– 409. https://doi.org/10.1016/j.soilbio.2013.09.032

Kleber, M., & Johnson, M. G. (2010). Advances in understanding the molecular structure of soil organic matter: Implications for interactions in the environment. Advances in Agronomy, 106, 77– 143. https://doi.org/10.1016/S0065-2113(10)06003-7

Lal, R. (2011). Sequestering carbon in soils of agro-ecosystems. Food Policy, 36, S33– S39. https://doi.org/10.1016/j.foodpol.2010.12.001

Legendre P & Anderson MJ. 1999. Distance-based redundancy analysis: Testing multispecies responses in multifactorial ecological experiments, Ecological Monographs 69, 1– 24. https://doi.org/10.1890/0012-9615(1999)069[0001, DBRATM]2.0.CO;2

Manley, J., van Kooten, G. C., Moeltner, K., & Johnson, D. W. (2005). Creating carbon offsets in agriculture through no-till cultivation: A meta-analysis of costs and carbon benefits. Climatic Change, 68, 41– 65. https://doi.org/10.1007/s10584-005-6010-4

Manning, D. A. C., & Renforth, P. (2013). Passive sequestration of atmospheric CO_2 through coupled plant-mineral reactions in urban soils. Environmental Science Technology, 47, 135– 141. https://doi.org/10.1021/es301250j

Minasny, B., et al. (2017). Soil carbon 4 per mille. Geoderma, 292, 59– 86. https://doi.org/10.1016/j.geoderma.2017.01.002

Narasimhan, T. N. (2009). Hydrological cycle and water budgets. In G. E. Likens (Ed.), Encyclopedia of inland waters (pp. 714– 720). Oxford, U.K.: Elsevier.

Nebbioso, A., & Piccolo, A. (2011). Basis of a Humeomics science: Chemical fractionation and molecular characterization of humic biosuprastructures. Biomacromolecules, 12, 1187– 1199. https://doi.org/10.1021/bm101488e

Nuzzo A, Madonna E, Mazzei P, Spaccini R, Piccolo A. 2016. In situ photo-polymerization of soil organic matter by heterogeneous nano-TiO2 and biomimetic metal-porphyrin catalysts. Biology Fertility Soils, 52, 585– 593. https://doi.org/10.1007/s00374-016-1102-z

Nuzzo, A., & Piccolo, A. (2013a). Enhanced catechol oxidation by heterogeneous biomimetic catalysts immobilized on clay minerals. Journal Molecular Catalysis A Chemistry, 371, 8– 14. https://doi.org/10.1016/j.molcata.2013.01.021

Nuzzo, A., & Piccolo, A. (2013b). Oxidative and photo-oxidative polymerization of humic suprastructures by heterogeneous biomimetic catalysis. Biomacromolecules, 14, 1645–1652. https://doi.org/10.1021/bm400300m

Ostle, N. J., Levy, P. E., Evans, C. D., & Smith, P. (2009). UK land use and soil carbon sequestration. Land Use Policy, 26S, S274– S283. https://doi.org/10.1016/j.landusepol.2009.08.006

Piccolo, A. (2002). The supramolecular structure of humic substances. A novel understanding of humus chemistry and implications in soil science. Advances in Agronomy, 75, 57– 134. https://doi.org/10.1016/S0065-2113(02)75003-7

Piccolo, A. (2016). In memoriam Prof. F.J. Stevenson and the question of humic substances in soil. Chemical and Biological Technologies in Agriculture, 3, 23. https://doi.org/10.1186/s40538-016-0076-2

Piccolo, A., Conte, P., & Cozzolino, A. (2001). Chromatographic and spectrophotometric properties of dissolved humic substances compared with macromolecular polymers. Soil Science, 166, 174– 185. https://doi.org/10.1097/00010694-200103000-00003

Piccolo, A., Conte, P., & Tagliatesta, P. (2005). Increased conformational rigidity of humic substances by oxidative biomimetic catalysis. Biomacromolecules, 6, 351– 358. https://doi.org/10.1021/bm0495203

Piccolo, A., Conte, P., Trivellone, E., Van Lagen, B., & Buurman, P. (2002). Reduced heterogeneity of a lignite humic acid by preparative HPSEC following interaction with an organic acid. Characterization of size-separates by PYR-GC-MS and 1H-NMR spectroscopy. Environmental Science Technology, 36, 76– 84. https://doi.org/10.1021/es010981v

Piccolo, A., Spaccini, R., Nebbioso, A., & Mazzei, P. (2011). Carbon sequestration in soil by in situ catalyzed photo-oxidative polymerization of soil organic matter. Environmental Scence Technology, 45, 6697– 6702. https://doi.org/10.1021/es201572f

Piccolo, A., & Spiteller, M. (2003). Electrospray ionization mass spectrometry of terrestrial humic substances and their size-fractions. Analalytical Bioanalytical Chemistry, 377, 1047– 1059. https://doi.org/10.1007/s00216-003-2186-5

Piccolo, A., Cozzolino, A., Conte, P., & Spaccini, R. (2000). Polymerization by enzyme-catalyzed oxidative couplings of supramolecular associations of humic molecules. Naturwissenschaften, 87, 391– 394. https://doi.org/10.1007/s001140050747

Pittelkow, C. M., Liang, X., Linquist, B. A., vanGroenigen, K. J., Lee, J., Lundy, M. E., vanGestel, N., … vanKessel, C. (2015). Productivity limits and potentials of the principles of conservation agriculture. Nature, 517, 365– 368. https://doi.org/10.1038/nature13809

Powlson, D. S., Bhogal, A., Chambers, B. J., Coleman, K., Macdonald, A. J., Goulding, K. W. T., & Whitmore, A. P. (2012). The potential to increase soil carbon stocks through reduced tillage or organic additions–an England and Wales case study. Agriculture Ecosystems Environment, 146, 23– 33. https://doi.org/10.1016/j.agee.2011.10.004

Puglisi, E., & Trevisan, M. (2012). Effects of methods of carbon sequestration in soil on biochemical indicators of soil quality. In A. Piccolo (Ed.), Carbon sequestration in agricultural soils (pp. 179– 207). Heidelberg, Germany: Springer.

Read D & May R. 2001. The role of land carbon sinks in mitigating global climate change. Policy Document 10/01, Royal Society, pp 27.

Renforth, P. J., Washbourne, C. L., & Taylder, J. (2011). Silicate production and availability for mineral carbonation. Environmental Science Technology, 45, 2035– 2041. https://doi.org/10.1021/es103241w

Salem, H. M., Valero Ubierna, C., Muñoz-García, M. A., Gil Rodríguez, M., & Silva, L. L. (2015). Short-term effects of four tillage practices on soil physical properties, soil water potential, and maize yield. Geoderma, 237–238, 60– 70. https://doi.org/10.1016/j.geoderma.2014.08.014

Sannino, F., & Gianfreda, L. (2001). Pesticide influence on soil enzymatic activities. Chemosphere, 45, 417– 425. https://doi.org/10.1016/S0045-6535(01)00045-5

Schlesinger, W. H., & Lichter, J. (2001). Limited carbon storage in soil and litter of experimental forest plots under increased atmospheric CO2. Nature, 411, 466– 469. https://doi.org/10.1038/35078060

Schmidt, M. W. I., Torn, M. S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I. A., … Kögel-Knabner, I. (2011). Persistence of soil organic matter as an ecosystem property. Nature, 478, 49– 56. https://doi.org/10.1038/nature10386

Sheehy, J., Regina, K., Alakukku, L., & Six, J. (2015). Impact of no-till and reduced tillage on aggregation and aggregate-associated carbon in northern European agroecosystems. Soil Tillage Research, 150, 107– 113. https://doi.org/10.1016/j.still.2015.01.015

Sinasbaugh, R. L. (2010). Phenol oxidase, peroxidase and organic matter dynamics of soil. Soil Biology Biochemistry, 42, 391– 404. https://doi.org/10.1016/j.soilbio.2009.10.014

Six, J., Ogle, S. M., Breidt, F. J., Conant, R., Mosier, A. R., & Paustian, K. (2004). The potential to mitigate global warming with no-tillage management in only realized when practiced in the long-term. Global Change Biology, 10, 155– 160. https://doi.org/10.1111/j.1529-8817.2003.00730.x

Smejkalova, D., & Piccolo, A. (2005). Enhanced molecular dimension of a humic acid induced by photooxidation catalyzed by biomimetic metalporphyrins. Biomacromolecules, 6, 2120–2125. https://doi.org/10.1021/bm050112o

Smejkalova, D., & Piccolo, A. (2006). Rates of oxidative coupling of humic phenolic monomers catalyzed by a biomimetic iron-porphyrin. Environmental Science and Technology, 40, 1644–1649. https://doi.org/10.1021/es051760i

Smejkalova, D., & Piccolo, A. (2008). Aggregation and disaggregation of humic supramolecular assemblies by NMR diffusion ordered spectroscopy (DOSY-NMR). Environmental Science Technology, 42, 699–706. https://doi.org/10.1021/es071828p

Smejkalova, D., Piccolo, A., & Spiteller, M. (2006). Oligomerization of humic phenolic monomers by oxidative coupling under biomimetic catalysis. Environmental Science and Technology, 40, 6955–6962. https://doi.org/10.1021/es061127c

Smith, P., Martino, D., Cai, Z., Gwary, D., Janzen, H., Kumar, P., … Ogle, S. (2007). Policy and technological constraints to implementation of greenhouse gas mitigation options in agriculture. Agriculture Ecosystem Environment, 118, 6–28. https://doi.org/10.1016/j.agee.2006.06.006

Smith, P., Bustamante, M., Ahammad, H., Clark, H., Dong, H., Elsiddig, E. A., … House, J. (2014). In O. R. Edenhofer, et al. (Eds.), Climate change 2014: Mitigation of climate change. Contribution of working group III to the fifth assessment report of the intergovernmental panel on climate change (IPCC) (pp. 816–887). Cambridge: Cambridge University Press.

Spaccini, R., & Piccolo, A. (2013). Effects of field managements for soil organic matter stabilization on water-stable aggregates distribution and aggregate stability in three agricultural soils. Journal Geochemical Exploration, 129, 45–51. https://doi.org/10.1016/j.gexplo.2012.10.004

Spaccini, R., Piccolo, A., Conte, P., Haberauer, G., & Gerzabek, M. H. (2002). Increased soil organic carbon sequestration through hydrophobic protection by humic substances. Soil Biology Biochemistry, 34, 1839–1851. https://doi.org/10.1016/S0038-0717(02)00197-9

Stevenson, F. J. (1994). Humus chemistry: Genesis, composition, reaction. New York: Wiley Interscience.

Tanford, C. (1991). The hydrophobic effect: Formation of micelles and biological membranes. Malabar: Krieger.

Tisdall, J. M., & Oades, J. M. (1982). Organic matter and water-stable aggregates in soils. Journal Soil Science, 33, 141–163. https://doi.org/10.1111/j.1365-2389.1982.tb01755.x

West, T. O., & Post, W. M. (2002). Soil organic carbon sequestration rates by tillage and crop rotations. Soil Science. Society American Journal, 66, 1930–1946. https://doi.org/10.3334/CDIAC/tcm.002

Zavattaro, L., Costamagna, C., Grignani, C., Bechini, L., Spiegel, A., Lehtinen, T., … Krüger, J. (2015). Long-term effects of best management practices on crop yield and nitrogen surplus. Italian Journal Agronomy, 10, 47–50. https://doi.org/10.4081/ija.2015.643