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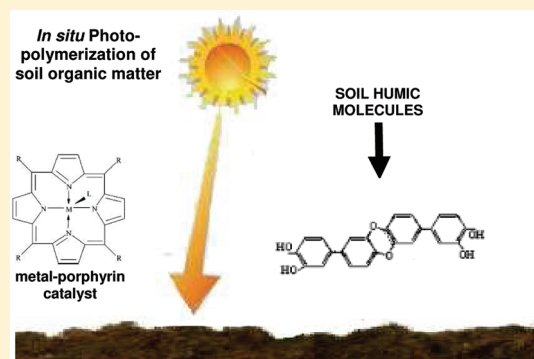
Carbon Sequestration in Soil by in Situ Catalyzed Photo-Oxidative Polymerization of Soil Organic Matter

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ABSTRACT: Here we describe an innovative mechanism for carbon sequestration in soil by in situ photopolymerization of soil organic matter under biomimetic catalysis. Three different Mediterranean soils were added with a synthetic water-soluble iron-porphyrin, irradiated by solar light, and subjected first to 5 days incubation and, then, 15, and 30 wetting and drying (w/d) cycles. The *in situ* catalyst-assisted photopolymerization of soil organic carbon (SOC) increased water stability of soil aggregates both after 5 days incubation and 15 w/d cycles, but not after 30 w/d cycles. Particle-size distribution of all treated soils confirmed the induced soil physical improvement, by showing a concomitant lower yield of the clay-sized fraction and larger yields of either coarse sand- or fine sand-size fractions, depending on soil texture, though only after 5 days incubation. The gain in soil physical quality was reflected by the shift of OC content from small to large soil aggregates, thereby suggesting that photopolymerization stabilized OC by both chemical and physical processes. A further evidence of the carbon sequestration capacity of the photocatalytic treatment was provided by the significant reduction of CO₂ respired by all soils after both incubation and w/d cycles. Our findings suggest that “green” catalytic technologies may potentially be the bases for future practices to increase soil carbon stabilization and mitigate CO₂ emissions from arable soils.



INTRODUCTION

Agricultural lands under food and bioenergy crops, managed grass and permanent crops including agro-forestry, occupy about 40–50% of the Earth's land surface, and accounted for an estimated emission of 5.1–6.1 GtCO₂-eq/yr (10–12% of total global anthropogenic emissions of GHGs).¹ However, actual measures to mitigate GHGs emission from agricultural soils are limited to improved cropland practices such as crop rotation, nutrient management, tillage/residue management, agroforestry, and return to natural vegetation.² These practices are not only far from substantially reducing GHGs emissions from soils or permanently stabilizing soil organic matter (SOM),^{3–5} but are also predicted to hardly match more than a maximum of 25% of the GHGs reductions required by the Kyoto Protocol within 2050.⁶ Despite the knowledge that GHGs release from soil largely derives from biochemical transformations of plant litter and SOM,^{7–9} no new and much wished biotechnological measures are adopted so far to augment mitigation.¹

The humified organic matter in soil (70–80% of SOM)¹⁰ represents the most persistent pool in OC accumulation with mean residence time of several hundreds of years,¹¹ and, thus, the principal potential C sink in the biosphere, whose advanced comprehension may help to mitigate CO₂ emissions from soil.¹² Soil humus is composed by the hydrophobic and heterogeneous aliphatic and aromatic molecules progressively surviving the microbial transformation of dead biological tissues.¹³ Recent scientific evidence shed new light on the chemical nature of

humus by describing humic molecules as heterogeneous but relatively small in mass (≤ 1000 Da),^{14,15} rather than the previously assumed macropolymers.¹⁶ Humic molecules were shown to be tightly associated in supramolecular structures, which are prevalently stabilized by noncovalent hydrophobic bonds.¹³

Based on this new understanding, we reasoned that small aromatic humic molecules could be covalently linked to each other by oxidative coupling reactions under appropriate catalysis, thereby enhancing their molecular size and complexity. We have already shown that larger and more chemically stable humic molecules were obtained by treating solutions of humus extracts in oxidative (H₂O₂) conditions, with a phenoloxidase enzyme, such as peroxidase,¹⁷ or a biomimetic catalyst, such as a water-soluble iron-porphyrin.¹⁸ Both the enzymatic and biomimetic catalysts accelerate the oxidative coupling of phenols via a free-radical mechanism.¹⁹ Moreover, we found that the biomimetic catalysis increased the molecular dimension of humic molecules in solution simply by photo-oxidation under solar radiation, without the need of an additional oxidant.²⁰ By applying photo-oxidative catalyzed conditions on lignin-derived phenolic monomers (similar to those found in soil humus), we proved the

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rapid formation of new intermolecular C—C and C—O—C bonds leading to several identified (up to tetramers) and unidentified oligomers.^{21–23}

The catalyzed photo-oxidative formation of covalent bonds among phenolic molecules in situ in soil is expected to chemically stabilize SOM by increasing the content of chemical energy in soil humic structures. The induced greater stabilization of soil humus would have the consequence to enhance the metabolic threshold required by soil microorganisms to mineralize SOM, thus resulting in soil carbon sequestration and reduction of CO₂ emission from soil. Moreover, increasing the mass of humus molecules would facilitate soil particles association into larger soil aggregates and thus improving soil physical quality.^{24–26} The positive effect of the photopolymerization process was already hinted by a preliminary work, in which the catalyst was used on a microcosm controlled experiment, and determined a reduced respiration from one bare soil.²⁷

Here, we aimed to verify on three different Mediterranean soils whether the new approach based on a catalyst-assisted photopolymerization reaction may stabilize in situ soil organic carbon against microbial mineralization. The persistence of the effect on SOC was studied by subjecting the soils added with the biomimetic iron-porphyrin catalyst subjected to subsequent severe disaggregating processes such as 15 and 30 wetting and drying cycles. SOM stabilization was evaluated by assessing soil physical quality, distribution of organic carbon in soil particle sizes and soil respiration.

MATERIALS AND METHODS

Soil Samples and Characterization. Soil samples were collected from the surface layers (0–30 cm) of three agricultural plots from south-central Italy: 1. Porrara (Avellino), 2. Colomabaia (Caserta), 3. Itri (Latina). Samples were air-dried, sieved through a 4.75 mm sieve, and used for characterization and incubation experiments. Soil texture was measured by the pipet method. Total organic carbon (TOC) was determined by the standard Walkley–Black titration method. Clay mineralogy was determined on oriented samples by X-ray diffraction (XRD) using a Rigaku Geigerflex D/MaxB diffractometer with Fe-filtered CoK α -radiation generated at 40 kV and 30 mA.

Biomimetic Catalyst. The preparation procedure of the *meso*-tetra-(2,6-dichloro-3-sulfonatophenyl)porphyrinate of Fe(III) [Fe(TDCPPS)Cl], have been previously described.¹⁸ Briefly, a *meso*-Tetra(2,6-dichlorophenyl)porphyrin (H₂TDCPP) was first synthesized and then treated for 12 h under argon atmosphere with a 100 mL of a water solution containing 100 mg of Fe(II)SO₄. The solution was then filtered and purified through a cationic exchange resin Dowex 50W X8–100 (50–100 mesh), previously conditioned with a 10% HCl solution. The column was eluted with water and the recovered material dried upon vacuum. The final Fe(TDCPPS)Cl product, was recrystallized in methanol-acetone in order to purify the sample from residual salts.

Photopolymerization Experiments. For each replicate ($n = 3$), 60 g of air-dried soil sample was placed on a Petri dish (12 cm diameter) and soil moisture was kept at 40% of water holding capacity (WHC) by adding X mL of water ($X = 20, 17, 10$ mL, for Porrara, Colomabaia and Itri soil, respectively) in order to obtain a control series. The polymerized series were similarly prepared ($n = 3$) and added with 0.24 μ mol of synthetic water-soluble Fe-(TDCPPS)Cl dissolved in the X mL of water pertaining to

WHC of each soil. After preparation, both control and polymerized series were left under natural solar radiation throughout the following treatments: (i) covered with a Petri dish and incubated for 5 days; (ii) submitted to 15 wetting/drying (w/d) cycles; (iii) submitted to 30 w/d cycles. During w/d cycles, samples were uncovered after a 5 day incubation and distilled water was added, whenever samples became dry (approximately once a week), to re-establish WHC.

Soil Aggregate Stability. Separation of water-stable aggregates was done by a wet sieving method.²⁸ An air-dried subsample (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. Recovered aggregate fractions were oven-dried at 60 °C, weighed, and stored at room conditions.

The mean weight diameter index in water (MWDw), used for the determination of aggregate stability, was calculated according to the following equation:

$$\text{MWDw} = \sum_{i=1}^n X_i W_i$$

where X_i is the mean diameter of each aggregate fraction and W_i is the proportion of the total sample weight occurring in the i th fraction.

The amount of OC (%) in each aggregate fraction was normalized to the weight of each fraction: OC content in fraction (g kg^{-1}) \times mass of recovered fraction (g kg^{-1})/total OC recovered (g kg^{-1}).

Particle-Size Fractionation. All samples were fractionated by applying low-energy sonication, followed by sieving and centrifugation separations as previously described.²⁹ Since the dispersion efficiency is significantly affected by the output of the sonication energy, the applied energetic was calibrated.³⁰ In brief, 9 g of air-dried subsample (<2 mm) were dispersed in 60 mL of distilled water using a probe-type ultrasonic disaggregator (Sonicator Ultrasonic liquid processor, Misonix Incorporated) applying an energy of 267 J \cdot mL⁻¹. To avoid reduction in cavitation, the temperature of soil suspension was kept below 35 °C.³⁰ Coarse sand (2000–200 μ m) and fine sand (200–63 μ m) were separated by manual wet sieving with about 250 mL of cooled distilled water, while silt (63–2 μ m), and clay (<2 μ m) fractions were separated by centrifugation at 1000 and 5000 rpm, respectively. The separated fractions were oven-dried at 60 °C and stored at room conditions.

Soil Respiration. Soil respiration was evaluated by a dynamic absorption method. Briefly, 9 g of air-dried and rewetted soil sample (<2 mm) were placed on a airtight soil respiration flask in which a CO₂-free air was continuously fluxed by a peristaltic pump. The CO₂ emitted from soil was then captured in a trap containing a 0.01 M NaOH solution. The amount of CO₂ absorbed in this solution was determined after 27 days by back-titration with 0.01 M HCl after addition of 7 mL of 0.5 M BaCl₂. The ambient CO₂ concentration was determined by inserting blank samples (i.e.: no soil) into the respiration system.

Statistical Analysis. A coupled two-tailed Student's t test was used to compare values obtained for control and treatments, and difference was considered to be significant at the level of $P \leq 0.05$. All values are based on triplicate samples from which the mean and the standard error of the mean (s.e.m) were calculated.

Table 1. Properties of Soils (mean \pm s.e.m)

property	Porrara	Colombaia	Itri
coarse sand (g kg^{-1})	119 \pm 6	180 \pm 7	80 \pm 3
fine sand (g kg^{-1})	237 \pm 10	230 \pm 9	440 \pm 11
silt (g kg^{-1})	227 \pm 8	350 \pm 7	380 \pm 10
clay (g kg^{-1})	417 \pm 12	240 \pm 5	100 \pm 4
organic C (g C kg^{-1})	9.1 \pm 0.4	13.1 \pm 0.9	4.0 \pm 0.2
pH-H ₂ O	8.3 \pm 0.1	8.2 \pm 0.1	8.4 \pm 0.1
minerals ^a	C+++, F++, I++, K++, M++, Q+++, S+++	C+++, F+++, I++, K++, M++	D+++, F+, LCK+

^a C = calcite, D = dolomite, F = feldspar, I = Illite, K = kaolinite, LCK = low crystalline kaolinite, M = mica, Q = quartz, S = smectite; +++ = predominant, ++ = present, + = traces.

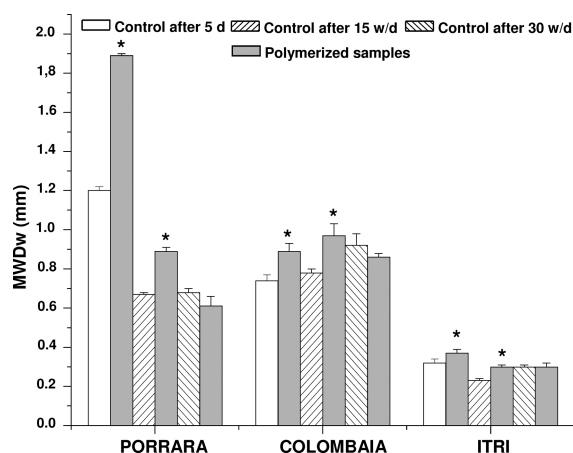


Figure 1. Mean weight diameter in water (MWDw) for the three soils, Porrara, Colombaia, Itri, before and after the photopolymerization treatment for a 5 day incubation, and 15 and 30 wetting and drying cycles (w/d). Error bars indicate standard error ($n = 3$). The asterisks denote significant differences between control and treatment at the level of $P \leq 0.05$.

RESULTS AND DISCUSSION

While the pH and mineralogy of the selected agricultural soils indicate their similar calcareous origin, large variations were noted in both textural and organic carbon properties (Table 1). The textural composition of soils affected the values of the mean-weight diameter of soil aggregates in water (MWDw), an index of aggregate stability.²⁸ This was large in the control sample of the most clayey and well structured Porrara soil, while progressively decreased with increase of sand content in Colombaia and Itri soil.

The catalyst-assisted photopolymerization treatment significantly increased the MWDw over control values for all the three soils after a 5 day incubation (Figure 1). Again, it was the originally well-structured Porrara soil that showed the most dramatic increase. The gain in aggregate stability indicates that the photopolymerization treatment was effective in increasing the molecular mass and cross-linking of soil humic molecules, thereby promoting a tighter association among soil particles and formation of larger water-stable aggregates. The persistence of the aggregating effect induced by the SOM photopolymerization in all samples is suggested by MWDw values, which remained larger than the respective control samples even after the soils had been subjected to 15 w/d cycles (Figure 1). The beneficial effects of the catalyzed photopolymerization were lost for all soils after

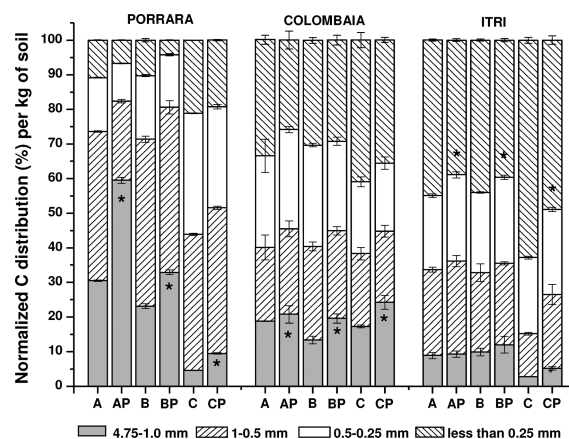


Figure 2. Percent distribution of organic carbon in different fractions of water-stable aggregates for the three soils, Porrara, Colombaia, Itri, before and after photopolymerization treatment for 5 days incubation, and 15 and 30 wetting and drying cycles (w/d). A = control soil after 5 days incubation; AP = photopolymerized soil after 5 days incubation; B = control soil after 15 w/d cycles; BP = photopolymerized soil after 15 w/d cycles; C = control soil after 30 w/d cycles; CP = photopolymerized soil after 30 w/d cycles. Error bars indicate standard error ($n = 3$). The asterisks denote significant differences between control and treatment at the level of $P \leq 0.05$.

30 w/d cycles, whereby the MWDw values were no longer different from the relative control.

The molecular modification of SOM following the photopolymerization treatment was reflected by the percent distribution of OC in the separated water-stable aggregates (Figure 2). After only 5 days of incubation, a significant decrease of OC in small-sized aggregates (<0.25 mm) was noted for all three soils, whereas OC significantly increased in the largest macro-aggregates (4.75–1.0 mm) only by the most clayey Porrara soil. The OC accumulation in the largest aggregates increased also with w/d cycles, becoming significant over control after 15 and 30 cycles for the silty Colombaia soil, and after 30 cycles for the sandy and least stable Itri soil.

These results suggest that the catalytic photopolymerization of SOM was effective in forming macro-aggregates at the expenses of microaggregates, thereby improving the soil physical quality. Moreover, the process of SOM photopolymerization not only remained active within the period of the w/d experiments and progressively increased the mass of humic molecules, but also enabled a greater OC content into larger aggregates, due to aggregation of stabilized microaggregates. This implies that, despite the generalized loss of physical stability under the severe

Table 2. Percent (mean \pm s.e.m.) of Yield and Content of Total Organic Carbon (TOC) for Particle-Size Fractions (μm) in Soils, Following 5 Day Incubation, And 15 and 30 Wetting and Drying Cycles (w/d).^a

	coarse sand 2000–200	fine sand 200–63	silt 63–2	clay <2	toc ^b
PORRARA					
5 Day Incubation					
A	18.7 \pm 1.2b	20.3 \pm 2.2a	43.7 \pm 2.2a	17.3 \pm 1.9a	0.84a
AP	22.0 \pm 2.0a	22.2 \pm 2.7a	42.4 \pm 2.1a	13.3 \pm 0.3b	0.80a
15 w/d Cycles					
A	14.8 \pm 0.7a	12.3 \pm 0.9a	51.7 \pm 1.5a	21.2 \pm 1.3a	0.78b
AP	15.4 \pm 1.5a	12.9 \pm 1.1a	50.4 \pm 3.6a	21.4 \pm 2.9a	0.83a
30 w/d Cycles					
A	14.4 \pm 0.8a	12.0 \pm 1.0a	52.3 \pm 1.3a	21.3 \pm 1.4a	0.70b
AP	15.0 \pm 1.3a	12.7 \pm 1.2a	50.2 \pm 2.2a	22.1 \pm 1.7a	0.81a
COLOMBAIA					
5 Day Incubation					
A	27.3 \pm 2.6a	17.6 \pm 1.2b	47.8 \pm 1.1a	7.3 \pm 0.4a	1.43a
AP	27.6 \pm 2.6a	19.7 \pm 0.4a	46.8 \pm 1.9a	5.9 \pm 0.3b	1.40a
15 w/d Cycles					
A	26.4 \pm 2.1a	17.8 \pm 0.8a	47.8 \pm 2.2a	8.0 \pm 0.5a	1.08b
AP	27.3 \pm 1.9a	19.1 \pm 1.1a	45.5 \pm 3.2a	8.2 \pm 0.3a	1.23a
30 w/d Cycles					
A	26.2 \pm 1.8a	18.0 \pm 0.7a	47.6 \pm 2.0a	8.2 \pm 0.4a	0.92b
AP	27.4 \pm 2.0a	19.2 \pm 1.2a	45.0 \pm 2.3a	8.5 \pm 0.2a	1.15a
ITRI					
5 Day Incubation					
A	12.4 \pm 0.8a	71.4 \pm 0.5a	11.0 \pm 0.9a	5.3 \pm 0.1a	0.43a
AP	12.7 \pm 1.7a	72.2 \pm 0.4b	11.0 \pm 1.1a	4.2 \pm 0.1b	0.42a
15 w/d Cycles					
A	10.3 \pm 1.5a	70.2 \pm 0.7a	13.8 \pm 1.6a	5.7 \pm 0.7a	0.42a
AP	10.4 \pm 1.4a	71.4 \pm 1.1a	13.8 \pm 1.9a	4.4 \pm 0.4b	0.41a
30 w/d Cycles					
A	10.2 \pm 1.1a	70.1 \pm 0.7a	13.7 \pm 1.0a	5.9 \pm 0.8a	0.40a
AP	10.3 \pm 1.0a	71.5 \pm 0.9a	13.6 \pm 1.6a	4.6 \pm 0.3b	0.40a

^a Different letter in columns indicate statistical difference ($P \leq 0.05$) between control soil (A) and treated soil (AP). ^b s.e.m. = $\pm 0.1\%$ ($n = 3$).

degradation induced by w/d cycles (Figure 1), the soil particles aggregated by the photopolymerized SOM were able to resist excessive slacking in water and OC redistribution into small-sized aggregates, as it was instead observed for control samples.

The effects of the SOM photopolymerization treatment were also observed when a distribution of soil particle-size fractions was obtained by sonication (Table 2). The 5 day incubation provided a statistically significant difference in particle-size distribution between control and treated samples for all three soils. While the yield of the clay-size ($<2 \mu\text{m}$) fraction decreased, that of the coarse sand-size (2000–200 μm) increased concomitantly for the most clayey Porrara soil. For the increasingly sandy soils of Colombaia and Itri, the noted significant decrease in yield of the clay-size ($<2 \mu\text{m}$) fraction was compensated by an increase of the fine sand-size (200–63 μm) fraction. However, the 15 and 30 w/d cycles canceled the differences in yield distribution of particle-sizes noted after the 5 day incubation for both Porrara and Colombaia soils. For the treated Itri soil, the yield reduction in clay-size ($<2 \mu\text{m}$) was still appreciated after

15 and 30 w/d cycles, although, possibly because of the applied sonication energy, the yield increase of the fine sand-size (200–63 μm) fraction was no longer statistically different from control (Table 2).

Moreover, the catalyzed photo-oxidative stabilization of soil particle-sizes was also reflected by the sum of OC in particle-size fractions (Table 2). While the 5 day incubation did not show a statistical difference between control and treated samples for all soils, the 15 and 30 w/d cycles showed a significant increase of particle-size TOC in treated samples of the most clayey and OC-rich Porrara and Colombaia soils. This suggests that the induced mass increase of humic molecules and consequent physical stabilization of soil samples were able to promote both a chemical and physical sequestration of SOM, that was not reverted even by the continuous and harsh w/d soil disrupting cycles.

A stronger chemical and physical stabilization of OC after the catalytic photopolymerization of soils can be also inferred by the amount of CO_2 respired by soil samples (Figure 3). The microbial mineralization of SOM was significantly inhibited in

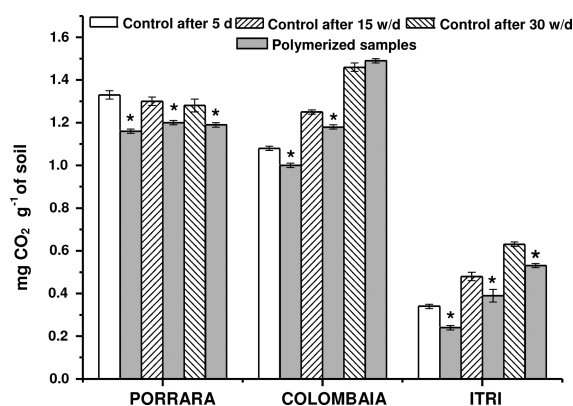


Figure 3. Soil respiration ($\text{mg CO}_2\text{g}^{-1}$ of soil) from the three soils, Porrara, Colombaia, Itri, before and after photopolymerization treatment for 5 day incubation, and 15 and 30 wetting and drying cycles (w/d). Error bars indicate standard error ($n = 3$). The asterisks denote significant differences between control and treatment at the level of $P \leq 0.05$.

the photopolymerized samples, as compared to control, for all soils after 5 day incubation and even 15 w/d cycles. The reduction of CO_2 emission was significant for Porrara and Itri soils also after 30 w/d cycles, whereas no significant difference was noted for this treatment for the Colombaia soil. The CO_2 respiration behavior of Porrara and Itri soils confirmed that the catalytic photopolymerization had strongly stabilized their SOM, thereby inhibiting microbial mineralization of OC.

The different result for the Colombaia soil after 30 w/d cycles may be attributed to its relatively large amount of OC (Table 1) and specific humic molecules. Although the phenolic components of this soil must have been photopolymerized as shown by the increased OC content in larger aggregates even after 30 w/d cycles (Figure 2), other unreacted humic molecules (presumably medium or long-chain alkyls)³¹ may have been released from clay-humic aggregates, made fragile by the repeated w/d cycles, and microbially mineralized as in control. Nevertheless, the OC stabilization obtained in soil samples of Porrara, Colombaia, and Itri mitigated the CO_2 emission by, respectively, 12.8, 7.4, and 29.4% after a 5 day incubation, 8.3, 5.6, 18.7% after 15 w/d cycles, and 7.0, -2.0, 15.9% after 30 w/d cycles (Figure 3). Such mitigation corresponded to 0.34, 0.16, and 0.20 Tons of $\text{CO}_2\text{Ha}^{-1}$ for Porrara, Colombaia, and Itri soils, respectively, after 5 days of incubation, and still to 0.18 and 0.20 Tons of $\text{CO}_2\text{Ha}^{-1}$ for Porrara and Itri soils, respectively, even after the severe disaggregation of 30 w/d cycles.

The implications of this work are 2-fold. First, only one addition (corresponding to 10 kg.Ha^{-1}) of a water-soluble biomimetic catalyst for the in situ photopolymerization of SOM reduced CO_2 emissions from soils to a larger extent than that uncertainly estimated with mathematical models from European agricultural soils.³² This is to be attributed to the increased intermolecular covalent bonds established among SOM components, resulting into a greater energy requirement for microorganisms to mineralize OM. The soil humic molecules most susceptible to a catalyst-assisted photopolymerization are the phenols derived from the microbial degradation of lignin. Since these phenols are also rapidly mineralized in soil,³³ their photo-oxidative coupling catalyzed by iron-porphyrin provides a means to sequester their carbon in soil.

Second, the cross-coupling of soil phenolic molecules by the photocatalytic oxidation improved soil physical quality by enhancing the stability of soil microaggregates and promoting their aggregation into larger sizes. In fact, the resulting more hydrophobic polyphenols were more easily adsorbed on solid soil surfaces than single phenols, thus favoring interparticle aggregation and their stabilization into macro-aggregates. These combined effects produced a chemical and physical sequestration of SOM, and both contributed to mitigate CO_2 emission from soils. The innovative catalytic technology described here may be potentially employed to control SOM dynamics, as well as physical quality, in agricultural soils.

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