

MITIGATION OF GHGs EMISSION FROM SOILS BY A CATALYZED *IN SITU* PHOTO-OXIDATIVE POLYMERIZATION OF SOIL ORGANIC MATTER

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Agricultural lands under food and bio-energy crops, managed grass and permanent crops including agro-forestry, occupy about 40-50% of the Earth's land surface¹. In 2005, agriculture accounted for an estimated emission of 5.1 to 6.1 GtCO₂-eq/yr (10-12% of total global anthropogenic emissions of greenhouse gases (GHGs))¹. However, 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 vegetation². These practices are not only far from substantially reducing GHGs emissions from soils or permanently stabilizing soil organic matter¹⁻⁴, but are also predicted to hardly match more than a maximum of 25% of the GHGs reductions required by the Kyoto Protocol within 2050⁵. Despite the knowledge that GHGs release from soil largely derives from biochemical transformations of plant litter and soil organic matter (SOM)⁶⁻⁸, no new and much wished biotechnological measures are adopted so far to augment mitigation¹. Here we propose an innovative approach to mitigate GHGs emissions from soils based on the *in situ* photo-polymerization of SOM under biomimetic catalysis. Three Mediterranean soils of different physical and chemical properties were added with a synthetic water-soluble iron-porphyrin, irradiated by solar light, and subjected to 15, and 30 wetting and drying cycles. We found that the *in situ* catalysed photo-polymerization of SOM increased soil physical aggregation, shifted OC into larger soil aggregates, and reduced CO₂ released by microbial respiration. Our findings suggest that "green" catalytic technologies can become viable soil management practices to enhance mitigation of GHGs emission from arable soils and contribute to match the expectations of the post-Kyoto Protocol in the agricultural sector.

The humified organic matter in soil (70-80% of SOM)⁹ represents the most persistent pool in OC accumulation with mean residence time of several hundreds of years¹⁰, and, thus, the principal potential C sink in the biosphere, whose advanced comprehension may help to mitigate CO₂ emissions from soil¹¹. Soil humus is composed by the hydrophobic and heterogeneous aliphatic and aromatic molecules progressively surviving the microbial transformation of dead biological tissues¹². 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)¹³, rather than the previously assumed macropolymers¹⁴. Humic molecules were shown to be tightly associated in supramolecular structures, which are prevalently stabilized by noncovalent hydrophobic bonds¹². 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 peroxidase¹⁵, or a biomimetic catalyst, such as an iron-porphyrin¹⁶. Both the enzymatic and biomimetic catalysts accelerate the oxidative coupling of phenols via a free-radical mechanism^{15, 16}. Moreover, we found that the biomimetic catalysis increased the molecular dimension of HS in solution simply by photo-oxidation under solar radiation, without the need of an additional oxidant¹⁷. By applying photo-oxidative catalysed conditions on lignin-derived phenolic monomers (similar to those found in soil humus), we proved a rapid formation of new intermolecular C-C and C-O-C bonds leading to several identified (up to tetramers) and unidentified oligomers¹⁸⁻²⁰.

We thus conceived that it could be possible to stabilize SOM by applying the photopolymerization technology *in situ* on soils. The catalyzed photo-oxidative formation of covalent bonds among soil phenolic molecules would chemically stabilize SOM by increasing the content of chemical energy in humic structures and consequently reducing the extent of SOM mineralization by microbes. Moreover, increasing the mass of humus molecules would result in linking together soil particles to larger soil aggregates and thus improving soil physical quality²¹⁻²³. We tested these hypotheses in a laboratory experiment aimed to verify SOM stabilization in treated soils even after severe disaggregating processes such as 15 and 30 wetting and drying (w/d) cycles.

The occurrence of SOM photo-polymerization in the studied soils was suggested by our results. The mean-weight diameter of soil aggregates in water (MWD_w), an index of aggregate stability, was significantly increased over control for all the three soils after the 5 d

incubation (Fig. 1), though to a different extent depending on the intrinsic composition of each soil (Table 1). This indicates that the photo-polymerization treatment, by increasing the molecular mass and cross-linking of humic molecules, promoted a tighter association among soil particles and formation of larger water-stable aggregates. An enhanced soil aggregate stability in the photo-polymerized samples was still kept after 15 w/d cycles, but it was lost for all soils after 30 w/d cycles.

The molecular modification of SOM following the catalyzed photo-polymerization was reflected by the percent distribution of OC in the separated water-stable aggregates (Fig. 2). After only 5 d of incubation, a significant decrease of OC in small-sized aggregates (< 0.25 mm) was noted for all three soils, whereas a OC significant enhancement in the largest macro-aggregates (4.75-1.0 mm) was shown only by the most clayey Porrara soil. The OC accumulation in the largest aggregates was increased with w/d cycles, becoming significant over control already after 15 cycles for the silty Colombaia soil, and after 30 cycles for the sandy and least stable Itri soil. These results suggest that the catalytic photo-polymerization of SOM not only remained active within the period of the w/d experiments, but the progressively modified humic molecules also determined the formation of more stable micro-aggregates that increased the fraction of larger aggregates. This implies that, despite the generalized loss of physical stability under the severe degradation induced by w/d cycles (Fig. 1), soil aggregates stabilized by the photo-polymerized humus were able to resist excessive slacking in water and OC re-distribution into small-sized aggregates, as observed for control samples.

A stronger chemical and physical stabilization of OC after the catalytic photo-polymerization of soils can be inferred by the amount of respired CO₂ (Fig. 3). The microbial mineralization of SOM was significantly inhibited in the photo-polymerized samples, as compared to control, for all soils after 5 d incubation and even after 15 w/d cycles. While the reduction of CO₂ emission was significant for Porrara and Itri soils also after 30 w/d cycles, no significant difference was noted for this treatment for the Colombaia soil. The CO₂ respiration behaviour of Porrara and Itri soils confirmed that the catalytic photo-polymerization had strongly stabilized their SOM. The different result for the Colombaia soil after 30 w/d cycles may be attributed to its larger amount of OC (Table 1). Although the phenolic components of this soil must have been photo-polymerized as shown by the increased OC content in larger aggregates even after 30 w/d cycles (Fig. 2), unreacted humic molecules (presumably medium or long-chain alkyls)²⁴ 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 Porrara, Colombaia, and Itri soils

mitigated the CO₂ emission by, respectively, 12.8, 7.4, and 29.4 % after 5 d incubation, 8.3, 5.6, 18.7 % after 15 w/d cycles, and 7.0, -2.0, 15.9 % after 30 w/d cycles. Such mitigation corresponded to 0.34, 0.16 and 0.20 Tons of CO₂.Ha⁻¹ for Porrara, Colombaia, and Itri soils, respectively, after 5 d of incubation, and still to 0.18 and 0.20 Tons of CO₂.Ha⁻¹ for Porrara and Itri soils, respectively, even after the severe disaggregation of 30 w/d cycles.

The implications of this work are two-fold. First, only one addition (about 10 Kg.Ha⁻¹) of a water-soluble biomimetic catalyst for the *in situ* photo-polymerization of SOM reduced CO₂ emissions from soils to a larger extent than that indecisively estimated with models from European agricultural soils²⁵. 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. Second, the cross-coupling of SOM by the photo-catalytic oxidation improved soil physical quality by enhancing the stability of soil micro-aggregates and promoting their aggregation into larger sizes. These combined effects produced a chemical and physical sequestration of SOM, and both contributed to mitigate CO₂ emission from soils. The innovative catalytic technology described here may become a relatively inexpensive and easy soil management practice to control SOM dynamics as well as physical quality in agricultural soils.

METHODS SUMMARY

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, Colombaia (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 pipette method. Organic carbon was calculated as difference between the soil total carbon content, as determined by dry combustion using EA 1108 (Fisons Instruments) elemental analyzer, and the total carbon determined by standard Walkley–Black titration. Clay mineralogy was determined by X-ray diffraction of oriented samples.

Photo-polymerization 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, Colombaia and Itri soil, respectively) in order to obtain a control series. The polymerized series were similarly prepared (n = 3) and added with 0.24 μmol of synthetic water-soluble iron-porphyrin (*meso*-tetra(2,6-dichloro-3-sulfonatophenyl)porphyrinate of iron(III) chloride, Fe-(TDCPPS)Cl)¹⁶ 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 d; (ii) submitted to 15 wetting/drying cycles; (iii) submitted to 30 wetting/drying cycles. During wetting/drying cycles, samples were uncovered after 5 d incubation and distilled water was added, whenever samples became dry (approximately once a week), to reestablish WHC.

Aggregate stability. Separation of water-stable aggregates was done by a wet sieving method²⁶. 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. Recovered aggregate fractions were oven-dried at 60°C, weighed, and stored at room conditions.

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

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

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}).

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 air-tight 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 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 $P \leq 0.05$.

References

1. Smith, P., D. Martino, Z. Cai, D. Gwary, H. Janzen, P. Kumar, B. McCarl, S. Ogle, F. O'Mara, C. Rice, B. Scholes & Sirotenko O. in *Agriculture, Climate Change 2007: Mitigation. Contribution of Working Group III to the Fourth Assessment Report of the*

- Intergovernmental Panel on Climate Change* (eds B. Metz *et al.*) 497-540 (Cambridge University Press 2007).
2. Paustian, K. *et al.* Agricultural Mitigation of Greenhouse Gases: Science and Policy Options. *CAST (Council on Agricultural Science and Technology) Report R141*, 120 pp. (2004)
 3. Schlesinger, W. H. Carbon sequestration in soils: some cautions amidst optimism. *Ag. Ecosys. Environ.* **82**, 121–127 (2000).
 4. Schlesinger, W. H. & Lichter J.. Limited carbon storage in soil and litter of experimental forest plots under increased atmospheric CO₂. *Nature*, **411**, 466-469 (2001).
 5. Read, D. & May R. The role of land carbon sinks in mitigating global climate change. *Policy Document 10/01, Royal Society*, 27 pp. (2001).
 6. Smith, P. Engineered biological sinks on land. in *The Global Carbon Cycle. Integrating humans, climate, and the natural world* (eds C.B. Field & M.R. Raupach) 479-491 (Island Press 2004).
 7. Janzen, H.H. Carbon cycling in earth systems - a soil science perspective. *Ag. Ecosys. Environ.* **104**, 399-417 (2004).
 8. Fontaine, S. *et al.* Stability of organic carbon in deep soil layers controlled by fresh carbon supply. *Nature* **450**, 277-280 (2007).
 9. Stevenson, F.J. *Humus chemistry: genesis, composition, reaction.* (Wiley 1994)
 10. Andreux, F. in *Humic substances in terrestrial ecosystems* (ed Piccolo A.) 45-100 (Elsevier 1996).
 11. Paustian, K., *et al.* Agricultural soils as a sink to mitigate CO₂ emissions. *Soil Use Manag.* **13**, 230– 244 (1997)
 12. Piccolo, A. The Supramolecular structure of humic substances. A novel understanding of humus chemistry and implications in soil Science. *Adv. Agron.* **75**, 57-134 (2002).
 13. Piccolo, A. & Spiteller, M. Electrospray ionization mass spectrometry of terrestrial humic substances and their size-fractions. *Anal. Bioanal. Chem.* **377**, 1047-1059 (2003).
 14. Piccolo, A., Conte P. & Cozzolino A. Chromatographic and spectrophotometric properties of dissolved humic substances compared with macromolecular polymers. *Soil Sci.* **166**, 174-185 (2001).
 15. Piccolo, A., Conte P. & Cozzolino A. Polymerization by enzyme-catalyzed oxidative couplings of supramolecular associations of humic molecules. *Naturwissenschaften*, **87**, 391-394 (2000).

16. Piccolo, A., Conte, P. & Tagliatesta, P. Increased conformational rigidity of humic substances by oxidative biomimetic catalysis. *Biomacromolecules* **6**, 351-358 (2005).
17. Smejkalova, D. & Piccolo, A. Enhanced molecular dimension of a humic acid induced by photooxidation catalyzed by biomimetic metalporphyrins. *Biomacromolecules* **6**, 2120-2125 (2005).
18. Smejkalova, D. & Piccolo, A. Rates of oxidative coupling of humic phenolic monomers catalyzed by a biomimetic iron-porphyrin. *Environ. Sci. Technol.*, **40**, 1644-1649 (2006).
19. Smejkalova, D., Piccolo, A. & Spiteller, M. Oligomerization of humic phenolic monomers by oxidative coupling under biomimetic catalysis. *Environ. Sci. Technol.*, **40**, 6955-6962 (2006).
20. Smejkalova D., Conte P. & Piccolo, A. Structural characterization of isomeric dimers from the oxidative oligomerization of catechol with a biomimetic catalyst. *Biomacromolecules*, **8**, 737-743 (2007).
21. Tisdall, J.M. & Oades, J.M. Organic matter and water-stable aggregates in soils *J. Soil Sci.* **33**, 141-163 (1982).
22. Piccolo, A., & Mbagwu, J.S.C. Role of hydrophobic components of soil organic matter on the stability of soil aggregates. *Soil Sci. Soc. Am. J.* **63**, 1801-1810 (1999).
23. Baldock, J. A. & Skjemstad, J. O. Role of the matrix and minerals in protecting natural organic materials against biological attack. *Org. Geochem.* **31**, 697-710 (2000).
24. Fiorentino, G., Spaccini, R. & Piccolo, A. Separation of molecular constituents from a humic acid by solid-phase extraction following a transesterification reaction. *Talanta* **68**, 1135-1142 (2006).
25. Freibauer, A. *et al.* Carbon sequestration in the agricultural soils of Europe. *Geoderma* **122**, 1-23 (2004)
26. Kemper, D.W. & Rosenau, R.C. in *Methods of Soil Analysis, Part I* (ed Klute A.) 425-442 (ASA and SSSA 1986).

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Author contributions

All authors designed and carried out research and sample analysis, analysed the data and wrote the paper.

FIGURES CAPTIONS

Figure 1. Mean Weight Diameter in water (MWD_w) for the three soils, Porrara, Colombaia, Itri, before and after photo-polymerization treatment for 5 d incubation, and 15 and 30 wetting and drying cycles. Error bars indicate standard error (n = 3). The asterisks denote a significant difference between control and treatment at the level of $P \leq 0.05$.

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

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

Table 1. Properties of soils based on triplicates for each determination (mean±s.e.m).

Property	Porrara	Colombaia	Itri
Coarse sand (g kg ⁻¹)	119±6	180±7	80±3
Fine sand (g kg ⁻¹)	237±10	230±9	440±11
Silt (g kg ⁻¹)	227±8	350±7	380±10
Clay (g kg ⁻¹)	417±12	240±5	100±4
Organic C (g C kg ⁻¹)	9.1±0.4	13.1±0.9	4.0±0.2
pH-H ₂ O	8.3±0.1	8.2±0.1	8.4±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

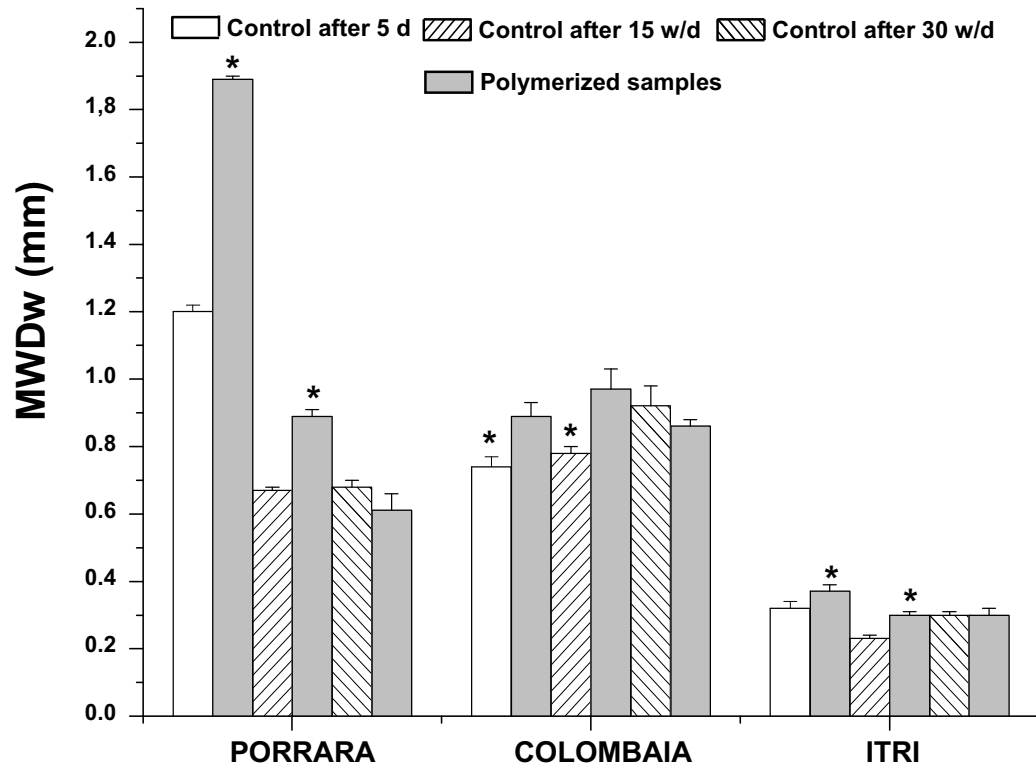


Figure 2

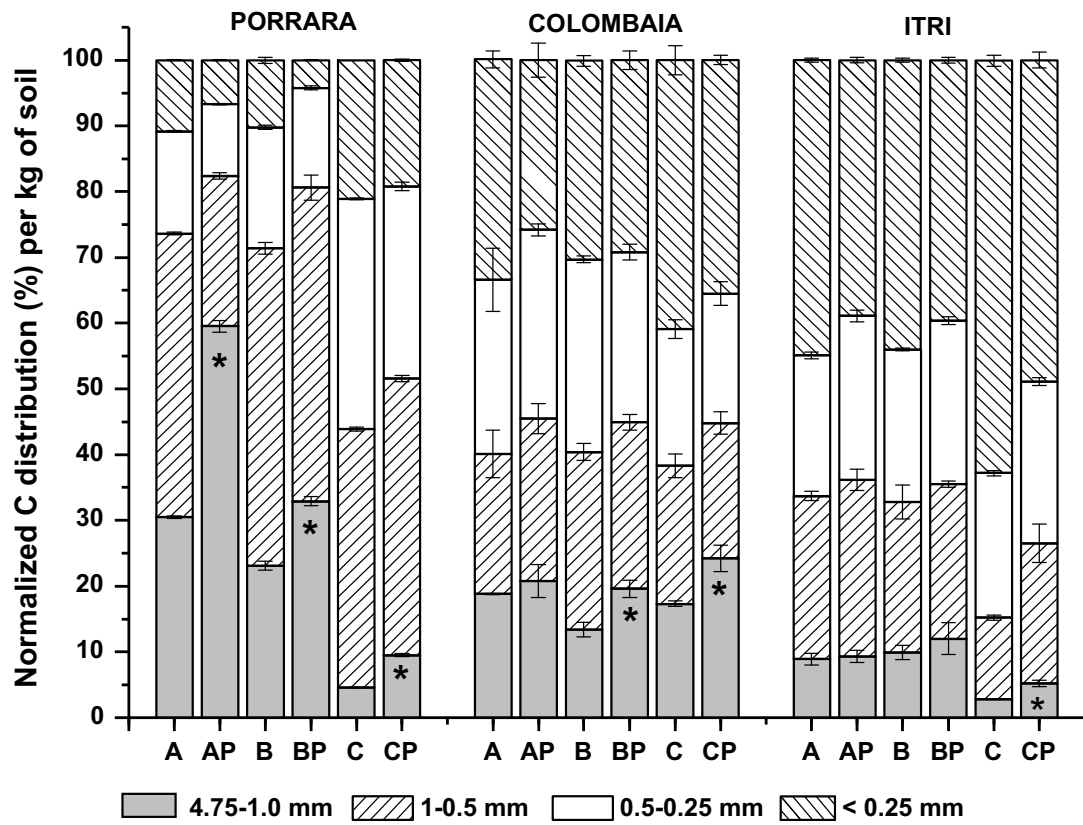


Figure 3

