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In situ polymerization of soil organic matter by oxidative biomimetic catalysis

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Abstract

Background: Agricultural practices that enhance organic matter content in soil can play a central role in sequestering soil organic carbon (SOC) and reducing greenhouse gases emissions.

Methods: We used a water-soluble iron-porphyrin to catalyze directly in situ oxidative polymerization of soil organic matter in the presence of H₂O₂ oxidant, with the aim to enhance OC stabilization, and, consequently, reduce CO₂ emissions from soil. The occurred SOC stabilization was assessed by monitoring soil aggregate stability, OC distribution in water-soluble aggregates, soil respiration, and extraction yields of humic and fulvic acids.

Results: Soil treatment with H₂O₂ and iron-porphyrin increased the physical stability of water-stable soil aggregates and the total OC content in small aggregates, thereby suggesting that the catalyzed oxidative polymerization increased OC in soil and induced a soil physical improvement. The significant reduction of CO₂ respired by the catalyst- and H₂O₂-treated soil indicated an enhanced resistance of polymerized SOC to microbial mineralization. The catalyzed oxidative polymerization of SOC also significantly decreased the extraction yields of humic and fulvic acids from soil.

Conclusions: The oxidative catalytic technology described here may become an efficient agricultural practice for OC sequestration in soils and contribute to mitigate global changes.

Keywords: Soil carbon sequestration, Soil respiration, Oxidative biomimetic catalysis, Soil organic matter, CO₂ emissions

Background

The recognition of the agricultural activities in the production of greenhouse gases (GHG) is intensively debated at international level. The Kyoto Protocol states that subscribing countries should promote a sustainable policy in order to reduce the impact of agriculture on climate change, and, thus, invite to draft national or regional programs to reduce GHG emissions also from agriculture [1]. One strategy may rely on the improvement of practices or new methods for carbon sequestration in soil.

Soil organic matter (SOM) is a basic soil component and plays a major role in maintaining soil functions and quality [2, 3]. The organic matter management practices

currently adopted to sequester organic carbon in croplands are mainly focused on the physical protection of SOM by combining minimum or zero tillage with crop rotation and the beneficial use of crop, and exploiting plant residues such as in green manuring and mulching. The stable incorporation of soil organic carbon (SOC) is closely related to both the physical protection in soil aggregates and the biochemical recalcitrance of clay-humic complexes. Even though SOC content may be enhanced by reduced tillage, a persistent carbon sequestration is not warranted since incorporation in soil of plant-derived biolabile components is nullified when soil management is reversed to conventional tillage and organic carbon is rapidly mineralized again [4]. Moreover, while the mineral properties of a soil are constant, SOC stabilization is a function of the variable and still poorly understood molecular characteristics of the humified organic matter.

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The humified organic matter is the most important SOM pool and represents the largest reservoir of OC in the biosphere [5]. Humic matter is a complex mixture of heterogeneous aliphatic and aromatic molecules of plant and microbial origin [6, 7], whose hydrophobic components progressively separate from the soil solution for thermodynamic reasons [8, 9], and accumulate on soil particles, thus giving rise to chemical and physical protection of SOC [10]. Recent experimental evidence changed the paradigm on the chemical nature of humus as being composed by relatively small (≤ 1000 Da) heterogeneous molecules [11], rather than the previously assumed macropolymers de novo synthesized in soil [12, 13]. Soil humic molecules were shown to be self-assembled in supramolecular structures, which are prevalently stabilized by weak non-covalent bonds and may be easily disrupted by interactions with organic acids [14, 15]. A relevant implication towards carbon sequestration in soil is that the self-assembled small humic molecules may be coupled together into larger molecular weight materials by catalytic technologies that form stable intermolecular covalent bonds. Based on the proved activity of the heme prosthetic group of oxidative enzymes in the oxidative couplings of humic phenols [16, 17], the use of much more resilient biomimetic catalysts, such as biocompatible metal-porphyrins, can be employed to promote an effective oligo-/polymerization of the phenolic components of humus [18, 19]. The oxidative coupling among humic molecules can be catalyzed by either water-soluble or immobilized metal-porphyrins and induced either by an oxidizing agent such as hydrogen peroxide (H_2O_2) [18, 20] or photo-chemically [21, 22].

Metal-porphyrins were also employed to catalyze in situ photopolymerization of soil humic matter. In fact, both a water-soluble iron-porphyrin and a manganese-porphyrin immobilized on spacer-functionalized montmorillonite were found to couple humic phenolic molecules in the soil complex matrix by photo-oxidation under solar radiation [23–26]. However, up to our knowledge, no investigation has been reported so far on the in situ oxidative polymerization of soil humic molecules under biomimetic catalysis in the presence of a chemical oxidant. Therefore, the objective of this work was to investigate the enhancement in chemical stability of SOM when a soil was subjected to oxidative catalysis, by applying in situ a water-soluble iron-porphyrin and H_2O_2 as oxidants.

Methods

Soil sample and characterization

The soil sample was a Fluventic Xerochrept (FAO Soil Classification) which was collected from the surface layer (0–20 cm) of an agricultural area near Naples, Italy. After air drying and sieving through a 2-mm sieve, the

soil texture was measured using pipet method, and total organic carbon (TOC) was determined by an EA 1108 Elemental Analyzer (Fisons Instruments). Soil properties are reported elsewhere [27].

Biomimetic catalyst

The synthesis of *meso*-tetra(2,6-dichloro-3-sulfonatophenyl)porphyrinate of Fe(III) (Fe-Pha) has been previously described [18]. A *meso*-tetra(2,6-dichlorophenyl)porphyrin (H_2TDCPP) was first synthesized and then dissolved in steaming H_2SO_4 (25 mg mL^{-1}) and stirred for 12 h at 160 °C under argon atmosphere. The product was recovered with cool water in a flask immersed in ice. The pH of the solution was increased up to 7 with a saturated NaOH solution and then evaporated at 60–70 °C under vacuum. The residue was recovered with methanol, again evaporated under vacuum, re-dissolved in methanol, and recrystallized in ethyl ether. The product was obtained by filtration and then purified through a cationic exchange resin Dowex 50W X8–100 (50–100 mesh), previously conditioned with a 10% HCl solution. The product was eluted with water and the recovered fraction-dried under vacuum. The final material, *meso*-tetra(2,6-dichloro-3-sulfonatophenyl)porphyrin ($H_2TDCPPS$), was recrystallized in a methanol–acetone solution with a final reaction yield of 75%. The $H_2TDCPPS$ (200 mg) and 100 mg of $Fe(II)SO_4$ were dissolved in 100 mL of water. The solution was degassed and left for 12 h under argon atmosphere. Then the solution was vacuum-evaporated and the residue re-dissolved in water. This solution was 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-Pha product was recrystallized in methanol–acetone in order to purify the sample from residual salts. The yield of the reaction was 80%.

In situ oxidative polymerization experiments

Two control series were obtained by placing either 40 or 200 g of air-dried soil samples on a Petri dish (12 cm diameter) and kept at 40% of water-holding capacity (WHC) by adding 20 and 100 mL of water, respectively, and either 0 or 3 mL of H_2O_2 8.6 M. Soil samples subjected to oxidative polymerization were similarly prepared and added with 0.1 mg mL^{-1} of synthetic water-soluble Fe-Pha and H_2O_2 8.6 M. All series were prepared in 6 replicates, covered with a Petri dish and incubated in the dark at room temperature for 5 days.

Soil aggregate stability

Separation of water-stable aggregates was conducted by a wet sieving method [28]. Twenty g of air-dried subsample 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. Afterward, the sieves were manually oscillated (up and down 4 cm) 30 times for 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}).

Soil respiration

Soil respiration was evaluated by a static absorption method [29]. In brief, 9 g of a 2-mm sieved, air-dried, and rewetted soil sample, a beaker containing 20 mL of distilled water (blank), and another one added with 20 mL of 0.05 M NaOH were placed in a tightly closed 1-L jar. The beaker with distilled water served to maintain a constant humidity in the closed system. The amount of CO_2 emitted from the respiration of soil sample and adsorbed by NaOH solution was determined every 3–4 days (longer incubation periods could result in anaerobic conditions) by back titration with 0.05 M HCl after addition of 7 mL of 0.5 M BaCl_2 in both water blank and NaOH solutions. The estimation of evolved CO_2 in the closed system was referred to 23 days of soil incubation.

Soil respiration measurements were then repeated after soil fumigation treatment [29]. Briefly, for the fumigation, the soil sample was placed in a glass beaker and put in a large desiccator lined with moist paper. The desiccator contained a beaker with alcohol-free CHCl_3 and a few anti-bumping granules. The desiccator was evacuated until the CHCl_3 boiled vigorously. Finally, the tap was closed and the desiccator was left in the dark at 25 °C for 18–24 h. The beaker with the CHCl_3 and the paper were then removed and CHCl_3 vapor was removed from the soil by repeated evacuation in the desiccator. Six 3-min evacuations, three with a water pump and three with a high-vacuum oil pump, were usually sufficient to remove the smell of CHCl_3 from the soil; two additional evacuations were then carried out with the high-vacuum pump.

Humic and fulvic acids extraction

At the start and end of the experiment, humic (HA) and fulvic (FA) acids were extracted and isolated from soil sample as described elsewhere [30]. Two different amounts of soil (40 and 200 g) were extracted with 0.1 M NaOH and 0.01 M $\text{Na}_4\text{P}_2\text{O}_7 \cdot 10\text{H}_2\text{O}$ 1:8 extraction (soil:solution), under N_2 atmosphere. The solutions were then centrifuged at 7000 rpm for 20 min and the supernatants were filtered with glass fibers and acidified to pH 1 with 12 M HCl to promote precipitation of HA. The acidified solutions were allowed to stand on a bench overnight, and then centrifuged at 7000 rpm for 20 min to recover the HA fraction. The supernatants containing FA were separated as such, while the precipitated HAs were treated with a solution of 0.06 M HF and 0.03 M HCl to remove the co-extracted silicates. Thereafter, the solution was centrifuged at 7000 rpm for 20 min and the HA re-dissolved in water. Both solutions of HA and FA were dialyzed against water until it is Cl-free and freeze-dried.

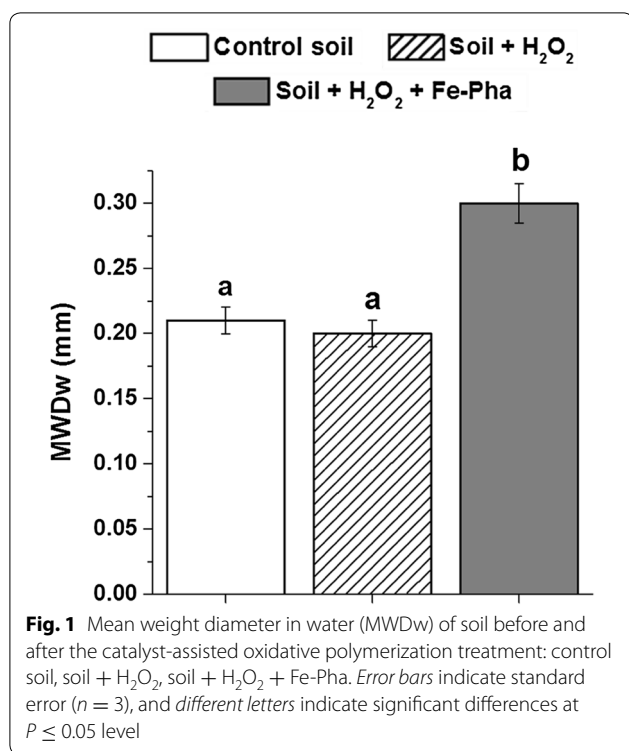
Statistical analysis

The Tukey's test was used to compare values obtained for control and treatments, and the difference was considered to be significant at the level of $P \leq 0.05$. All values were based on triplicate samples from which the mean and the standard error of the mean were calculated.

Results and discussion

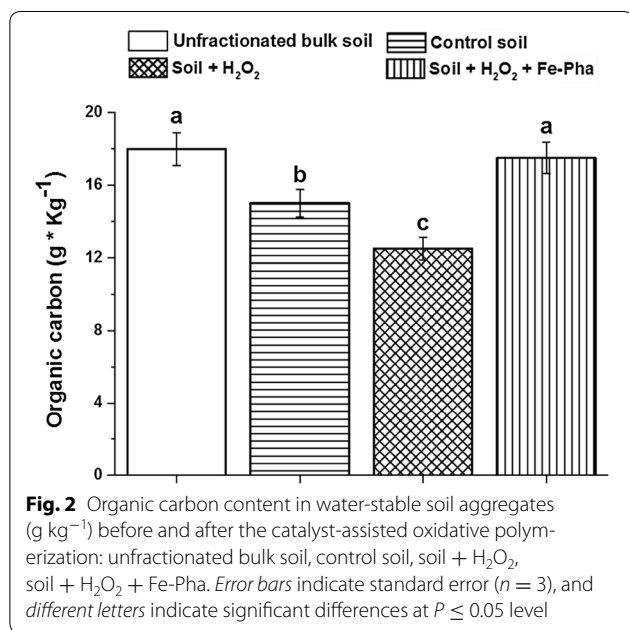
The physical effect on soil of the in situ polymerization of SOM by oxidative catalysis was assessed by the mean weight diameter in water (MWDw) of soil aggregates and the consequent aggregate structural stability [28]. While the catalyst-assisted oxidative treatment significantly increased the MWDw value as compared to both control and H_2O_2 -treated samples (Fig. 1), no significant differences were observed between the MWDw values of the latter two samples. The gain in aggregate stability indicates that the catalyzed oxidative polymerization of SOM had been effective in cross-linking of humic molecules and increasing their molecular mass, thereby promoting a tighter association among soil particles and formation of larger water-stable aggregates [23].

Further evidence of in situ oxidative coupling among soil humic molecules under Fe-Pha catalysis was provided by the amount of total organic carbon (TOC) in the water-stable aggregates (Fig. 2). The TOC content for the sum of aggregates of both control and H_2O_2 -added soils was significantly less than for the bulk soil, due to release of water-soluble humic matter released during the procedure of aggregates separation. Conversely, the TOC of the sum of aggregates for the soil sample subjected to oxidative polymerization remained almost unchanged as compared to the bulk unfractionated soil. This resistance



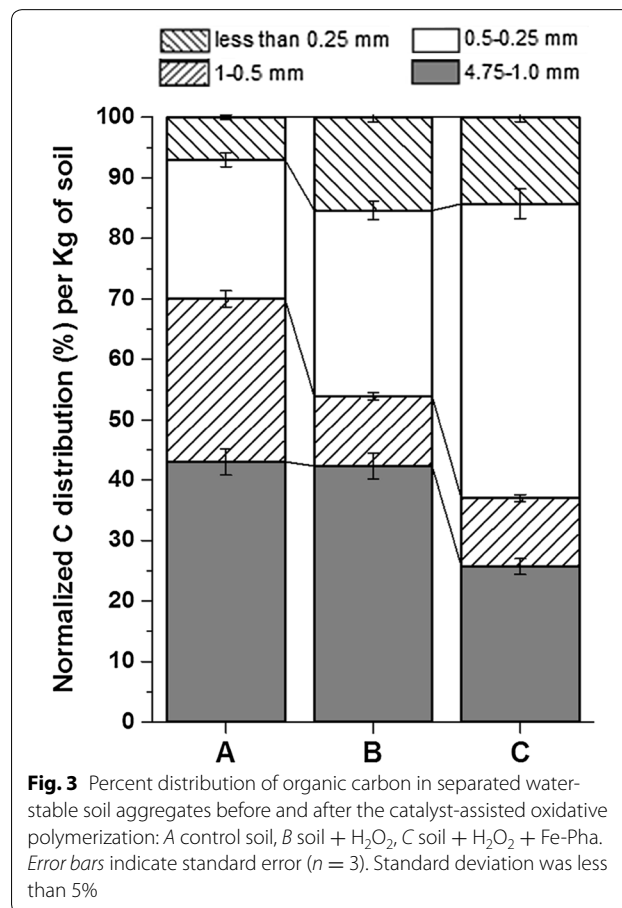
separated water-stable aggregates (Fig. 3). The significant decrease of OC content in the largest macro-aggregates (4.75–1 mm) and its concomitant enhancement in the 0.5–0.25 and <0.25 mm ranges indicate that the reaction products of the oxidative catalytic treatment were stabilized preferentially in the small size aggregates. This shift of the aggregating humic molecules from the macro- to the meso-aggregates, combined with the greater TOC accumulation in the treated soil (Fig. 2), confirmed the stronger aggregate stability conferred to soil by the catalytic treatment (Fig. 1).

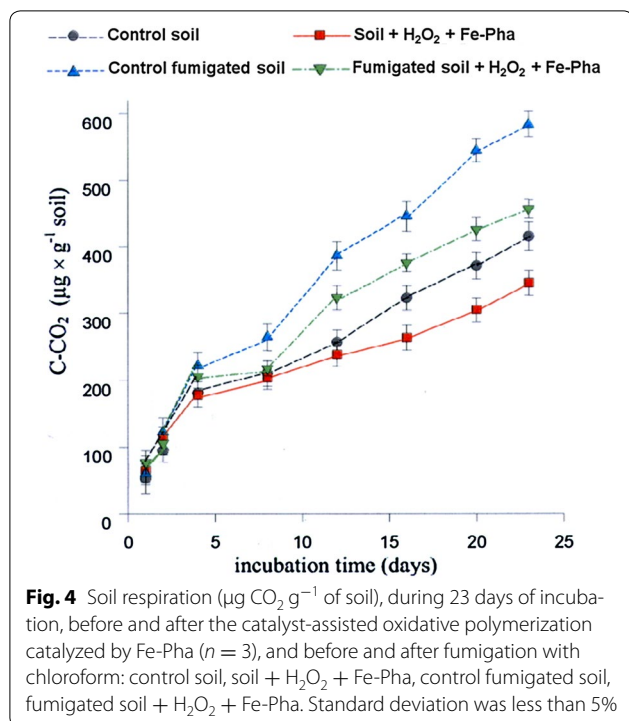
The stabilization of OC after the in situ catalytic polymerization of SOM was further evaluated by measuring the soil CO₂ respiration during 23 days of incubation (Fig. 4). The catalyzed oxidative reaction significantly decreased CO₂ emission from soil compared to the untreated control soil, starting from about 15th day of incubation. This implies that the increase of intermolecular covalent bonds among the soil phenolic humic molecules promoted by the addition of the biomimetic catalyst and H₂O₂ significantly limited the microbial mineralization of lignin and phenolic components of SOM, which conversely display larger decomposition rate and shorter turnover time if not



of soil particles to excessive slacking in water is evidence that the Fe-Pha treatment increased the masses of humic molecules, thereby preventing their cementing action among soil particles and confirming the effect on soil physical quality.

The effects of the catalyzed treatment on SOM were also reflected by the percent distribution of OC in the

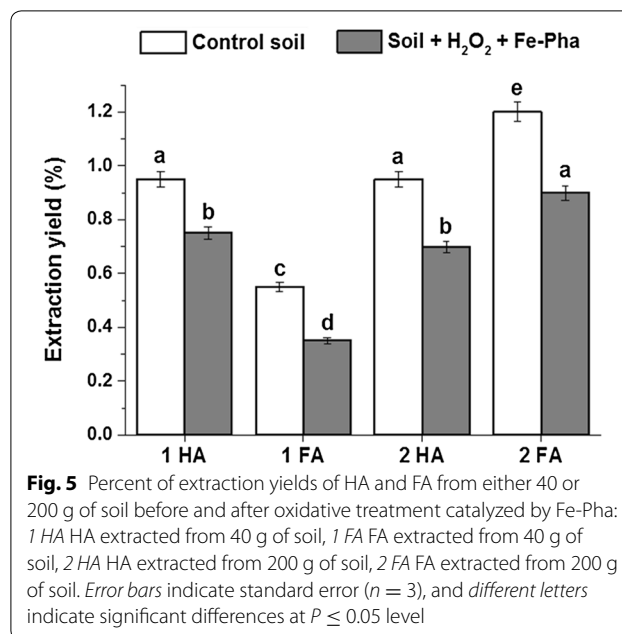




involved in physical/chemical stabilization processes [31, 32]. This may be attributed, on one hand, to the increased energy threshold in polymerized humic molecules to be overcome by the microbial metabolism, and, on the other hand, by the already noted physical protection of humic matter in stronger soil meso- and micro-aggregates [23].

Soil respiration measurements were repeated after the chloroform fumigation treatment of soil. Chloroform is toxic towards soil microbial communities, and, due to the death and lysis of soil microbes, the presence of additional metabolic carbon derived by dead cells resulted in a significant increase of respired CO_2 from soil [29]. In fact, the re-emergent microbial activity had more accessible carbon to respire. Despite the enhanced respiration due to fumigation, the oxidative treatment with water-soluble Fe-Pha catalyst still enabled a reduction of about 50% of CO_2 emission respect to control. Again, this finding confirms that the catalyzed oxidative polymerization of SOM phenolic components reduced CO_2 emission from soil because of the increased difficulty of soil microbial communities to mineralize SOM, due to either an enhanced chemical stabilization or physical protection in less accessible micro-aggregates.

The effect of the biomimetic catalysis in promoting the in situ polymerization of soil humic molecules could be also inferred by the extraction yields of HA and FA fractions from two different amounts of soils (40 and 200 g) before and after the catalytic treatment (Fig. 5). For both soil quantities, the amount of humic matter extracted



from soil was significantly smaller than that for the untreated control soil. This suggests that the catalyzed oxidative treatment induced a chemical modification in SOM molecules that decreased their solubility in the alkaline extracting solution. The coupling of phenolic components of SOM through a free radical mechanism sustained by the Fe-Pha catalysis [18] and the consequent increase of SOM molecular mass should have promoted a stronger adsorption of humic matter on soil mineral particles and its reduced availability to extraction.

Conclusions

Our findings indicate that the in situ oxidative coupling of humic molecules under biomimetic catalysis effectively increased their molecular mass and content of chemical energy. This resulted in less microbial mineralization of SOM, because the new intermolecular covalent bonds either required more energy from soil microorganisms for their breakage or favored a tighter stabilization of clay-humic complexes and a reduced accessibility to the microflora. This is in line with the improved soil physical quality observed after the catalyzed soil treatment that favored interparticle aggregation and stabilization of humus carbon into meso- and micro-aggregates. A soil management practice based on the use of biomimetic oxidative catalysts may represent an innovative strategy for the control of SOM aimed to limit GHG emissions from soils and, hence, mitigate global climate changes.

Authors' contributions

All authors have contributed equally to the work. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

Consent for publication

All authors give their personal consent for publication.

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