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ORIGINAL PAPER

In situ photo-polymerization of soil organic matter by heterogeneous nano-TiO_2 and biomimetic metal-porphyrin catalysts

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Abstract Two oxidative catalytic systems, titanium dioxide (TiO_2) nanoparticles and a biomimetic Mn-porphyrin linked to montmorillonite through a spacer (MnP-M), were used to photo-polymerize soil organic matter (SOM) directly in situ on soil, with the aim to sequester organic carbon (OC) and reduce CO_2 emissions from soil. We first validated, by infrared spectroscopy, thermogravimetric analysis (TGA), and proton spin–lattice relaxation time ($T_{1\rho}H$) in ^{13}C cross-polarization magic angle spinning (^{13}C CPMAS) nuclear magnetic resonance (NMR) spectra, the capacity of the nano-TiO_2 catalyst to photo-oxidatively couple humic molecules together by covalent bonds, as already proved under MnP-M. Both catalysts turn the loosely associated humic superstructures into more stable and larger molecular masses through a photo-oxidative free radical coupling mechanism. Then, the addition of either nano-TiO_2 or MnP-M to soil induced an in situ photo-polymerization of SOM, since exposure of catalyst-treated soils to solar light determined a significant reduction of respired CO_2 and an increase in thermal stability of humic components and, consequently, in SOC content. This suggests that both nano-TiO_2 and immobilized metal-porphyrin photo-catalysts effectively promote OC sequestration in soil and reduction of CO_2 emissions from soil to atmosphere. Soil treatments with heterogeneous photo-catalysts may become a promising technology for the development of future agricultural practices aimed to increase SOM stabilization and contribute to mitigate global climate change.

Keywords Heterogeneous biomimetic catalysis · TiO_2 nanoparticles · Photocatalysis · Soil organic matter · CO_2 emissions · Soil carbon sequestration

Introduction

The increase of global temperature due to uncontrolled anthropogenic emissions of greenhouse gases (GHG) and consequent changes in climate is the most alarming concern for mankind (Bergoglio 2015). Agricultural activity contributes considerably to global warming due to an increasing transfer of C from soil organic matter (SOM) to atmosphere that is caused by extensive deforestation and intensive soil management (Schlesinger 1997; Lal 2008). Moreover, since SOM is closely related to chemical, physical, and biochemical soil properties, its decrease also signifies loss of soil quality and, thus, soil fertility and productivity. Following recommendation from the Kyoto Protocol, C sequestration in terrestrial pools should be enhanced by adopting soil management practices which improve SOM incorporation and accumulation, thereby counteracting both GHG emissions and soil degradation processes (Jandl et al. 2007).

The humified organic matter in soil represents the largest C reservoir in terrestrial ecosystems (Schlesinger 1997) and, thus, it is essential in the processes of transformation, stabilization, and accumulation of SOM. A body of experimental evidence has recently indicated humic substances as

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supramolecular associations of heterogeneous and relatively small (<1000 Da) molecules (Piccolo and Spiteller 2003; Piccolo et al. 2010), which are held together by relatively weak forces, such as hydrogen and hydrophobic bonds, in only apparently large molecular sizes (Piccolo 2002) and whose conformations can be disrupted by the action of weak organic acids (Piccolo et al. 2002; Smejkalova and Piccolo 2008). Within the supramolecular understanding, the stability of humic conformations and their content of chemical energy can be enhanced by forming intermolecular covalent bonds among aromatic humic molecules through catalyzed oxidative reactions (Piccolo et al. 2000; Cozzolino and Piccolo 2002). It is assumed that such increased chemical stabilization of soil humus would consequently enhance its microbial recalcitrance, due to a greater energy threshold to further biotic or abiotic degradation, and ultimately result in both C sequestration and reduction of CO_2 emission from soil. Moreover, the increased molecular mass of humic matter following a catalyzed oxidative reaction would promote soil particle association into larger soil aggregates, thus improving soil structural stability and resistance to erosion (Piccolo and Mbagwu 1999; Baldock and Skjemstad 2000).

Previous works have shown that larger and more chemically stable humic molecules were successfully obtained by treating solutions of natural organic matter (NOM) with either a water-soluble or immobilized metal-porphyrin as biomimetic catalyst, under both chemi- (Nuzzo and Piccolo 2013a; Piccolo et al. 2005) and photo-oxidation (Smejkalova and Piccolo 2005; Nuzzo and Piccolo 2013b). Metal-porphyrins promote oxidative coupling reactions between humic phenols via a free radical mechanism induced by an oxygen donor (Sheldon 1994). When the system is exposed to light, molecular dioxygen $^3O_2$ is acting as an oxygen donor. Light causes an electron intramolecular transfer which leads to the formation of metal(II)porphyrin that can coordinate O_2 and form highly reactive metal-oxo species with strong oxidizing capacity (Maldotti et al. 2002). These species oxidize the surrounding organic substrates to give unstable free radicals which are rapidly stabilized by spontaneous mutual coupling, without any further catalyst involvement (Dec et al. 2001). However, it has been found that when metal-porphyrins are free in solution, side reactions, such as self-destruction or formation of μ-oxo porphyrin dimers, limit considerably their catalytic activity (Meunier 1994).

The immobilization of metal-porphyrin on adequate supports is known to be a winning strategy to reduce both its oxidative degradation (Nuzzo and Piccolo 2013a; Zucca et al. 2009; Zucca et al. 2010; Zucca et al. 2014) and loss by leaching to the undersurface soil. We previously showed that a manganese-porphyrin catalyst immobilized on spacer-functionalized kaolinite or montmorillonite clay significantly increased the rate of oxidative coupling of catechol, a humic phenolic precursor, as compared to the free catalyst (Nuzzo and Piccolo 2013a). We also proved that the manganese-porphyrin immobilized on kaolinite successfully promoted the oxidative and photo-oxidative polymerization of humic molecules in NOM solutions (Nuzzo and Piccolo 2013b). The photo-polymerization reaction of humic molecules under the homogeneous biomimetic catalysis was also shown to occur within the soil complex matrix either at the lab or the field-plot scale. In fact, a water-soluble iron-porphyrin was found to couple humic phenolic molecules directly in soils simply by photo-oxidation under solar radiation, thus resulting in a significant reduction of soil respiration and CO_2 emissions to the atmosphere (Piccolo et al. 2011; Spaccini and Piccolo 2012; Spaccini and Piccolo 2013).

Another photo-catalyst that may become useful to increase NOM stability and accumulation directly in soil is the nano-structured titanium dioxide (TiO_2). TiO_2 nanoparticles have attracted much attention in the last decades owing to its use for photo-assisted degradation of organic molecules and pollutants in different environmental compartments (Drosos et al. 2015; Kibanova et al. 2009; Yan et al. 2006). TiO_2 is a semiconductor whose photo-catalytic activity derives from the redox alteration of its surface following adsorption of photons with energy greater (or equal) than the band gap of the semiconductor (Herrmann 1999). Such photo-excitation promotes charge transfer reactions through the interface between the semiconductor and the reaction medium, and the irradiated TiO_2 semiconductor catalyzes the oxidation of dissolved molecules.

The first objective of this work was to verify whether the TiO_2 nanoparticles are capable to catalyze the photo-oxidative coupling of humic molecules in NOM solutions, as it does the manganese-porphyrin when immobilized on clay minerals. The second objective was to investigate the enhancement in chemical stability of SOM when a soil is subjected to a photo-oxidative heterogeneous catalysis by either TiO_2 nanoparticles or Mn-porphyrin immobilized on a montmorillonite support through a molecular spacer. The reduced microbial mineralization from the soil treated with the two heterogeneous nano and biomimetic catalysts was evaluated by following soil respiration, thermogravimetric behavior, and changes in total organic C content.

Materials and methods

Materials

Anatase TiO_2 powder (CristalActiv^{TM} PC105) and other chemicals (Reagent Grade by Sigma-Aldrich) were used without further purification. The synthesis of meso-tetra(2,6-dichloro-3-sulfonatophenyl)porphyrinate of Mn(III) chloride [Mn(TDCPPS)Cl] has been previously described (Piccolo et al. 2005). The montmorillonite (M) clay mineral originated from Crook County, Wyoming (USA) and was supplied by the Source Clay Minerals Repository (University of Missouri, USA). The humic acid (HA) was extracted from North Dakota leonardite (Mammoth, Int. Chem. Co., USA), purified, and turned into potassium humate, as reported earlier (Fontaine and Piccolo 2012). The soil sample was a Haplic Luvisol (FAO Soil Classification) from Roskilde, Denmark (mean annual temperature 8.4 °C), whose surface (0–20 cm) was air-dried and sieved through a 2-mm sieve. The soil had the following properties: pH(H_2O), 6.5; organic C (%), 1.4; clay (%), 12.3; silt (%), 31.7; and sand (%), 56 (Spaccini et al. 2000). The elemental composition (C, N, H) of both control and catalyst-treated soils was carried out using an EA 1108 Elemental Analyzer (Fisons Instruments).

Mn(TDCPPS)Cl immobilized on spacer-functionalized montmorillonite

A M sample (30 g) was first protonated by suspending in 1 L of a 2 M HCl solution and stirred for 48 h. After proton saturation, M was first dialyzed (3500 Da dialysis tubes) against water until pH 5–6 and, then, freeze-dried and pulverized. The molecular spacer, 3-(1-imidazolyl)propylcarbamoyl-3′-aminopropyl-triethoxysilane (Imi-APTS), was synthesized as described elsewhere (Zucca et al. 2007). Briefly, 260 μL (10 mmol) of (3-isocyanatopropyl)-triethoxysilane and 140 μL (11 mmol) of *N*-(3-aminopropyl)imidazole were mixed in 2 mL of dioxane and allowed to react overnight at room temperature under magnetic stirring. Protonated M (1 g) was then added to this reaction mixture, and the resulting slurry was refluxed at 80 °C overnight. The slurry was centrifuged (7000×*g*), and the residue was washed sequentially with dioxane, deionized water, and then freeze-dried.

Imi-APTS-functionalized M (100 mg) was added with 5 mL of deionized water containing 0.40 mg of Mn(TDCPPS)Cl, and the suspensions were stirred overnight at room temperature. The solid residue was separated by centrifugation and exhaustively washed with a 1 M NaCl solution and deionized water for removing unbounded Mn(TDCPPS)Cl. The amount of Mn(TDCPPS)Cl leached from the support was quantified by measuring the amount of Mn-porphyrin (MnP) removed in the successive washings through spectrophotometric measurements with a UV–vis spectrophotometer (Perkin-Elmer Lambda 25) at λmax = 467.4 nm. The adduct between Imi-APTS functionalized M and MnP was finally freeze-dried.

Photo-oxidative coupling reaction of HA by TiO_2

The control humic solution was prepared by dissolving 100 mg of potassium humate in 200 mL of Milli-Q water, while reaction mixtures were obtained by adding 1, 10, 20, and 40 mg of TiO_2, to the humic solution. The humic mixtures were stirred at room temperature and exposed to solar light for 72 h. Finally, they were centrifuged at 10,000×*g* and the supernatant was freeze-dried.

Attenuated total reflectance infrared Fourier transform (ATR-IR) spectroscopy

ATR-IR spectra were recorded with a Perkin-Elmer Frontier FT-IR/NIR Spectrometer, equipped with a Perkin-Elmer Attenuated Total Reflectance Sampling Accessory, by accumulating up to ten scans with a resolution of 4 cm^{-1}.

Thermal analysis

Thermogravimetric measurements were obtained by a Perkin-Elmer Simultaneous Thermal Analyzer (STA 6000). Freeze-dried humic matter before and after a TiO_2 treatment (5 mg) and soil samples (30 mg) before and after a photo-polymerization reaction under either biomimetic manganese-porphyrin immobilized on montmorillonite (MnP-M) or TiO_2 nanoparticles were heated continuously from 30 to 900 °C with a constant heating rate of 10 °C min^{-1}, under a 50-mL min^{-1} flow of air. All samples were run in triplicate.

Solid-state nuclear magnetic resonance (NMR) spectroscopy

NMR spectra were acquired with a 300-MHz (7.0 T) Bruker Avance magnet (Bruker Biospin, Rheinstetten, Germany), composed by a wide-bore system and equipped with a cross-polarization magic angle spinning (CPMAS) probe, working at the ^{13}C frequency of 75.47 MHz. Samples (60–80 mg) were loaded into 4-mm zirconia rotors, closed with KelF caps and spun at a rate of 13,000 ± 1 Hz. Two kinds of NMR experiments were conducted and consisted in (i) ^{13}C CPMAS and (ii) proton spin–lattice relaxation time ($T_{1\rho}H$) (carbon-detected) experiments. In particular, the latter was performed by acquiring an array of 24 spectra wherein the spin lock time was linearly and progressively incremented within 0.01 and 10 ms in 24 experiments. All spectra consisted in 1814 time domain points, a spectral width of 300 ppm (22,727.3 Hz), a recycle delay of 2 s, 8000 scans for CPMAS, and 2000 scans for $T_{1\rho}H$ experiment. The optimal ^{1}H-^{13}C contact time for CPMAS and $T_{1\rho}H$ experiments was set to 1 ms. In all cases, a high power proton decoupling was performed by using a time proportional phase modulation (TPPM15) decoupling scheme. Free induction decays (FIDs) were processed by Bruker Tospin (v2.1) and MestreC (v. 4.9.9.9, Mestrelab Research) software. In detail, spectra were Fourier transformed by applying a 4-k zero filling and adopting an exponential filter function, with a line broadening of 250 Hz, prior to be phase and baseline corrected. Spectra were integrated by the available software in the 150–100-ppm range to evaluate the aromatic signal area $I(t)$. $T_{1\rho}H$ values were calculated by using the following equation:

$$I(t) = I_0 \exp\left[-t_{SL} T_{1\rho} H^{-1}\right]$$

where t_{SL} is the variable spin lock time, $I(t)$ is the signal area at t-th spin lock time, and I_0 is the signal area in absence of any relaxation.

In situ photo-polymerization of soil organic matter under heterogeneous catalysts

A control series was obtained by placing air-dried soil samples (7 g) on Petri dishes (12 cm diameter) and kept at 45 % of water holding capacity (WHC) by adding 1 mL of distilled water. The series subjected to photo-oxidative polymerization were similarly prepared and added with either 2.8×10^{-8} mol of Mn(TDCPPS)Cl immobilized on spacer-functionalized montmorillonite (9.44 mg containing 3.85×10^{-3} $mg^{MnP}/mg^{Imi\text{-}APTS\text{-}M}$) or TiO_2 (15 and 50 mg, respectively). All series were prepared in triplicates, covered with a Petri dish, and incubated for 5 days under natural solar radiation.

Soil respiration

Respiration of both control and catalyst-treated soils was evaluated by a static absorption method. The experimental arrangement of static absorption method followed that of Alef (1995). In brief, 7 g of a 2-mm sieved, air-dried, and rewetted soil sample (45 % WHC), 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 $BaCl_2$ on both water blank and NaOH solutions. The total estimation of evolved CO_2 in the closed system was referred to 27 days of soil incubation.

Statistical analysis

The Tukey's test was used to compare values obtained for control and polymerized soils, and the 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.

Results and discussion

Heterogeneous biomimetic catalysis was found to successfully catalyze the oxidative polymerization of humic molecules in aqueous media (Nuzzo and Piccolo 2013b), whereas no previous attempts have been reported up to now on the capacity of TiO_2 nanoparticles to catalyze the oxidative coupling reaction among humic phenolic molecules. We thus investigated the effects of a heterogeneous TiO_2 catalysis under solar light irradiation on a dissolved humic solution, and we followed the changes in humic molecules by both infrared and ^{13}C-CPMAS-NMR spectroscopies and by thermogravimetric analysis.

ATR-IR spectra of humic matter before and after the photo-oxidative treatment catalyzed by different amounts of TiO_2 nanoparticles are reported in Fig. 1. Spectra showed the typical absorptions of humic material: OH stretching vibrations

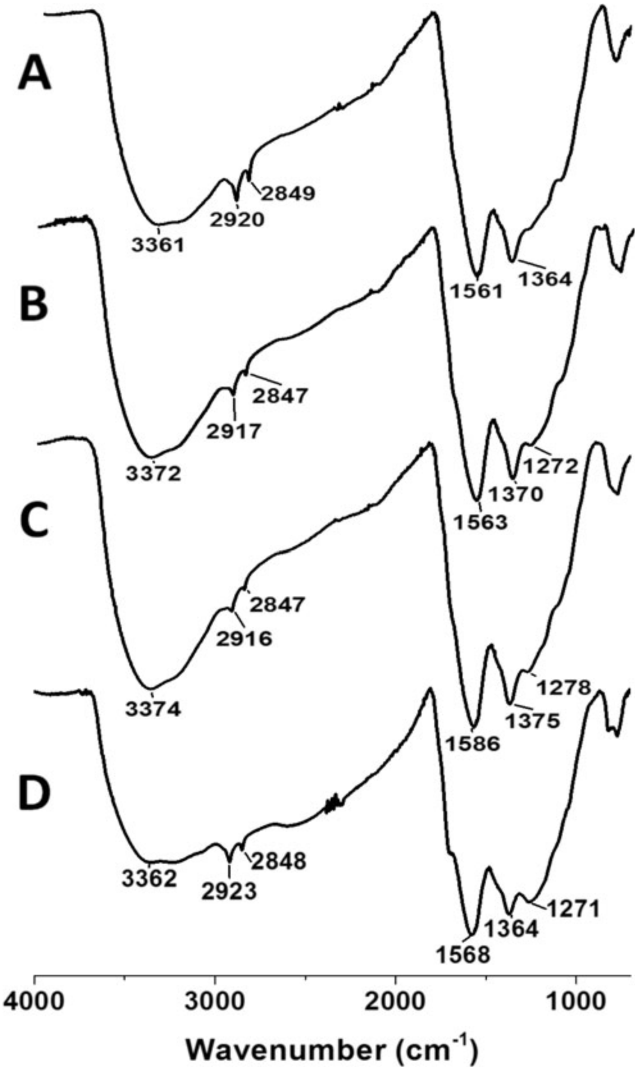

Fig. 1 ATR-IR spectra of humic matter after 72 h of exposure to solar light: *A* = control HA, *B* = HA + 1 mg of TiO_2, *C* = HA + 10 mg of TiO_2, *D* = HA + 40 mg of TiO_2

near 3400 cm^{-1}, aliphatic C–H stretching bands at about 2920 and 2850 cm^{-1}, the band near 1600 cm^{-1} due to aromatic C=C conjugated with COO^{-} and COO^{-} symmetric stretching frequencies, bands in the range between 1400 and 1300 cm^{-1} attributed to OH deformation, and COO^{-} anti-symmetric stretching (Piccolo et al. 2005). The photo-oxidation of humic matter catalyzed by progressively larger amounts of TiO_2 provided infrared spectra with increasingly intense absorption at about 1270–1230 cm^{-1}. These bands may be assigned to C–O phenol stretchings and account for an enhanced content of oxidized aromatic rings (Piccolo et al. 2005; Bellamy 1975).

A further evidence of the occurred photo-oxidative coupling of humic molecules by nano-TiO_2 catalysis was provided by thermogravimetric analysis (TGA) of humic samples before and after the catalytic treatment. Only the TGA curves of humic matter before and after addition of the largest amount (40 mg) of TiO_2 are shown in Fig. 2, since no difference was detected in the TGA curve for the small TiO_2 addition. The treated humic matter showed a mass loss at about 100 °C, that is attributed to evaporation of residual water (Janos and Kozler 1995), followed by continuous mass losses up to 400 °C due to decarboxylation, extensive decomposition of aliphatic structures and degradation of alcoholic, phenolic, and amino groups (Ioselis et al. 1985; Kucerik et al. 2004), and, finally, by mass losses in the 400–600 °C range, attributable to the thermal breakdown of aromatic structures (Nuzzo and Piccolo 2013b). While the TGA curves for catalyst-treated and untreated humic matter displayed a similar profile, the former showed a smaller rate of weight loss than for the latter (Fig. 2), thereby indicating a greater thermal stability for the catalyst-treated sample. This result suggests that the TiO_2-catalyzed photo-oxidative reaction effectively stabilized the chemical structure of humic matter, and a larger thermal energy was required for its degradation.

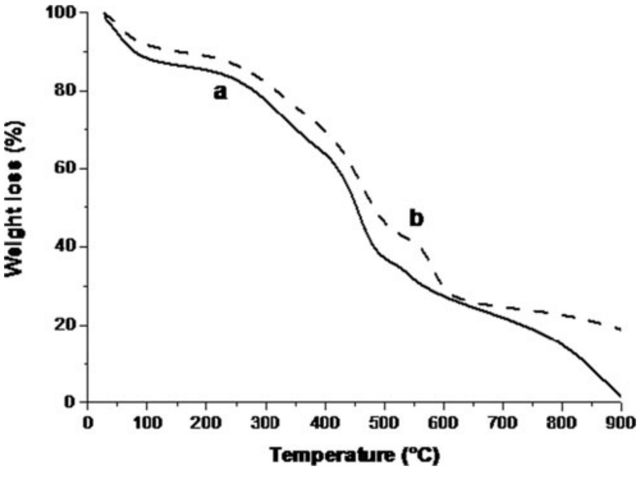

Fig. 2 TGA curves of humic matter after 72 h of exposure to solar light: *a* (*solid line*) = control HA, *b* (*dotted line*) = HA + 40 mg of TiO_2. Standard deviation was less than 5 %

The photo-polymerization of dissolved humic molecules by TiO_2 catalysis was also evaluated by solid-state MAS-NMR spectroscopy that is often used to characterize the molecular rigidity of solid samples, including humic materials (Piccolo et al. 2005; Peuravuori et al. 2003; Cao et al. 2014). Even though the MAS technique was conceived to minimize the phenomena that prevent acquisition of meaningful NMR spectra in the solid-state (e.g., homonuclear dipolar interactions, chemical shift anisotropy, quadrupolar interactions, and enhanced magnetic susceptibility), several MAS-NMR applications take advantage from strong dipolar interactions deriving from heteronuclear ^{1}H-^{13}C couplings through the process of cross-polarization (CP) (Duer 2002). This consists in a through-space polarization transfer, commonly from ^{1}H to surrounding nuclei, that (i) increases the sensitivity of nuclei with poor natural isotopic abundance and low gyromagnetic ratio (γ), such as ^{13}C, and (ii) provides indirect information on nanoscale morphology of molecular segments (Duer 2002; Stejskal and Memory 1994). While no significant evidence of catalyst-induced polymerization was detected in 1D-^{13}C-CPMAS-NMR spectra (not shown), the $T_{1\rho}H$ in the rotating frame (carbon-detected via CP) revealed diagnostic variations ascribable to the TiO_2 treatment. In fact, $T_{1\rho}H$ depends strictly on the rate of magnetization transfer from many proton nuclei, namely spin diffusion, that, in turn, may be relevantly influenced by intermolecular distance, as well as by the degree of overall molecular motions and the rigidity of a molecular system (Cao et al. 2014; Duer 2002; McCall 1971; Stejskal et al. 1981).

The $T_{1\rho}H$ values calculated from spectral signals of the aromatic region (100–150 ppm) for humic samples before and after the photo-oxidative treatment by the TiO_2 catalysis are shown in Fig. 3. The $T_{1\rho}H$ value for control was 5.4 ms, whereas the values for the catalyst-treated humic samples progressively increased with catalyst amount and reached 7.9 ms for the largest TiO_2 addition (Fig. 3). The significant increase in $T_{1\rho}H$ values indicates that the catalyst-treated humic matter underwent a relevant and gradual modification in conformational stability and that covalent intermolecular bonds were formed among humic molecules, with a consequent reduced mobility and increased motional rigidity of the overall humic supramolecular structure (Piccolo et al. 2005). This result is in line with those obtained by infrared spectroscopy and TGA, which suggested that the catalytic treatment increased both the oxidation state and the chemical stability of the treated humic samples. Since it is plausible that the larger $T_{1\rho}H$ values with increasing catalyst additions were due to changes in the mutual spatial relations of proton-rich humic segments, when passing from a loosely bound humic suprastructures into more rigid conformations, these results show the TiO_2 catalyst capacity to covalently link humic molecules together, as already found in humic matter subjected to catalysis by the oxidative biomimetic metal-porphyrin (Piccolo et al. 2005).

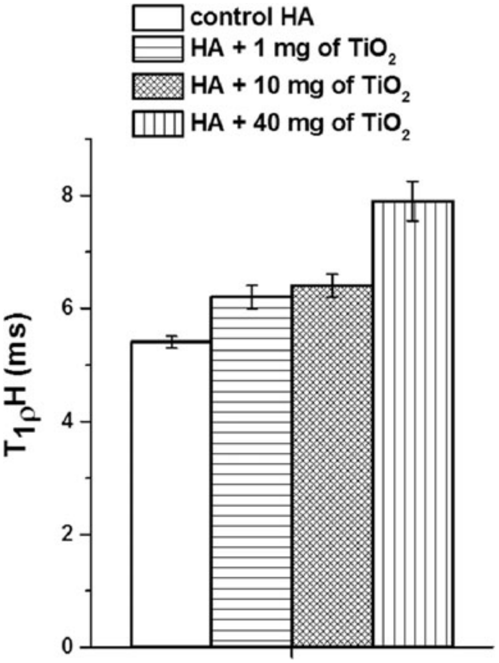

Fig. 3 $T_{1\rho}H$ NMR values calculated for aromatic signals of humic samples after 72 h of exposure to solar light: control HA, HA + 1 mg of TiO_2, HA + 10 mg of TiO_2, HA + 40 mg of TiO_2

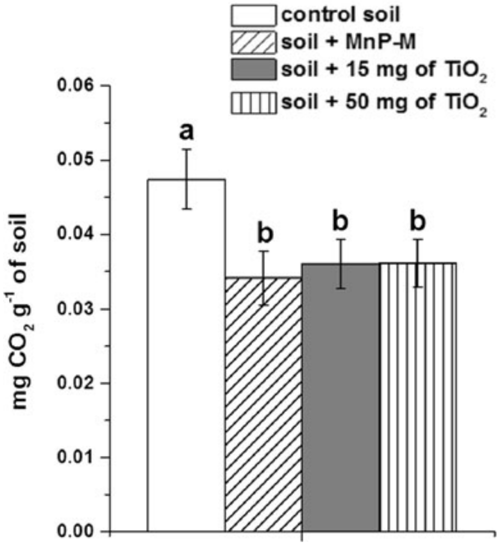

Fig. 4 Soil respiration (mg CO_2 g^{-1} of soil) before and after catalyst-assisted in situ photo-polymerization treatment: control soil, soil + 9.44 mg of MnP-M, soil + 15 mg of TiO_2, soil + 50 mg of TiO_2. *Error bars* indicate standard error ($n = 3$), and *different letters* indicate significant differences by the Tukey's test at $P \leq 0.05$

As for the second objective of this work, we verified whether the heterogeneous nano-TiO_2 and MnP-M could exert their photo-oxidative coupling catalysis directly on humic molecules in situ in the soil matrix. Previous findings have shown that despite the complex chemical and biochemical heterogeneity of soils, the in situ catalyzed oxidative coupling of humic molecules is to be considered among the abiotic photochemical reactions in soils (Sannino et al. 2013; Piccolo et al. 2011). As in the case of the homogeneous biomimetic catalysis when the water-soluble iron-porphyrin was added directly to soil (Piccolo et al. 2011), the efficacy of the two heterogeneous catalysts in favoring an in situ photo-polymerization of SOM was also evaluated here by measuring the total CO_2 respiration of soil samples during an incubation period (Fig. 4). The catalyst-assisted in situ photo-oxidative modification of SOM significantly decreased CO_2 emission from all treated soils, as compared to the untreated control soil. The observed reduced CO_2 respiration from soils treated with the heterogeneous catalysts suggests that the photo-polymerization reaction had also occurred in situ in the soil matrix. In fact, the stabilization of SOM through an increase of intermolecular covalent bonds promoted by the photo-catalytic reaction must have significantly limited the capacity of soil microbes to mineralize the soil organic carbon, thereby reducing soil respiration. However, no significant differences were noted between the two heterogeneous catalysts and between the two rates of TiO_2. Nevertheless, the biomimetic heterogeneous MnP-M catalyst enabled the same reduction of CO_2 emission as the TiO_2 nanoparticles, despite the active manganese-porphyrin catalyst was only 2.8×10^{-8} mol in the MnP-M adduct. This suggests that the photo-oxidative coupling among the susceptible phenol molecules in soil is catalyzed much more efficiently by the biomimetic than the nano-structured catalyst. An alternative explanation for the reduction in soil respiration by toxicity of the biomimetic catalyst on soil microbial activity should be ruled out based on previous findings (Gelsomino et al. 2010; Puglisi and Trevisan 2012). Similarly, a microbial toxicity by TiO_2 nanoparticles should be excluded since their free radical mechanism of action is the same as that of metal-porphyrins, already proven not to be toxic. However, even assuming a toxicity of TiO_2 nanoparticles towards soil microbial communities, the presence of additional metabolic carbon derived by dead cells would rather result in either a significant increase of respired CO_2 (Alef and Nannipieri 1995) or a total lack of respiration due to TiO_2 toxicity. Conversely, CO_2 respiration from TiO_2-treated soil samples was neither increased nor nullified but rather significantly decreased in respect to control. It is thus plausible to explain the effect of the TiO_2 nanoparticles with an increased chemical stability of organic carbon molecules and their consequent reduced availability to microbial mineralization.

Both control and catalyst-treated soil samples were then investigated by thermogravimetric analysis (Fig. 5). No significant differences were observed among thermogravimetric curves of samples added with different amounts of either TiO_2 or MnP-M, whereas the thermal degradation curves of all catalyst-treated samples were significantly different from control. The shift to higher temperatures than control, for TGA

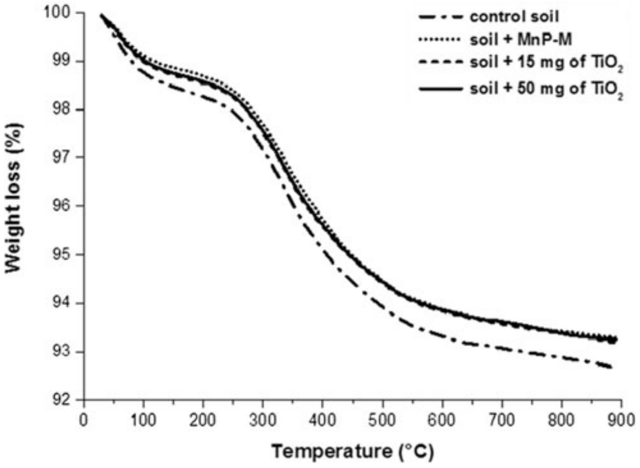

Fig. 5 TGA curves of soil samples before and after in situ photo-oxidative polymerization catalyzed by either MnP-M or TiO_2 ($n = 3$): control soil, soil + 9.44 mg of MnP-M, soil + 15 mg of TiO_2, soil + 50 mg of TiO_2. Standard deviation was less than 0.1 %

curves of samples subjected to heterogeneous catalysts, indicates a larger thermal stability of the catalysts-treated soils, thereby matching with findings that showed a reduced respiration in the same soils.

A further evidence of the increased SOM chemical energy in soils treated with heterogeneous catalysts was shown by the total organic C found in soil after the incubation period (Fig. 6 and Table S1 in Supplementary Material). The content of soil organic C was significantly different between the control soil before and after the incubation period. In fact, the organic C content of the untreated control soil decreased after 27 days due to microbial respiration, whereas the OC content in the catalyst-treated soils remained unchanged. This difference cannot be accounted to the addition of the MnP-M catalyst, whose carbon corresponded to only 0.022 g per 100 g of soil. Moreover, the behavior of OC in soils well agreed with results on CO_2 emission (Fig. 4) and thermal stability (Fig. 5) after incubation, thereby confirming that soil phenolic humic components can become recalcitrant to microbial degradation when coupled together in more complex and larger-sized conformations by the action of photo-oxidative catalysts (Smejkalova and Piccolo 2005; Smejkalova et al. 2006). The fact that these SOM components can conversely be rapidly mineralized and the C content decreased in the untreated soil (Fig. 6) suggests that the in situ photo-oxidative treatment with heterogeneous catalyst is effective in sequestering organic C in soil.

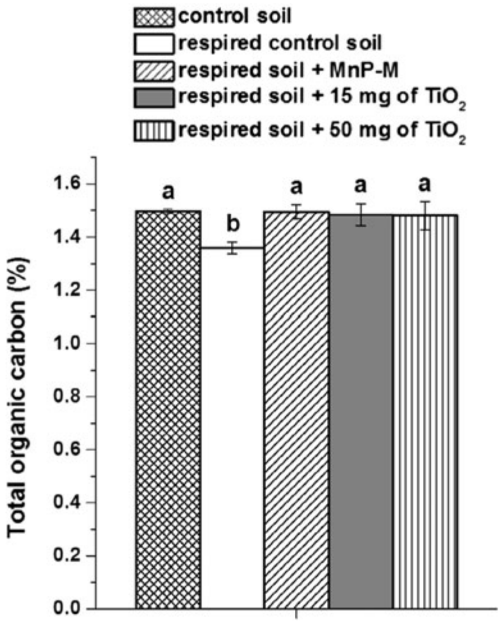

Fig. 6 Percent of total organic C content (mean ± s.e.m.) in soil samples without and with in situ photo-polymerization catalyzed by either MnP-M or TiO_2 ($n = 3$), following 27 days of incubation: control soil, respired control soil, respired soil + 9.44 mg of MnP-M, respired soil + 15 mg of TiO_2, respired soil + 50 mg of TiO_2. Values followed by the *same letter* within columns are not significantly different by Tukey's test at the level of $P \leq 0.05$

Conclusions

Our findings indicate that the soil treatment with heterogenous photo-oxidative catalysts, whether solid TiO_2 nanoparticles or biomimetic catalysts immobilized on a clay mineral, represents an innovative and useful technology for C sequestration in soil and for reducing GHG emission from soil to the atmosphere. The in situ photo-polymerization of soil humic molecules promoted by these heterogeneous catalysts enhances the number of intermolecular covalent bonds among SOM components and, thus, requires from soil microorganisms a greater energy to mineralize organic matter. This enhanced metabolic threshold limits the overall degrading activity of soil microbes, thereby making the modified SOM less bioaccessible and, ultimately, the soil C more protected from mineralization. Such practice based on the use of heterogeneous catalysts may represent a promising management strategy to contribute to limit greenhouse gas emissions from agroecosystems and mitigate global changes.

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