



Carbon deposition in soil rhizosphere following amendments with compost and its soluble fractions, as evaluated by combined soil–plant rhizobox and reporter gene systems

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ABSTRACT

We determined the organic carbon released by roots of maize plants (*Zea mays* L.) when grown in soils amended with compost and its soluble fractions. In rhizobox systems, soil and roots are separated from the soil of a lower compartment by a nylon membrane. Treatments are applied to the upper compartment, while in the lower compartment luminescent biosensors measure the bioavailable organic carbon released by roots (rhizodeposition). The rhizobox–plants systems were amended with a compost (COM), its water extract (TEA), the hydrophobic (HoDOM) and hydrophilic (HiDOM) fractions of the dissolved organic matter (DOM) extracted from the compost. After root development, the lower untreated compartments were sampled and sliced into thin layers. The bioavailable organic carbon in each layer was assessed with the *lux*-marked biosensor *Pseudomonas fluorescens* 10586 pUCD607, and compared with total organic carbon (TOC) analyses. The TOC values ranged between 8.4 and 9.6 g kg⁻¹ and did not show any significant differences between bulk and rhizosphere soil samples in any treatment. Conversely, the biosensor detected significant differences in available C compounds for rhizosphere soils amended with various organic materials. Concentrations of available organic compounds in the first 2 mm of soil rhizosphere were 1.69 (control), 1.09 (COM), 2.87 (HiDOM), 4.73 (HoDOM) and 2.14 (TEA) μmol C g⁻¹ soil g⁻¹ roots. The applied rhizobox–biosensor integrated method was successful in detecting and quantifying effects of organic amendments on organic carbon released by maize plant roots. This approach may become important in assessing the carbon cycle in agricultural soils and soil–atmosphere compartments.

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1. Introduction

The rhizosphere is a biologically active zone in soil where complex interactions occur among plant roots, soil particles and microbes. Investigations on the rhizosphere help to gain a better understanding on a variety of processes such as nutrient cycling, ecosystem functioning, carbon dynamics, and to evaluate the impacts of anthropogenic activities on agricultural and natural ecosystems.

An important rhizosphere process is rhizodeposition, that involves the transfer of C from plant roots to soil in the form of water-soluble exudates (low molecular weight molecules such as organic acids, amino acids and sugars), secretions of higher molecular weight substances, lysates from cells after autolysis, gases

such as ethylene, carbon dioxide, and hydrogen cyanide (Grayston et al., 1996). Through rhizodeposition, plants supply soil microbes with readily available organic C, which increases microbial biomass around roots and affects activity and composition of microbial communities. On the other hand, microbes mobilize nutrients, primarily nitrogen, and render them available to plants, as described by the “microbial loop” model (Paterson, 2003; Bonkowski, 2004). Rhizosphere nutrient flow also plays an important role in organic C mobilization and immobilization processes, with important consequences on carbon dioxide fluxes involved in the greenhouse effect (Cheng and Johnson, 1998). In particular, it has been estimated that about 15–25% of carbon fixed below-ground is exuded from roots into soil, including root turnover (Kuzakov, 2002). Several environmental factors influencing plant growth and physiology have been reported to affect rhizodeposition, including light intensity, atmospheric CO₂ concentration, presence of specific microorganisms, soil texture, and organic matter composition (Meharg and Killham, 1995; Paterson et al., 1997). Knowl-

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edge of rhizodeposition processes is still incomplete, particularly due to the limited methods available for studying rhizosphere C deposition and flow under realistic conditions. Moreover, methods with complementary, cross-disciplinary (physics, chemistry and biology) strengths have not yet been effectively combined.

Rhizobox systems were introduced to study root-induced changes in soil properties (Kuchenbuch and Jungk, 1982; Youssef and Chino, 1989; Wenzel et al., 2001). They are based on a soil-root compartment and one or more adjacent soil compartments separated by porous membranes, thus allowing the monitoring of gradients of rhizosphere characteristics at high spatial resolution. Rhizobox systems are composed of two physically separated compartments: an upper plant-roots soil compartment, and a lower rhizosphere compartment, where the soil is separated from roots by a nylon membrane. This enables treatment applications to the upper compartment and evaluation of consequent effects in the untreated lower compartment.

The development of reporter gene technologies such as biosensors has improved research not only on rhizosphere C depositions, but also on the fate of other associated nutrients (Killham and Yeomans, 2001). The most common biosensors are microorganisms into which reporter genes (e.g., *lux*, encoding luciferase; *lacZ*, encoding β -galactosidase and *luc*, encoding firefly luciferase) are introduced and eventually controlled by promoters activated by specific compounds. *Pseudomonas* spp. are ubiquitous in many agricultural soils and, as such, serve as useful recipient organisms for bioreporting genes. *Pseudomonas fluorescens* 10586 pUCD607 (Hamin-Hanjani et al., 1993) is a luminescence-based biosensor constructed by genetic transformation involving the insertion of *lux* genes from the natural marine bacterium *Vibrio fischeri*. These genes are inserted upstream of a general metabolic promoter and thus reveal the organism's general metabolism by luminescence. If the biosensor is placed under starving conditions, the emitted light intensity is reduced to a minimum. However, the emitted light can be restored quantitatively when C sources are available. This feature has been successfully exploited for revealing organic C deposition under different conditions, by using a protocol developed by Yeomans et al. (1999), and subsequently applied to non-sterile soils by Standing et al. (2005). The biosensor may be directly applied *in situ* on soil samples and the light emission becomes a function of the concentration of readily bioavailable organic compounds that are commonly present in root exudates.

In recent years, recycled organic wastes have been widely used to increase soil fertility and as an economical waste disposal strategy. An important ecological role may be played by the soluble or readily available fractions from compost materials. The hydrophobic organic fraction (HoDOM) of compost was shown to increase the adsorption and hydrolysis of pesticides (Said-Pullicino et al., 2004) and to be involved in the stabilization of organic carbon in soils by hydrophobic protection (Piccolo et al., 2004). Aerated compost tea (TEA) is becoming a widely used commercial fertilizer and it was shown to positively affect the production of fruits such as raspberries (Hargreaves et al., 2008). While many studies focused on the interactions between xenobiotics and compost, humic acids, and dissolved organic matter (DOM) (Barriuso et al., 1995; Totsche et al., 1997; Piccolo et al., 1998), little is known about the effects of these materials on plant rhizodeposition processes. Swinnen et al. (1995) demonstrated that more C tended to be transferred to barley (*Hordeum vulgare* L.) roots under conventional management with high fertilizer additions than in integrated management with reduced inputs. Addition of a composted material increased water-soluble C and enzymatic activities in rhizosphere of *Olea europea*, thus suggesting an increase microbial activity induced by plant roots (Caravaca et al., 2002). Conversely, synthetic nitrogen fertilization was shown to cause a significant decrease in the amount of organic C transferred to roots (Nguyen, 2003).

The main objective of this study was to evaluate the rhizodeposition of maize plants (*Zea mays* L.) amended with different well-characterized organic materials by a combined application of rhizobox and reporter gene systems. The rhizosphere compartment was sliced into thin layers using microtome techniques and the available fraction of organic C in each layer was assessed with the *lux*-marked *P. fluorescens* 10586 pUCD607, and compared to total organic C (TOC) analyses.

2. Materials and methods

2.1. Characterization of organic materials

The urban waste compost used in this work was produced mechanically on an industrial scale at the Gesenu SpA composting facility in Pietramelina (Perugia, Italy). The feedstock was composed of source-separated municipal solid waste (55% w/w), yard trimmings from pruning activities (30%) and foliage residues from tobacco agro-industry (15%). Composting was carried out under aerobic conditions and involved a thermophilic phase of approximately 28 d during which the feedstock was daily turned, followed by a curing phase of approximately 3 additional months in piles. The chemical composition of the compost used is reported in Table 1.

Dissolved organic matter (DOM) was extracted from compost as described by Gigliotti et al. (2002). Briefly, compost was placed in contact with deionized degassed water in an extraction ratio of 1:10 w/v for 24 h at room temperature and subsequently centrifuged at 2500g. The supernatant was collected and filtered through a 0.7 μ m glass microfibre filter and a 0.45 μ m membrane filter to obtain the total DOM extract. pH of the DOM extract was around 8.2.

This extract was divided into two fractions by means of Amberlite XAD-8 and XAD-4 resins. The DOM extract was acidified to pH 2 with 0.1 N HCl and passed through the XAD-8 and XAD-4 resins. The organic fraction retained by the XAD-8 resin was eluted with 0.1 N NaOH, passed through a AG MP-50 cation exchange resin and subsequently freeze-dried. This fraction was defined as the hydrophobic fraction (HoDOM). The organic fraction retained by the XAD-4 resin was eluted with a water:acetonitrile (1:3) mixture. Acetonitrile was removed from the eluate by rotary evaporation at 35 °C and the remaining aqueous solution freeze-dried. This fraction was defined as the hydrophilic fraction (HiDOM). The HoDOM and HiDOM fractions had an organic C content of 499 mg g⁻¹ and 523 mg g⁻¹, respectively.

The aerated compost tea (TEA) fraction was obtained by suspending compost in deionized water (1:5 w/v ratio) and leaving it under stirring for 10 d at 20 °C. In order to ensure aerobic con-

Table 1
Characteristics of municipal waste compost^a

Moisture content (g kg ⁻¹)	239	±17
Ash content (g kg ⁻¹)	446	±10
pH	8.0	±0.1
Total organic carbon (g OC kg ⁻¹)	188	±2
Total extractable carbon (g OC kg ⁻¹)	128	±2
Humic acids (g OC kg ⁻¹)	71.3	±1.4
Fulvic acids (g OC kg ⁻¹)	7.0	±0.3
Non-humic fraction (g OC kg ⁻¹)	49.3	±2.0
Dissolved organic carbon (g OC kg ⁻¹)	15.0	±0.1
Hydrophobic DOM (g OC kg ⁻¹)	8.8	±0.1
Hydrophilic DOM (g OC kg ⁻¹)	6.3	±0.1
SOUR _{max} (mg O ₂ g ⁻¹ VS h ⁻¹) ^b	1.9	±0.3

^a Average of triplicate analysis ± standard error; all data except moisture content and SOUR_{max} are expressed on a compost dry weight basis.

^b Maximum specific oxygen uptake rate normalized to the content of volatile solids (VS) and measured as described by Adani et al. (2003).

ditions, the fermentation vessel was equipped with air diffusers and an oxygen probe to constantly monitor dissolved oxygen. A software driven control system helped to maintain dissolved oxygen concentrations above 2.0 mg L^{-1} , by means of intermittent air bubbling through the suspension. After 10 days the compost suspension was filtered through a cellulose filter paper, and stored at 4°C . Organic C concentration of aerated compost tea was 1.19 g L^{-1} . A fraction of the compost tea was freeze-dried to obtain a solid sample for chemical characterizations.

2.2. CPMAS-NMR spectroscopy

The molecular compositions of organic materials were evaluated by Cross-Polarization Magic Angle Spinning carbon-13 Nuclear Magnetic Resonance spectroscopy (CPMAS- ^{13}C NMR). Experiments were conducted on a Bruker AV300 instrument operating at a carbon-13 resonating frequency of 75.475 MHz maintaining the rotor spin rate at 13000 Hz . All experiments were conducted with pulse sequence CP with RAMP, a recycle time of 1.5 s , and an acquisition time of $20 \mu\text{s}$. Further details on sample treatment and other operational conditions are reported in Šmejkalová et al., 2008. The different chemical-shift regions of the spectra were automatically integrated to obtain the hydrophilic C/hydrophobic C (HI/HB) ratio for each material.

2.3. Lipids fractionation

Aliquots of each organic material (350 mg of compost, TEA and HoDOM, 150 mg of HiDOM) were oven dried at 40°C for 1 h . Lipids were fractionated according to their extractability from the relative original matrix. Structurally unbound (SU) compounds were extracted using a dichloromethane and methanol mixture ($2:1 \text{ v/v}$) for 2 h at room temperature. After extraction of structurally unbound lipids, the weakly-bound compounds (WB) were extracted by treating the resulting air-dried residue with 15 ml of 12% boron–trifluoride–methanol ($\text{BF}_3\text{--CH}_3\text{OH}$) solution at 90°C for 12 h in a polyethylene bottle. The details of extraction procedures and GC–MS determination method are described in Fiorentino et al., 2006. Compounds still strongly-bound (SB) to the air-dried residue resulting from the $\text{BF}_3\text{--CH}_3\text{OH}$ transesterification were further extracted in 100 ml of 1 M KOH in methanol for 1 h at 70°C as reported by Nierop et al., 2003. The sequentially extracted fractions were classified as unbound (SU, freely dissolved in organic solvent), weakly bound (WB, soluble only after mild transesterification), and strongly bound (SB, soluble only after methanolic alkaline hydrolysis) material. Both WB and SB extracts were made up of an organic and an aqueous phase. The quantification of the extracted compounds was based on the sum of signal areas observed in the GC–MS chromatograms. The quantitative analysis was conducted by using calibration curves of different external standards from Aldrich: tridecanoic acid, octadecanol, 16-hydroxyhexadecanoic acid, docosandioic acid, β -sitosterol, and cinnamic acid. Tridecanoic acid was also used as an internal standard to evaluate derivatization yields and steadiness of the chromatographic response. All GC–MS analyses were conducted in duplicates over three individual extracted samples.

2.4. Soil

An orchard soil was collected at a field site near Matera, South-East Italy; soil samples (7 cm diameter cores) were randomly collected from the surface layer ($0\text{--}20 \text{ cm}$) at least 2 m far from trees, sieved through a 2 mm sieve, pooled on site and stored at 4°C before using for experiments. Soil had a loam texture (24.7% clay ($<0.002 \text{ mm}$), 33.8% silt ($0.05\text{--}0.002 \text{ mm}$), 41.5% sand ($>0.05 \text{ mm}$)), a pH (H_2O) of 6.7 , and total organic C of $8.3 \text{ mg C g}^{-1} \text{ d.w. soil}$.

2.5. Plant cultivation and microtome sampling

All experiments were conducted in the rhizobox system described by Wenzel et al. (2001) (Fig. 1), which comprises an upper soil–plant compartment and a physically separated lower rhizosphere compartment. Roots only vertically penetrate through a narrow slit from the upper to the lower compartment, where they are separated from the rhizosphere soil by a nylon membrane, thus forming a thin root plane. We employed Nylon membranes of $7 \mu\text{m}$ mesh width, which exclude the penetration of root hairs in the rhizosphere soil (Wenzel et al., 2001).

Amendments were applied only to the upper rhizobox compartments by homogeneously mixing organic materials with soil. The lower rhizosphere soil compartments were not amended. Five treatments were applied, with 3 replicates (3 rhizoboxes) per treatment (Table 2): (i) control without any addition; (ii) 11.5 g kg^{-1} of compost (COM); (iii) 67 mg kg^{-1} of the hydrophobic fraction of the DOM extracted from compost (HoDOM); (iv) 67 mg kg^{-1} of the hydrophilic fraction of the DOM extracted from compost (HiDOM); (v) 57.7 mL kg^{-1} of tea extracted from compost (TEA). All materials were dissolved in deionised water before being added to the upper rhizobox compartments. All solutions had a pH around 6. Although the total applied dose of compost was three orders of magnitude larger than that of extracts, treatments are fairly comparable considering that a dose of 11.5 g kg^{-1} of compost brought to soil 172.5 mg kg^{-1} of organic C as DOM. Furthermore, similar doses are

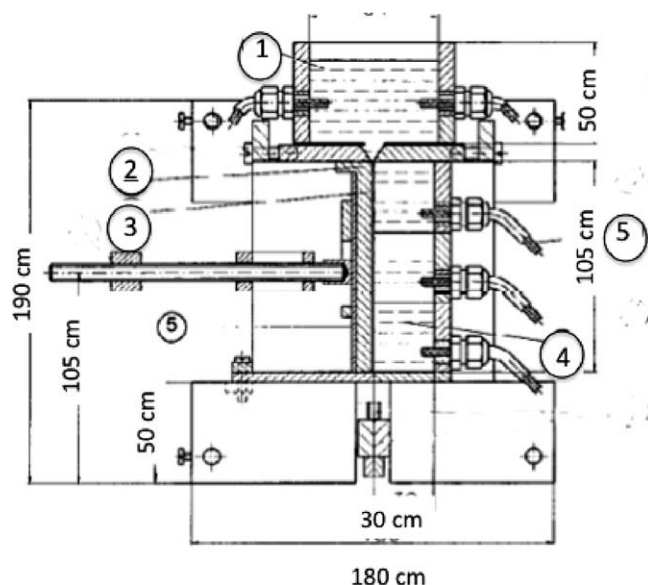


Fig. 1. Cross section of the rhizobox system, adapted from Wenzel et al. (2001). (1) soil–plant compartment; (2) transparent acrylic window; (3) nylon membrane; (4) rhizosphere soil compartment and (5) irrigation wicks.

Table 2

Treatments applied to each rhizobox and relative doses of each material applied to soil in the top compartment

Organic material	Applied dose	Applied dose expressed in compost equivalents (t ha^{-1})	Organic C added
Compost	11.5 g kg^{-1} Soil	6.3	2.16 g kg^{-1} Soil (172.5 mg kg^{-1} Soil as DOM)
HoDOM	67 mg kg^{-1} Soil	2.1	33.4 mg kg^{-1} Soil
HiDOM	67 mg kg^{-1} Soil	3.0	35.0 mg kg^{-1} Soil
TEA	57.7 mL kg^{-1} Soil	6.3	114.8 mg L^{-1} Soil

obtained when doses of extracts are expressed as compost equivalents, i.e., the amount of applied extract relative to applied compost (Table 2).

Maize seeds (*Zea mays* L.) were germinated in Petri dishes and pre-grown in the upper compartment of each rhizobox (6 seeds per each box). Ten days after germination, the upper and the lower compartment of each rhizobox were connected and the roots allowed penetrating through the slit. At 25 days after germination, the root plane was fully developed in each rhizobox, and the lower compartment was sampled using a microtome (Wenzel et al., 2001) in order to obtain soil samples at specific distances from the root plane (0–2, 2–4 and 6–4 mm). Soil samples from the upper compartment of the control treatment, i.e., bulk soil without any amendment, were also analysed. Root biomass yields for each treatment were, respectively, (g dry weight): 0.29 ± 0.03 (control), 0.36 ± 0.06 (COM), 0.18 ± 0.04 (HoDOM), 0.26 ± 0.08 (HiDOM), 0.35 ± 0.04 (TEA). All soil samples were frozen immediately after sampling and kept at -20°C before being analysed.

All experiments were conducted in a Binder KBWF 240 (Tuttlingen Germany) phytotron under the following controlled experimental conditions: 16 h light ($120 \mu\text{E s}^{-1} \text{m}^{-2}$) at 30°C , 8 h night at 20°C , 60% humidity.

2.6. Soil chemical analyses

Total organic carbon (TOC) concentrations were determined by oxidation to CO_2 with potassium dichromate according to the Walkley–Black method (Methods of Soil Analysis, 1996) and expressed as g kg^{-1} of soil on a dry weight basis.

2.7. Bacterial strain, growth conditions and bioassay protocol

Cells cultures and bioassays were carried out as described by Yeomans et al. (1999). Cultures of *P. fluorescens* pUCD607 (*lux* CDABE from *Vibrio fischerii*, *kanr*, *ampr*; Hamin-Hanjani et al., 1993) were grown in 250 ml Erlenmeyer flasks containing 100 ml of LB broth at 25°C , and rotated at 200 rpm. Late exponential cells were harvested by centrifugation (5 min at 4000 g) at OD_{550} of 2.0, which corresponds to a concentration of *P. fluorescens* pUCD607 cells of around 3.00×10^7 cells mL^{-1} (Yeomans et al., 1999). The supernatant was decanted and the cells resuspended in the same volume of C-free M9 minimal medium. The process was repeated and the final cell suspension shaken at 200 rpm in C-free M9 for 2 h at 25°C (starvation step). Kanamycin was added at $50 \mu\text{g mL}^{-1}$ to all cultures.

One millilitre aliquot of starved cells were added to 100 mg samples of soil collected from rhizoboxes in order to measure *in situ* carbon depositions. After 20 min of contact, 800 μL of soil and bacteria slurry was collected and measured for light emission. Bioassays were calibrated on a dilution series of glucose standard solutions in the range of concentrations between 1 and 10 mM of C content. Response of the biosensor was also tested on soil samples enriched with increasing concentrations of glucose.

2.8. Luminometry and biosensor response data

Light output was measured using a SystemSure luminometer Model 18172 (Nova Biomedical Waltham MA, USA). In order to compare results from different trial luminescence data were converted from relative light units (RLU; 1 RLU equivalent to 1 mV per 10 s) to relative glucose–carbon units (RGU), following interpolation with calibration curves of glucose standard solutions and expressed in terms of μmol of glucose–C equivalents per gram of soil. Biosensor response data were also normalized to the amount of Carbon added in each treatment, and thus expressed as mg of released C per gram of applied C.

2.9. Statistical analyses

All statistical analyses were performed using SPSS software. TOC and biosensor results from rhizoboxes soil samples were studied by means of fixed model analysis of variance (ANOVA). The following effects were studied:

- (1) Sampling effect (classification variable SAMP). The sampling types were: bulk 0 days (for control only), rhizosphere 0–2 mm, rhizosphere 2–4 mm, rhizosphere 4–6 mm.
- (2) Treatment effect (classification variable TREAT). The five treatments were: control, compost, HiDOM, HoDOM, and TEA.
- (3) The interaction between the two previous effects (classification variable TREAT*SAMP).

All significant effects were confirmed by Tukey's test (at $P < 0.05$) for comparison of means. Correlations between TOC and biosensor data were also analysed by means of linear regression.

Sum of different organic compounds obtained in sequential extracts of different fertilizers were also analysed by means of a fixed model analysis of variance (ANOVA). Two different effects were studied: the effect of the sequential extraction and the effect of the type of fertilizer.

3. Results

3.1. Characteristics of organic materials

The spectral information obtained from CPMAS-NMR spectra (Fig. 2) of the different compost materials are summarized in Table 3. The carbon distribution in the different intervals was used to define the HI/HB ratio, an index of hydrophilicity of complex organic materials (Spaccini et al., 2000). This ratio indicated, as expected, that the HiDOM fraction was the most hydrophilic of all fractions, whereas the HoDOM was the most hydrophobic. The compost itself and the TEA had similar HI/HB ratio.

The quantitative sum of compounds (as percent of initial organic carbon in the analysed sample) extracted from different

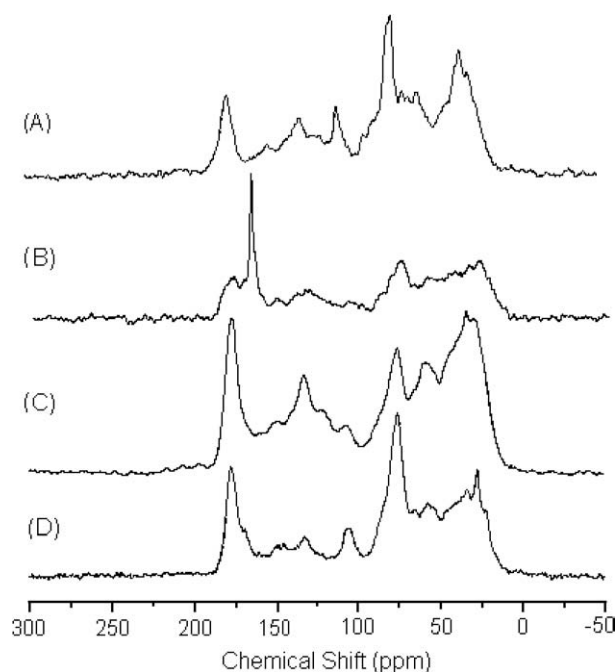


Fig. 2. CPMAS ^{13}C NMR spectra of (A) compost; (B) TEA; (C) HoDOM and (D) HiDOM.

Table 3
Percentage distribution of ^{13}C in resonance intervals (ppm) and hydrophilic-C/hydrophobic-C (HI/HB) ratios of organic materials according to CPMAS-NMR spectroscopy

Organic material	Alkyl-C	C–N	C–O	Aromatic-C	Carboxyl-C	Ketone-C	HI/HB ^a
	0–50	50–71	71–100	100–167	167–192	192–230	
Compost	31	17	21	23	8	0	0.85
TEA	28	14	16	25	16	1	0.89
HoDOM	31	14	14	27	12	1	0.71
HiDOM	32	13	21	14	14	5	1.16

^a HI/HB = [(50 – 71) + (71 – 100) + (192 – 230)] / [(0 – 50) + (100 – 167)].

compost and compost-derived samples are reported in Table 4. The SU compounds, mainly composed of lipids soluble in organic solvents, were more abundant in TEA than in the bulk compost, whereas HoDOM and HiDOM showed the lowest abundance of SU compounds. The WB compounds, extracted after transesterification, were predominant in the organic fraction from compost, and, to a lower extent, from TEA. The WB aqueous fraction was larger in HoDOM. On the other hand, a significantly lower amount of WB organic materials was detected in both the aqueous and organic fractions of HiDOM. A large amount of SB compounds was obtained after methanolic alkaline hydrolysis of compost, the majority of which were in the organic fraction. A lower amount of WB compounds was found in TEA, though they were equally distributed in the aqueous and organic fractions. The HoDOM sample produced little WB compounds in the organic fractions, whereas no strongly bound compounds (SB) were present in the HiDOM extract.

The result obtained for fractionation of compost-derived materials into progressively less available organic compounds, generally agrees well with the sample characteristics shown by CPMAS-NMR spectroscopy. The HiDOM, with the greatest hydrophilic character, also showed the lowest amount of material detected by GC–MS, thus implying a larger content of low molecular-weight hydrophilic compounds, such as simple carbohydrates and organic acids. Conversely, the hydrophobic character of HoDOM is related to the content of both SU and WB compounds in the sample matrix (Table 4).

3.2. Total organic carbon content

TOC organic values in all analysed samples were very similar, ranging from a minimum of 8 to a maximum of 10.2 g kg^{−1} soil. The two-way fixed ANOVA model showed that only the TREAT effect was significant for TOC values ($P < 0.05$). The control treatment had an average TOC content of 8.52 g kg^{−1} of soil, whereas all other organic matter treatments had significantly larger values (8.81,

9.13, 9.06 and 9.12 g kg^{−1} of soil, for COM, HiDOM, HoDOM, and TEA, respectively). However, no significant differences were found for SAMP and TREAT*SAMP effects. Moreover, TOC values for the different rhizosphere layers of all treatments were not statistically different, according to Tukey's test for comparison of means (Fig. 3a).

3.3. *Pseudomonas fluorescens* pUCD607 assay

Previous experiments (data not shown) demonstrated that the biosensor can be applied *in situ* on soil samples with different textures. The emission of light in response to the glucose standard solutions in the presence and absence of soil was linear in the range between 1 and 10 mM of glucose Carbon ($R^2 > 0.97$, $P < 0.05$), and the two calibration curves were not statistically different ($P = 0.097$). The linear regression obtained with glucose standards in the absence of soil was used to convert RLU values obtained into relative glucose–carbon units (RGU), that are defined as a μmol of glucose–C per gram of soil.

Results of the fixed two-way ANOVA model for biosensors RGU data showed significant differences ($P < 0.001$) for all studied effects (i.e., TREAT, SAMP, and TREAT*SAMP). Biosensor response at each sampling position for each treatment is plotted in Fig. 3b, together with the results of Tukey's of the TREAT*SAMP interaction. Carbon depositions regardless of the sampling position (TREAT effect), were significantly different in all treatments. Larger values were found for HoDOM (0.85 μmol glucose–C g^{−1} soil g^{−1}), followed by HiDOM (0.75 μmol glucose–C g^{−1} soil g^{−1}), TEA (0.74 μmol glucose–C g^{−1} soil), control (0.53 μmol glucose–C g^{−1} soil), and COM (0.40 μmol glucose–C g^{−1} soil). SAMP effect showed significant differences between the first layer (0–2 mm) of soil rhizosphere (0.92 μmol glucose–C g^{−1} soil), and the two following layers (2–4 and 4–6 mm) having RGU values of 0.48 and 0.57 μmol glucose–C g^{−1} soil, respectively.

Table 4
Percent (normalized to the organic carbon content in initial organic materials) of sum of organic compounds observed in GC–MS chromatograms of sequential extracts, as structurally unbound (SU), weakly-bound (WB), and strongly-bound molecules (SB), and of solid residue remaining after extractions

Organic materials	Compost	Tea	HoDOM	HiDOM	F value
SU Organic fraction	17.33 (±1.02) b B	33.97 (±0.88) a A	6.64 (±0.94) b C	7.69 (±0.78) a C	583***
WB Aqueous fraction	4.51 (±0.66) c B	1.39 (±0.38) c C	24.14 (±0.75) a A	0.57 (±0.12) b C	1280***
WB Organic Fraction	43.14 (±1.15) a A	6.28 (±0.42) b B	0.87 (±0.11) c C	0.44 (±0.08) b C	3335***
SB Aqueous fraction	0.00 d B	0.43±0.10 c A	0.00 c B	0.00 b B	52***
SB Organic Fraction	6.72 (±0.96) c A	0.37 (±0.18) c B	0.18 (±0.09) c B	0.00 b B	133***
Solid residue	17.64 (±0.79) b A	0.99 (±0.23) c B	0.14 (±0.03) c B	0.00 b B	1325***
F value	1002***	2672***	1125***	267***	

ANOVA significant differences are indicated by F values (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$) for comparisons between rows and columns. Data followed by the same minor letter on each column or by the same capital letter on each row are not statistically different from each other (Tukey's test, $P < 0.05$).

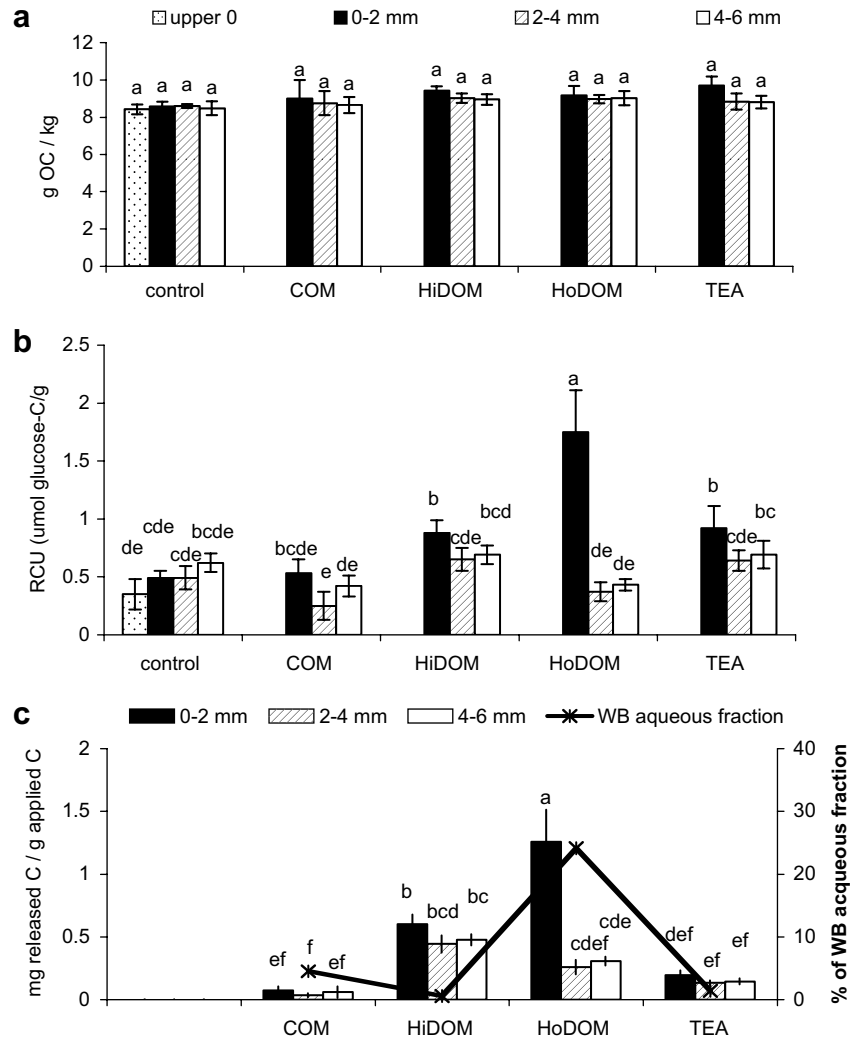


Fig. 3. Total and available amounts of organic carbon (means $\pm$ standard deviations, $n=3$) in the upper compartment of the control rhizobox and in the first three rhizosphere layers (0–2, 2–4, and 4–6 mm) of each studied treatment. Results of Tukey's test for comparison of means for the TREAT*SAMP effect are reported as minor letters above each bar. (a) Total organic carbon content (TOC) as measured with the potassium dichromate oxidation method; (b) available carbon estimated by the response of *P. fluorescens* pUCD 607 and expressed in terms of relative glucose units (RGU), which are μmol of glucose equivalents per gram of soil; (c) mg of released C per gram of applied C, calculated as the ratio between the amount of dissolved C applied in the upper compartments of the rhizoboxes and the RCU measured in the lower rhizosphere layers. The content of WB aqueous fraction is reported as well for each fertilizer.

In HoDOM, HiDOM and TEA treatments, a significant difference between the first and the following rhizosphere layers was found (TREAT*SAMP effect), but not in control and COM treatments. This difference among layers reflected a larger deposition of bioavailable compounds in the layer adjacent to roots.

In control, where no organic material was added to the upper rhizobox compartment, no significant effects on carbon deposition were measured. In fact, biosensor response, in terms of RGU, was similar in the bulk and in the rhizosphere soil samples. Similarly, no significant differences were found for the COM treatment between the three rhizosphere layers. Again, the RGU values were not significantly larger than those obtained for the bulk soil of control (TREAT*SAMP effect).

The HiDOM, HoDOM and TEA treatments showed a similar biosensor response. The first rhizosphere layer showed a significantly larger response than the following two layers, which were not statistically different between each other. The C deposition was also larger in the first layer of the above treatments, than in the bulk control soil. The largest RGU value ($9.73 \mu\text{mol glucose-C g}^{-1} \text{ soil}$) was obtained for the first rhizobox layer amended with HoDOM. It

was ascertained that this value was not due to an outlier since the variance for this sample (0.129, 2 degrees of freedom) was lower than the pooled variance (0.598, 46 degrees of freedom).

In order to have a better comparison among different treatments, data were also normalized to the amount of applied dissolved carbon, and thus expressed in terms of mg of released C per gram of applied C (Fig. 3c). By this approach, it was possible to overcome the slight differences of applied C among treatments (Table 2) and to estimate the ratio between applied and deposited C.

As for RGU data, also for ratio data the main effects TREAT, SAMP and the interaction TREAT*SAMP were significant to ANOVA ($P < 0.001$). For the TREAT effect, HoDOM and HiDOM (respectively, 0.61 and 0.51 mg released C per gram applied C) had significantly larger ratios than TEA and COM treatments (0.15 and 0.06 mg released C per gram applied C). SAMP effect showed the same significance as that found for RGU values, with the 0–2 mm layer (0.53 mg released C per gram applied C) significantly larger than the 2–4 mm and 4–6 mm layers (respectively, 0.25 and 0.22 mg released C per gram applied C). Tukey's test for comparison of means (Fig. 3c) among ratio data showed a higher level of stratifi-

cation than RGU (Fig. 3b). The 0–2 mm layer of HoDOM treatment had still the largest value, followed by the HiDOM treatment that had layer values larger than the TEA and COM treatments. The layers of the latter showed no significant differences among them.

The amount of organic compounds in the WB aqueous fraction for each added material were also reported on a secondary axis of Fig. 3b in order to allow a comparison between the ratio between released and added C, and the most available fraction of C in the organic materials. The HoDOM had the largest WB aqueous fraction and also stimulated the largest release of C in the first rhizosphere layer.

3.4. Correlations between biosensor and TOC values

Correlations between biosensor and TOC values were assessed either separately for each treatment or by pooling all data together, regardless of treatment type. Correlations using the pooled data was negligible ($R^2=0.09$, 46 degrees of freedom), whereas, when the correlations were assessed within each treatment, larger coefficients were found for the TEA treatment only ($R^2=0.78$, 7 degrees of freedom, $P<0.001$). This confirms that the assessment of rhizosphere C deposition by the *P. fluorescens* pUCD607 biosensor is sensitive to changes in the amount of bioavailable-C and, thus, more adequate than TOC measurements.

4. Discussion

We evaluated the rhizosphere C deposition in maize plants after soil treatment with different organic materials, by applying the rhizobox system developed by Wenzel et al. (2001). This system allowed the amendments of organic matter in the upper plant–roots–soil compartment and the evaluation of their effects on root exudation in the untreated lower compartment. Since the lower compartment is adjacent to the compartment containing only roots, the measured effects are restricted to plant roots activity. The 7 μm nylon membrane used in rhizoboxes prevented the penetration of roots hair into the lower compartment, and, thus, only the effect of root exudates composition was evaluated. These exudates, being labile substrates for fast growing heterotrophic soil microorganisms, induce a general flux of available C compounds which characterize the soil rhizosphere activity.

In our experiments, we followed the carbon changes of rhizosphere soil in such a controlled system by a biosensor assay method. The biosensor response was larger in the untreated rhizosphere compartments than in the control bulk soil. Comparing the sensitive biosensor results with those obtained by TOC analyses, it became clear that TOC determination fails to detect the significant changes occurring in rhizosphere bioavailable compounds. This finding seems to be in contrast with previous results on the comparison between *P. fluorescens* pUCD 607 response and TOC values for exudates of *Plantago lanceolata* plants exposed to different concentrations of Paraquat (Porteous et al., 2000). However, such previous findings were based on exudates collected from plants growing in a carbon-free artificial microcosm containing synthetic beads instead of soil. The only carbon source in that system was represented by plant root exudates, thereby justifying the high correlations between TOC and biosensor results. Conversely, in real non-sterile soil systems, the additional presence of soil organic C increases the difficulty of accurately evaluating the changes in plant C depositions by the less sensitive TOC method, especially during the early period of plant development. In such real conditions, we showed that the biosensor method, combined with the rhizobox system, successfully detected a significant root-derived deposition of readily bioavailable-C compounds in the soil rhizosphere following treatment with organic amendments.

Through the expression of data in terms of RGU (relative glucose–C units) and calculation of ratios between the amount of released and applied C, it was possible to estimate the partition of organic C applied in cultured soils. According to our estimates, maize plants grown in rhizobox systems released around $1 \mu\text{mol glucose-C g}^{-1} \text{ soil g}^{-1}$, in their first weeks of life. When this amount was related to the quantity of dissolved C in the organic materials, we showed that approximately one part per thousand of C applied in dissolved forms is released in the rhizosphere as available C.

After amendment of solid bulk compost on the upper rhizobox compartment, the amount of bioavailable-C in the rhizosphere was not statistically different from the control treatment. Conversely, the increased amount of bioavailable carbon substrates found in rhizobox systems treated with soluble compost fractions is in line with their chemical characteristics. In fact, the predominance of SU and WB fractions (Table 4) indicate that these materials are rich in labile compounds, which may serve as readily-available C and energy source to the microbial biomass. Moreover, the most active soluble extract from compost (HoDOM) (Fig. 2) had also the largest content of potentially bioavailable organic compounds represented by the WB (weakly-bound to the structural matrix) aqueous fraction (Table 4). Similar observations were made by Swinnen et al. (1995) and Caravaca et al. (2002), who reported an increased rhizodeposition in plants treated with organo-fertilizers or composted materials. The larger increase in rhizodeposition in the presence of soluble compost extracts, as compared to the bulk compost, implies that such fractions are far more active than the bulk compost, most probably because of the higher availability of the bioactive organic compounds contained in the soluble fractions. At similar levels of dissolved organic carbon, soluble extracts had an effect on plant physiology (at least in terms of rhizodeposition) that was more prominent than that of bulk compost from which they were extracted. This conclusion of our work may have an important consequence on the management of soil fertility and in the agricultural use of composted materials.

The successful combined application of rhizobox and gene reporter shown here might be extended to applications of other biosensors (e.g., gene reporters for nitrogen or phosphate) (Kragelund et al., 1995). Further research should also be conducted in order to investigate the biosensor response to specific chemical compounds (e.g., sugars, organic acids, phenols and amino acids) present in root exudates, and to strengthen the correlations between exudation processes and specific properties and chemical nature of organic amendments.

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