

Effect of a Compost and Its Water-Soluble Fractions on Key Enzymes of Nitrogen Metabolism in Maize Seedlings

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The growing concern on long-term productivity of agroecosystems has emphasized the need to develop management strategies to maintain and protect soil resources, particularly soil organic matter (SOM). Among these, the composting process allows both recycling of the increasing amount of organic waste materials and restoration of the content of organic matter in soil. A sequential chemical fractionation into structurally unbound (SU), weakly bound (WB) and strongly bound (SB) compounds was applied to a bulk compost, and its soluble fractions were extracted in water, either after oxidation of compost suspension with an oxygen flux (TEA), or without oxidation but separated into hydrophilic (HiDOM) and hydrophobic (HoDOM) components. The ratio of hydrophilic over hydrophobic compounds decreased in the order HiDOM > TEA > compost > HoDOM, while TEA and compost showed the largest content of SU and WB components, respectively. Such chemically characterized bulk compost and fractions were tested on maize seedlings grown in sand and in hydroponic conditions, and the effects on plant growth and nitrogen metabolism were measured. The structurally complex bulk compost and the hydrophobic HoDOM fraction negatively affected plant growth, whereas the hydrophilic and less-structured fractions (HiDOM and TEA) showed large positive effects on both growth and enzymatic activities of plants. These results suggest that composted organic matter can become useful to stimulate plant growth if the content of potentially bioavailable hydrophilic and poorly structured components is large. These components may be progressively separated from the compost matrix and contribute to the dynamics of natural organic matter in soil.

KEYWORDS: Maize; compost; water-soluble fractions; molecular fractionation; NMR; nitrogen; enzyme activity

INTRODUCTION

Naturally humified organic matter is known to improve physical and chemical properties of soil and be a source and sink for various nutrients (1). Nitrogen (N) is one of the most important and limiting factors for plant and crop yields (2). In fact, the sustainable increase of crop productivity requires the maximization of plant N availability and suppression of nitrate pollution from soil to groundwater (3). Humified organic matter exerts important direct influences on plant growth. Humic substances (HS) affect morphological, physiological and biochemical processes on seed germination, cell differentiation, ion uptake and overall plant metabolism (4, 5). As for the effects of HS on plant nitrate uptake, it has been suggested that humic acids isolated from earthworm compost induced the maize H⁺-ATPase activity by enhancing the content of the enzyme (6). More

recently, low molecular size HS from earthworm feces were found to stimulate the nitrate uptake in maize at a transcriptional level, possibly through the upregulation of mRNA synthesis of the major H⁺-ATPase form, Mha2, and induction of transcription of the nitrate ZmNrt2.1 transporter (7).

Since both the quantity and quality of soil humic matter (SHM) has been showed to contribute to plant growth (5), the long-term productivity of agroecosystems requires development of management strategies to maintain and protect soil resources, particularly soil organic matter (SOM) content. This is specifically important for rain-fed agricultural practices in the Mediterranean basin in which high summer temperatures promote the enhanced mineralization of SOM (8), and rainfall scarcity does not allow adequate vegetal development (9).

To counteract SOM losses, applications of manure or other organic amendments have been progressively introduced into agricultural practices and have been shown to be beneficial to soil fertility and crop yields (10). The application of organic

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amendments is useful also in recycling the increasing amount of the organic fractions of selected urban waste, green-woody material, residues from agroindustry, and sewage sludge waste. Compost consists of a controlled biological transformation of organic matter mediated by aerobic microorganisms that leads to a relatively biostable end-product substantially reduced in quantity as compared to the initial waste. The compounds obtained from a composting process are similar to those found in HS (11, 12). The similarity between HS and compost depends on the extent of decomposition undergone by compost. Moreover, the greater the degree of humification in compost, the larger is its agricultural value (12).

The aim of this work was to study the influence exerted by molecularly characterized compost and its water-soluble fractions on growth, root morphology and nitrogen metabolism of maize seedlings. In particular, assuming that plant enzyme activities reveal the effects of organic matter at the plant biochemistry level, we measured activities of nitrate reductase (NR), nitrite reductase (NiR), glutamine synthetase (GS), glutamate synthase (GOGAT), and aspartate amino transferase (AspAT), which are key enzymes in the ammonium assimilation.

The experiments were conducted under sand and hydroponics rather than soil condition first to reduce the studied system, and second because of the interest of sandy soils as these soils are well diffused in our region (Venezia and Rovigo provinces, North-East Italy).

MATERIALS AND METHODS

Compost and Its Water-Soluble Fractions. The compost used here 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 agroindustry (15%). Composting was carried out under aerobic conditions (13), and involved a thermophilic phase of approximately 28 days, during which the feedstock was daily turned, followed by a curing phase of approximately 3 additional months in piles. A dissolved organic matter (DOM) fraction was extracted from the bulk compost as described earlier (14). Briefly, the 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 μm glass microfiber filter and a 0.45 μm membrane filter to obtain the total DOM extract. The pH of the DOM extract was around 8.2.

The DOM extract was acidified to pH 2 with 0.1 N HCl and passed through Amberlite XAD-8 and XAD-4 resins. The organic fraction retained by the XAD-8 resin was eluted with 0.1 M NaOH, passed through an AG MP-50 strongly acidic 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. The 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) (15). The HoDOM and HiDOM fractions had an organic C content of 499 and 523 mg g⁻¹, respectively.

An aerated compost extract (TEA) was obtained by suspending compost in deionized water (1:5, w/v) and stirred for 10 days at 20 °C. The aerobic conditions were ensured by equipping the fermentation vessel 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 bubbling of air through the suspension. After 10 days, the compost suspension was filtered through a cellulose filter paper and stored at 4 °C. The organic C concentration of the TEA sample was 1.19 g L⁻¹. A fraction of TEA sample was freeze-dried to obtain a solid sample for further chemical characterization.

CPMAS-NMR Spectroscopy of Organic Materials. Cross-polarization magic-angle-spinning carbon-13 nuclear magnetic resonance

Table 1. Relative Distribution (%) of Signal Areas over Chemical Shift Regions (ppm) in CPMAS-¹³C NMR Spectra of Compost and Its Water-Soluble Fractions (Standard Deviation in Parentheses)

sample	200–160	160–110	110–60	60–50	50–0	HI/HB ^a
compost	10.1 (0.69)	14.7 (0.89)	33.3 (3.40)	8.5 (0.84)	33.4 (2.54)	1.08 (0.09)
TEA	23.6 (1.61)	13.3 (0.9)	23.1 (1.61)	9.0 (0.81)	31.0 (2.72)	1.26 (0.10)
HoDOM	14.0 (0.71)	19.3 (1.18)	19.5 (0.65)	12.6 (0.16)	34.6 (1.51)	0.85 (0.07)
HiDOM	14.0 (0.7)	9.1 (0.5)	36.8 (1.8)	9.8 (0.4)	30.3 (3.1)	1.54 (0.10)

^aHydrophilic carbons/hydrophobic carbons = [(50–110) + (160–200)]/[(0–50) + (110–160)].

spectra (CPMAS-¹³C NMR) were obtained on a Bruker AV300 instrument operating on carbon 13. The rotor spin rate was set at 13000 Hz. A contact time of 1 ms, a recycle time of 1.5 s and an acquisition time of 20 μs were used. All experiments were conducted with CP pulse sequence with the ¹H-RAMP pulse sequence to take into account the nonhomogeneity of the Hartmann–Hahn condition at high rotor spin rates. CPMAS-NMR spectra were done on triplicates for each sample. The different chemical-shift regions of the spectra were automatically integrated, and a ratio of hydrophilic (HI) over hydrophobic (HB) carbons (HI/HB) was obtained for each material (Table 1).

Chemical Fractionation of Organic Materials. *Structurally Unbound Components (SU).* An aliquot of each compost sample (350 mg for compost, TEA and HoDOM, and 150 mg for HiDOM) was oven-dried at 40 °C for 1 h. Structurally unbound compounds were extracted using a mixture of dichloromethane and methanol (2:1, v/v) for 2 h at room temperature. The sample was centrifuged for 25 min at 12000g, and the supernatant was removed and preserved. The residue was further re-extracted with a mixture of dichloromethane and methanol (2:1, v/v) for 12 h at room temperature. After centrifugation, the supernatants were combined and rotary evaporated to complete dryness. The dry extract was redissolved in 20 mL of dichloromethane/isopropanol (2:1, v/v), and 1 mL of this solution was adsorbed on an aminopropyl-bond solid-phase cartridge column (Strata NH₂ 500 mg (3 mL)⁻¹, Phenomenex) previously conditioned with hexane. The column was first eluted with 8 mL of dichloromethane/isopropanol (2:1), in order to obtain a neutral subfraction, and then with 8 mL of 2% (v/v) acetic acid in diethyl ether to obtain an acid subfraction (16). Both neutral and acid subfractions were derivatized and analyzed by GC–MS.

Weakly-Bound Components (WB). After the extraction of SU components, the air-dried residue was trans-esterified with 15 mL of 12% BF₃–CH₃OH complex at 90 °C for 12 h in a polyethylene bottle, in order to extract the weakly bound compounds (11). After centrifugation (15 min, at 12000g), the supernatant was recovered, and the residue was treated twice more with 10 mL of 12% BF₃–CH₃OH for 12 h. The combined supernatants were treated with an excess of water, in order to destroy the BF₃–CH₃OH complex, and then liquid–liquid extracted with chloroform. The total extract in chloroform (WB organic fraction) was dehydrated with anhydrous Na₂SO₄ and rotary-evaporated. The resulting dry extract was dissolved in 5 mL of dichloromethane/isopropanol (2:1, v/v), and 1 mL of this solution was loaded into a NH₂-bonded solid-phase cartridge column, in order to separate the neutral and the acid subfractions, as described above. Both neutral and acid subfractions were derivatized and analyzed by GC–MS. The extract in water (WB aqueous fraction) was dialyzed in dialysis tubes (3500 Da cutoff) against distilled water until conductivity was as low as 2 μS , and freeze-dried.

Strongly-Bound Components (SB). The air-dried residue from extraction of WB components was suspended in 1 M KOH in CH₃OH and refluxed for 1 h at 70 °C (16). After cooling, the reaction mixture was centrifuged (10 min, at 5000g) and the supernatant removed. The residue was extracted twice with 15 mL of CH₃OH and twice with 15 mL of dichloromethane. After each step, the suspensions were centrifuged (10 min, 5000g) and all supernatants combined. These were acidified to pH 2 using concentrated HCl (37%) and, after addition of water, extracted with dichloromethane in a separation funnel. The dichloromethane solution

was dehydrated with anhydrous Na_2SO_4 and filtered on a glass microfiber filter (Whatman) to remove residual salts. The final extract (SB organic fraction) was derivatized and analyzed by GC-MS. The water extract (SB aqueous fraction) was dialyzed in dialysis tubes (3500 Da cutoff) against distilled water until conductivity was as low as $2 \mu\text{S}$, and freeze-dried.

Derivatization and GC-MS Analysis. Each organic fraction was first methylated, by refluxing for 30 min at 60°C with 5 mL of MeOH and 0.5 mL of acetyl chloride. The solvent was evaporated to dryness under a gentle stream of nitrogen, and the resulting residue was silylated with 100 μL of BSTFA containing 1% TMCS, for 1 h at 70°C , added to 400 μL of hexane, and analyzed by GC-MS. The GC-MS analyses were conducted on a PerkinElmer Autosystem XL gas chromatograph, equipped with a PerkinElmer Turbomass Gold mass spectrometer. The injector was held at a constant temperature of 250°C , and a fused-silica capillary column (Restek Rtx-5MS, 30 m length \times 0.25 mm i.d. \times 0.25 μm film thickness) was used for analytical separation. Helium was the carrier gas with a flow rate of 1.6 mL min^{-1} . The oven was temperature-programmed from 100 to 300°C , at a rate of 4°C min^{-1} , and held there for 20 min. The mass spectrometer operated in full scan mode in the range of m/z 50–600 and by an electron impact ionization energy of 70 eV with a cycle time of 1.0 s. Due to the large variety of detected compounds with different chromatographic response, the quantitative analysis was conducted using calibration curves of different external standards from Aldrich: tridecanoic acid, octadecanol, 16-hydroxy hexadecanoic acid, docosandioic acid, beta-sitosterol, and cinnamic acid. Tridecanoic acid was also used as internal standard to evaluate derivatization yields and steadiness of chromatographic response.

Plant Material and Growth Conditions. Maize seeds (*Zea mays* L. var. DK 585) were soaked in running water for one night and germinated in the dark at 25°C for 60 h on filter paper wetted with 1 mM CaSO_4 (5). Two kinds of experiments were conducted: the first in a solid substrate (sand), and the second in hydroponic conditions. For the solid substrate experiment, 4 days old seedlings were moved to pots containing sand mixed with compost (1.2, 3.0, 6.0%, w/w). The quantities, corresponding to approximately 50, 100, and 200 t ha^{-1} , respectively, were chosen because they are typical application rates in agriculture (17). To the pots was added a modified Hoagland solution containing (μM) KH_2PO_4 (40), $\text{Ca}(\text{NO}_3)_2$ (200), KNO_3 (200), MgSO_4 (200), FeNaEDTA (10), H_3BO_3 (4.6), $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ (0.036), $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ (0.9), ZnCl_2 (0.09), $\text{NaMoO}_4 \cdot 2\text{H}_2\text{O}$ (0.01) (18). At the 14th day, plants were collected and analyzed for several growth parameters: length and width of the first leaf, diameter of the coleoptiles, length and fresh weight of shoot and root, and number of leaves per plant.

In order to test the effects of compost and its freeze-dried water-soluble fractions at the physiological level the second experiment was conducted under hydroponic conditions. Hydroponics is a plant culture technique which enables plant growth in a nutrient solution with the mechanical support of inert substrata. For the experiment, once germinated, seedlings were moved into hydroponic pots containing the same nutrient solution as the pot experiment. Sterile air was bubbled in the solution to avoid anoxic conditions. At day 12, plants were treated with a 0 (control), 1.2, 3 and 6% (w/v) compost, corresponding to 0.225, 0.564, and $1.13 \text{ g (100 mL)}^{-1}$ of organic carbon, respectively. The water-soluble fractions (TEA, HiDOM and HoDOM) were supplied at the concentration of 0 (control), 0.5, 1.0, and 5 mg CL^{-1} , for 48 h. The time and concentrations of treatments were chosen based on previous experiments (19) which showed that the maximum effect of a humic substances on the stimulation of growth parameters was between 24 and 72 h. At day 14, plants were analyzed for main growth parameters. In both experiments, plants were grown in a climate chamber for 14 days at 14 h light at 27°C and 60% relative humidity, and 10 h dark at 21°C and 80% relative humidity. Five replicates of each treatment were set up.

Scanning Electron Microscopy (SEM). Scanning electron microscopy (Stereoscan 250; Cambridge Instruments, Cambridge, U.K.) was performed on roots of maize seedlings grown in hydroponics and treated with compost, HiDOM, HoDOM, and TEA. Roots were prepared by treating the 0–20 mm region behind the root tips with glutaraldehyde (6%) in 0.1 M cacodylate buffer (pH 6.9). Samples were then postfixed in the same buffer solution with OsO_4 (1%) for 2 h at 4°C , dehydrated in graded acetone series, dried at the critical point, coated with gold and palladium and examined with SEM operating at 25 KV.

Enzyme Extraction and Assay Conditions. At the end of the maize growth period, leaves (1 g) were harvested immersed in liquid N_2 . The enzymes were solubilized from leaves by manually crushing tissues in a mortar supplemented with a 100 mM HEPES-NaOH solution at pH 7.5 containing 5 mM MgCl_2 , and 1 mM dithiothreitol (DTT). The ratio of plant material to mixture solution was 1:3. The extract was filtered through two layers of muslin and clarified by centrifugation at $20000g$ for 15 min. The supernatant was used for enzymatic analysis. All steps were carefully performed at 4°C . Nitrate reductase (NR, EC 1.7.1.1) activity was assayed (20) and expressed as unit g^{-1} fresh weight. One unit corresponds to the production of $1 \mu\text{mol of NO}_2^-$ per minute at 25°C . Nitrite reductase (NiR, EC 1.7.1.4) activity was determined on the basis of the drop in NO_2^- concentration in the reaction medium (21). After incubation at 30°C for 30 min, the NO_2^- content was determined colorimetrically at A_{540} and the activity was expressed as unit g^{-1} fresh weight. One unit corresponds to the consumption of $1 \mu\text{mol of NO}_2^-$ per minute at 25°C . To evaluate the glutamine synthetase (GS, EC 6.3.1.2) activity, the mixture for the assay contained 90 mM imidazole-HCl (pH 7.0), 60 mM hydroxylamine (neutralized), 20 mM Na_2KAsO_4 , 3 mM MnCl_2 , 0.4 mM ADP, 120 mM glutamine and the appropriate amount of enzyme extract. The assay was performed in a final volume of 750 μL . The enzymatic reaction was developed for 15 min at 37°C . The γ -glutamyl hydroxamate (gh) was colorimetrically determined by addition of 250 μL of a mixture (1:1:1) of 10% (w/v) $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ in 0.2 M HCl, 24% (w/v) trichloroacetic acid and 50% (w/v) HCl. The optical density was recorded at A_{540} (22). The activity was expressed as unit g^{-1} fresh weight. One unit corresponds to the production of $1 \mu\text{mol of gh}$ per minute at 37°C . NADH dependent glutamate synthase (NADH-GOGAT, EC 1.4.1.14) assay contained 25 mM HEPES-NaOH (pH 7.5), 2 mM L-glutamine, 1 mM α -ketoglutaric acid, 0.1 mM β -NADH, 1 mM Na_2EDTA and 100 μL of enzyme extract in a final volume of 1.1 mL. GOGAT was assayed spectrophotometrically by monitoring β -NADH oxidation at A_{340} . The activity was expressed as unit g^{-1} fresh weight. One unit corresponds to the oxidation of $1 \mu\text{mol of } \beta\text{-NADH}$ per minute at 37°C . The activity of aspartate aminotransferase (AspAT, EC 2.6.1.1) was measured spectrophotometrically by monitoring β -NADH oxidation at A_{340} at 30°C . The assay medium (final volume 2.4 mL) contained 100 mM Tris-HCl pH 7.8, 240 mM L-aspartate (Asp), 0.11 mM pyridoxal phosphate, 0.16 mM NADH, 0.93 kU malate dehydrogenase (MDH), 0.42 kU lactate dehydrogenase (LDH), 12 mM 2-oxoglutarate and 200 μL of enzyme extract. The activity was expressed as unit g^{-1} fresh weight. One unit corresponds to the oxidation of $1 \mu\text{mol of } \beta\text{-NADH}$ per minute at 37°C .

Determination of Protein Content. For the extraction of soluble protein, frozen foliar (0.5 g) were ground in liquid N_2 , vortexed with the extraction buffer (100 mM Tris HCl pH 7.5, 1 mM Na_2EDTA , 5 mM DTT) and centrifuged at $14000g$. The supernatants were mixed with 10%

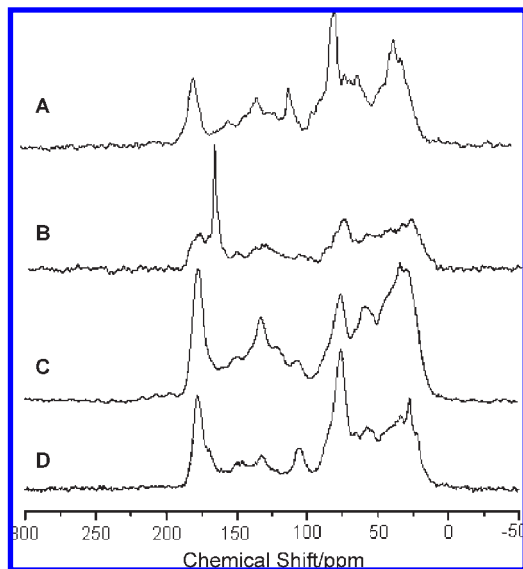


Figure 1. CPMAS ^{13}C NMR spectra of (A) compost; (B) TEA; (C) HoDOM; (D) HiDOM.

(w/v) trichloroacetic acid (TCA) and centrifuged. The pellets obtained were then resuspended in 0.1 N NaOH. The amount of total proteins was determined by the Bradford method using bovine serum albumin as a standard. The results were expressed as $\mu\text{g protein g}^{-1}$ fresh tissue.

Statistical Analysis. Sums of different organic compounds obtained in sequential extracts of different organic materials were analyzed first by the analysis of variance (ANOVA) using the XLSTAT program, and, then, the Duncan test was used to determine the difference among means, with $P < 0.05$ as statistical significance. All biochemical data were the means of five replicates, and the standard deviations did not exceed 5%. The results obtained were processed statistically with the Student–Newman–Keuls test (23).

RESULTS AND DISCUSSION

Chemical Characteristics of Organic Materials. Total N content was 2.9, 3.6, 4.7, and 3.8 g kg^{-1} in compost, TEA, HiDOM, and HoDOM respectively, whereas total C quantity was 44.8, 38.1, 40.7 and 36.6% in compost, TEA, HoDOM, and HiDOM, respectively. The substantial decrease in N and C during composting can be assigned to the substantial degradation of organic substances, particularly the most labile soluble organic moieties

(carbohydrates, amino sugars and low molecular weight organic acids) which characterize both HiDOM and TEA. In contrast, HoDOM, which was rich in aromatic moieties and consequently resistant to microbial degradation, showed a large content of both N and C. This trend has been earlier observed (13).

The CPMAS-NMR spectra of different compost materials are shown in **Figure 1**, while integrations of spectral intervals are reported in **Table 1**. The carbon distributions in the different intervals were used to define the HI/HB ratio, an index of hydrophilicity of complex organic materials (24). This ratio indicated, as expected, that HiDOM was the most hydrophilic fraction, whereas HoDOM was the most hydrophobic. HiDOM showed, in fact, the largest relative content of carboxyl groups and C–N carbon, presumably in proteinaceous material. The bulk compost and TEA had similar HI/HB ratio, though the former appeared somewhat more hydrophilic.

The quantitative sums of compounds extracted from the different compost and compost-derived samples are reported in **Table 2**. The structurally unbound (SU) compounds (mainly lipids soluble in organic solvents) were relatively larger in TEA

Table 2. 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 Solid Residues Remaining after Extractions^a

organic materials	compost	TEA	HoDOM	HiDOM	F
SU					
organic fraction	17.33 ± 1.02 bB	33.97 ± 0.88 aA	6.64 ± 0.94 bC	7.69 ± 0.78 aC	583***
WB					
aqueous fraction	4.51 ± 0.66 cB	1.39 ± 0.38 cC	24.14 ± 0.75 aA	0.57 ± 0.12 bC	1280***
organic fraction	43.14 ± 1.15 aA	6.28 ± 0.42 bB	0.87 ± 0.11 cC	0.44 ± 0.08 bC	3335***
SB					
aqueous fraction	0.00 dB	0.43 ± 0.10 cA	0.00 cB	0.00 bB	52***
organic fraction	6.72 ± 0.96 cA	0.37 ± 0.18 cB	0.18 ± 0.09 cB	0.00 bB	133***
solid residue	17.64 ± 0.79 bA	0.99 ± 0.23 cB	0.14 ± 0.03 cB	0.00 bB	1325***
F	1002***	2672***	1125***	267***	

^a 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 lowercase letter in each column or by the same uppercase letter in each row are not statistically different from each other (Tukey's test, $P < 0.05$).

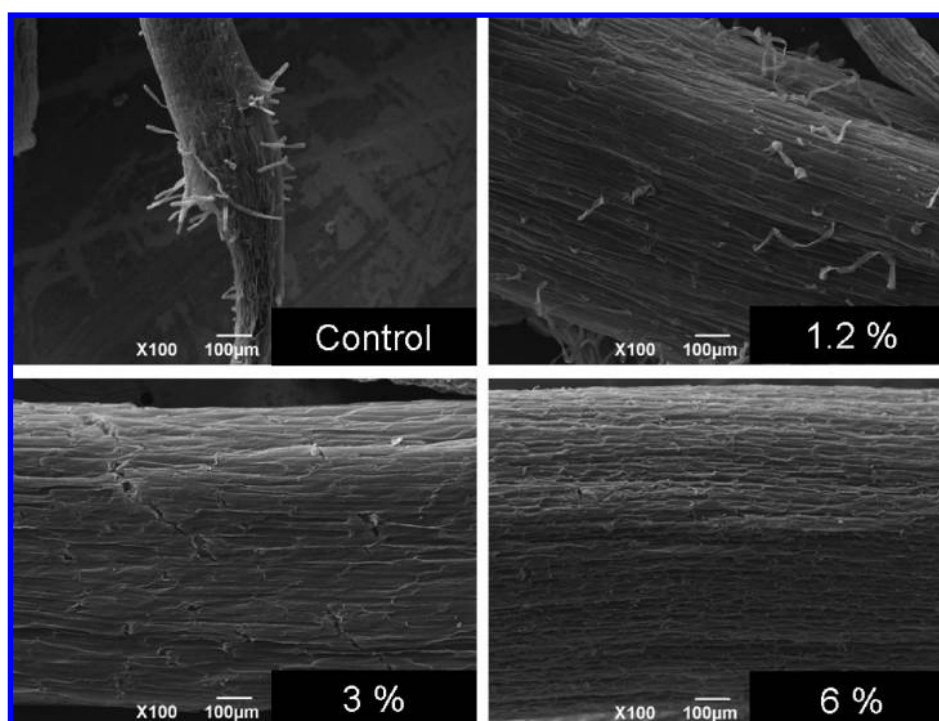


Figure 2. SEM micrographs of the 0–20 mm region behind the root tips of maize seedlings surface grown in Hoagland modified nutrient solution (control) and treated for the last 2 days with compost at 1.20, 3 and 6% (w/v).

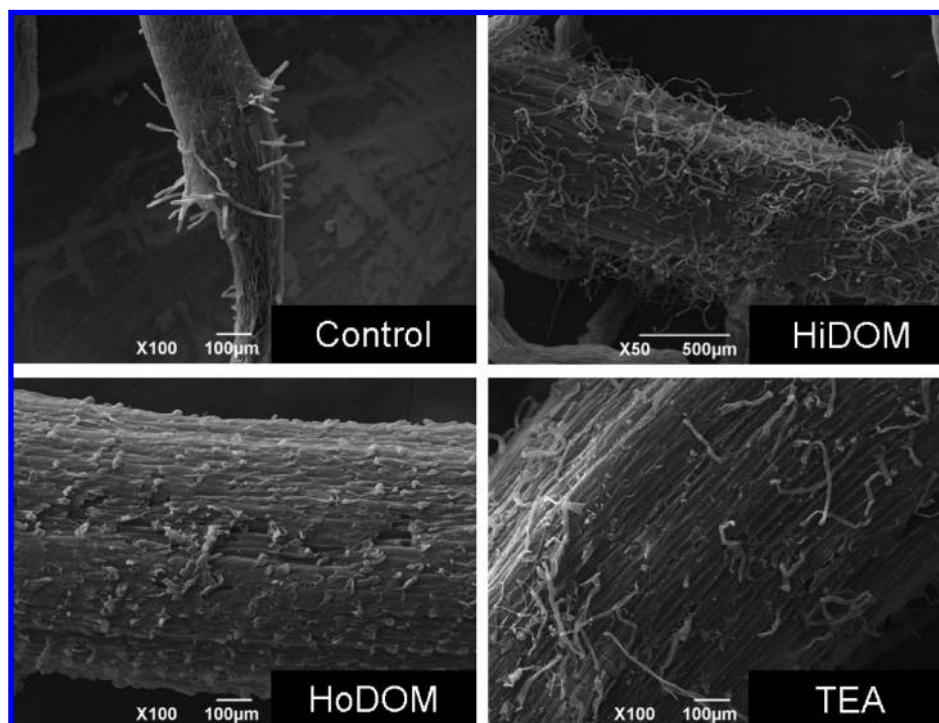


Figure 3. SEM micrographs of the 0–20 mm region behind the root tips of maize seedlings surface grown in Hoagland modified nutrient solution (control) and treated for the last 2 days with water extractable fractions stemming from compost HiDOM, HoDOM and TEA at 1 mg C L^{-1} .

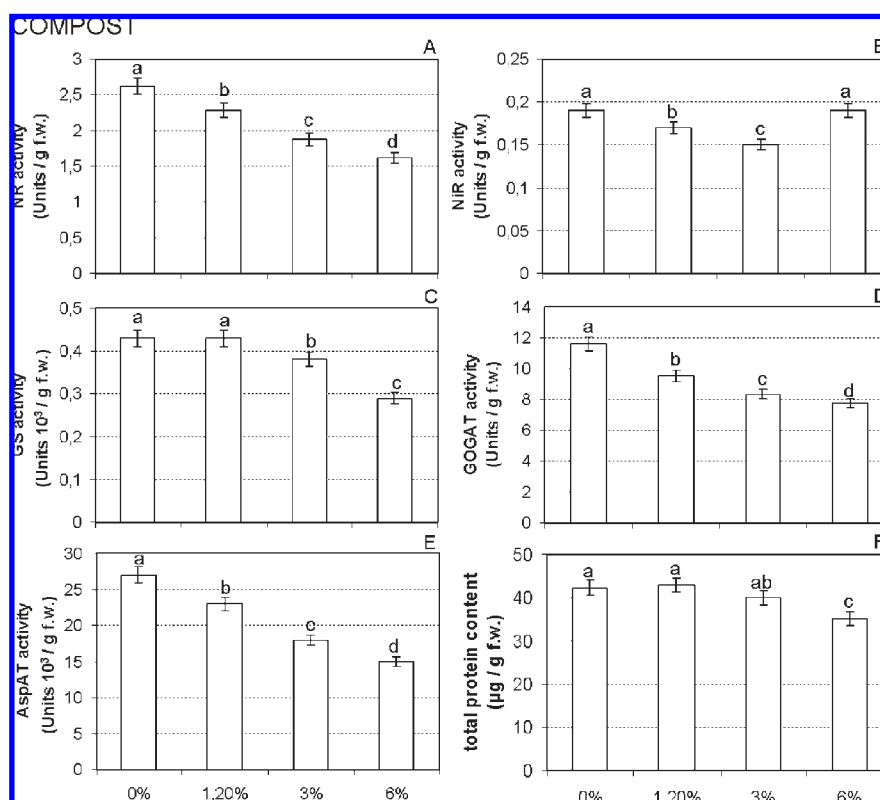


Figure 4. Effect of compost treatment on nitrate reductase (NR) (A), NiR nitrite reductase (NiR) (B), glutamine synthetase (GS) (C), glutamate synthetase NADH-dependent (NADH-GOGAT) (D) and aspartate aminotransferase (AspAT) (E) activities and total protein content (F) of maize leaves. Seedlings were grown for 14 days in a Hoagland modified nutrient solution (0) and treated for the last 2 days with compost (1.20, 3 and 6%). The activities were expressed in unit g^{-1} fresh weight. The protein concentration was expressed in μg of protein g^{-1} fresh weight. Values are the means of three independent determinations (\pm SE). Letters above bars indicates significant differences ($P < 0.05$).

than in the bulk compost, whereas they were similarly lower in both HoDOM and HiDOM. The weakly bound (WB) compounds (soluble only after mild transesterification) were large and

predominantly partitioned in the organic fraction for the bulk compost, and, to a lower extent, for the TEA. Conversely, HoDOM showed a much larger quantity of compounds in the

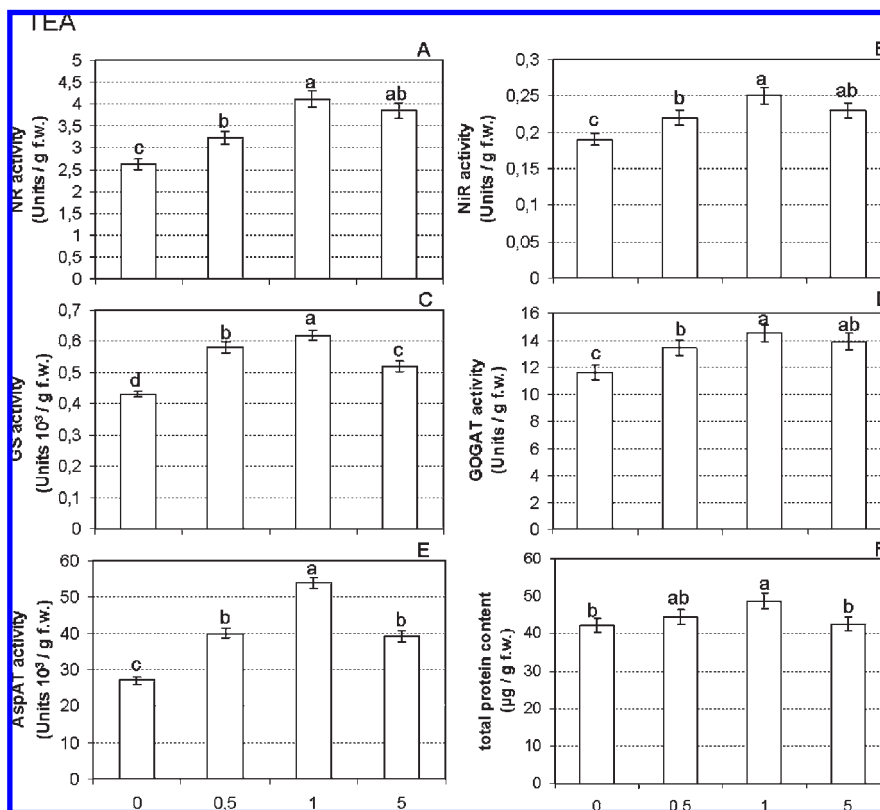


Figure 5. Effect of TEA fraction treatment on nitrate reductase (NR) (A), nitrite reductase (NiR) (B), glutamine synthetase (GS) (C), glutamate synthetase NADH-dependent (NADH-GOGAT) (D) and aspartate aminotransferase (AspAT) (E) activities and total protein content (F) of maize leaves. Seedlings were grown for 14 days in a Hoagland modified nutrient solution (0) and treated for the last 2 days with TEA (0.5, 1, and 5 mg CL⁻¹). The activities were expressed in unit g⁻¹ fresh weight. The protein concentration was expressed in µg of protein g⁻¹ fresh weight. Values are the means of three independent determinations (±SE). Letters above bars indicates significant differences ($P < 0.05$).

aqueous fraction, whereas little material was detected in both the aqueous and organic fraction for HiDOM. Considerable amounts of strongly bound (SB) compounds were further obtained by alkaline hydrolysis for the bulk compost, though they were all detected in the organic fraction. Lower contents of SB compounds (soluble only after methanolic alkaline hydrolysis) were found for TEA but equally distributed in aqueous and organic fractions. The HoDOM produced only very little SB compounds in the organic layer, whereas no such SB compounds were extracted from the HiDOM structure.

The sample fractionation of progressively less chemically available organic compounds, as relative to GC-MS determination, appears to generally correspond to the sample characteristics shown by CPMAS-NMR spectroscopy. HiDOM with the largest hydrophilic properties showed the lowest amount of GC-MS-detected compounds, thus implying a large content of low molecular weight hydrophilic compounds, such as simple carbohydrates and organic acids. Conversely, the most hydrophobic nature of HoDOM is related to the considerable content of both SU and WB extracted from this sample.

The chemical fractionation showed significant differences between the bulk compost and its water-soluble fractions in both their molecular composition and intramolecular interactions. The bulk compost was rich in phenolic compounds, long chain fatty acids, hydroxyl acids and alcohols. Conversely, TEA had a significant amount of oxidized products dominated by dehydroabietic acids (25), that have interesting effects as fungicide (26–29) and beneficial properties to human health (30). In general, TEA and HiDOM mostly contained hydrophilic compounds, while HoDOM had a larger content of hydrophobic compounds. However, TEA was also rich in SU hydrophobic compounds.

Moreover, while compost left a significant percentage of solid residue after the chemical fractionation, water-soluble materials were almost totally separated into the sequential fractions. These results suggest that the compost contained various and heterogeneous molecules which were differently associated and potentially available to a release in water-based solution with different hydrolyzing powers. In fact, the fractions readily soluble in water (HiDOM, HoDOM, and TEA) were significantly further dispersed during relatively mild hydrolysis reactions.

Effects of Compost and Its Fractions on Maize Seedlings.

Morphometric parameters of maize grown in sand mixed with compost were measured. Leaves of seedlings grown with 1.2% compost showed increments with respect to the control both in length and in width; on the contrary high concentrations (3 and 6%) caused decrements ($P < 0.05$). In the case of roots, length was strongly reduced as a function of the concentration. The same behavior was observed for both shoot and root fresh weight. For plants grown in hydroponics, the shoot and root fresh weight were measured. Although treatments lasted only 48 h, significant differences ($P < 0.05$) were observed. Compost in hydroponics caused a strong reduction of shoot fresh weight compared to the control on the order of 24, 30 and 35% for 1.2, 3 and 6% of compost, respectively. On the contrary, treatment with 1.2 and 3% compost determined a root fresh weight increment of 10 and 5%, respectively, while 6% compost caused a reduction of 10%.

Shoot biomass of TEA treated plants was increased with respect to the control. Similar effects were observed at roots. Treatment with HiDOM influenced the fresh weight of shoots in the same way as TEA. In fact, all HiDOM concentrations positively affected shoot fresh weight with increments of 12, 18 and 10%, respectively. Also root fresh weight was enhanced when

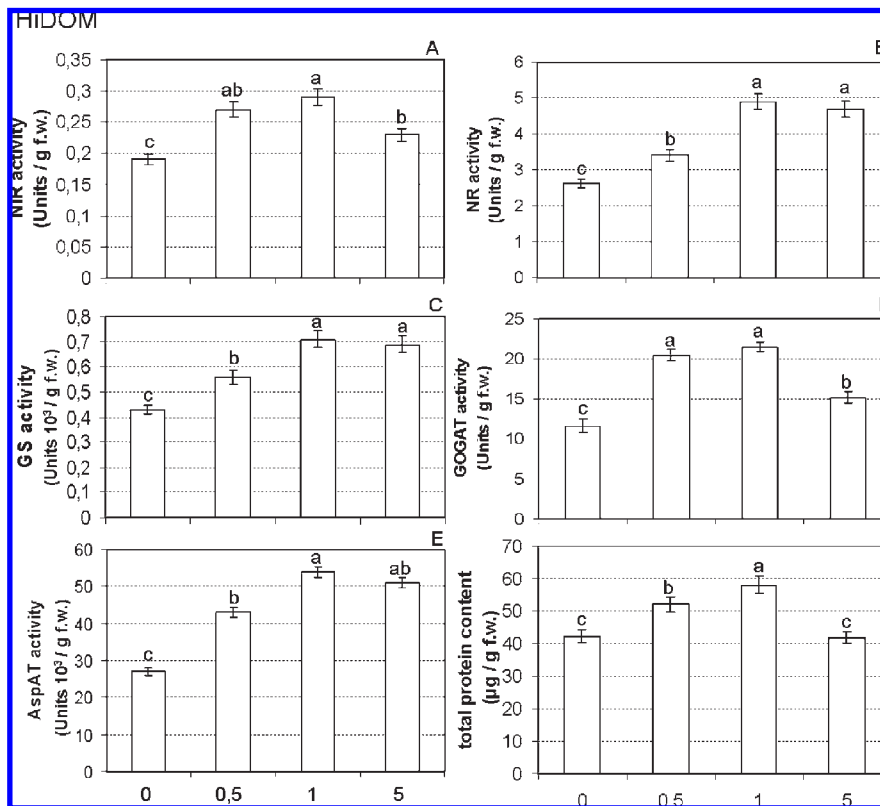


Figure 6. Effect of HiDOM fraction treatment on nitrate reductase (NR) (A), nitrite reductase (NiR) (B), glutamine synthetase (GS) (C), glutamate synthetase NADH-dependent (NADH-GOGAT) (D) and aspartate aminotransferase (AspAT) (E) activities and total protein content (F) of maize leaves. Seedlings were grown for 14 days in a Hoagland modified nutrient solution (0) and treated for the last 2 days with HiDOM (0.5, 1, and 5 mg C L⁻¹). The activities were expressed in unit g⁻¹ fresh weight. The protein concentration was expressed in µg of protein g⁻¹ fresh weight. Values are the means of three independent determinations (±SE). Letters above bars indicates significant differences ($P < 0.05$).

plants were treated with this fraction with increments with respect to the control of 5, 15 and 11%, respectively. On the contrary, HoDOM caused a general decrement in both shoot and root fresh weight.

SEM. The surface of apical root zone of maize grown in hydroponics and treated with compost was different in comparison to the control. High concentrations of compost (3 and 6%) showed no presence of root hair, while 1.2% compost determined a weak root hair presence (Figure 2). Differently from compost, TEA and HiDOM showed important effects on root hair abundance, while HoDOM treatment did not show any presence of root hair (Figure 3).

It is important to remember that the 0–20 mm root region is the dominant zone for nitrate uptake and transport in maize (31). Thus, a large root biomass and amount of root hair is advantageous in plant nutrient uptake (32). In fact, a change in root morphology is strictly related to the presence of soil nutrients (33), and thus contributes to increase the efficiency of both nitrogen uptake and assimilation (34). These results lead us to hypothesize that the hydrophobic organic materials might induce a stress condition through a phytotoxic effect or shortage of nutrient availability, thus causing the reduction of root hair. However, the mode of action of hydrophobic components remains to be elucidated.

Enzyme Activities. Because the compost mixed with sand treatment did not show a positive effect on the morphometric parameters, the enzyme activity was only performed with hydroponic conditions. In hydroponics, compost negatively affected the enzyme activities related to N metabolism. In particular, the reduction of nitrate to nitrite, catalyzed by NR, showed a dose-dependent decrease, as compared to control (Figure 4A). A

similar behavior was observed for NiR, especially when plants were supplied with 1.2 and 3% compost. GS activity, which is responsible for the first step of ammonium organization via glutamine synthesis, was affected by 3 and 6% compost. The production of glutamate and aspartate (GOGAT and AspAT activities, respectively) strongly decreased ($P < 0.05$) in compost treated plants (Figures 4D and 4E). Moreover, both 3 and 6% compost treatments caused a decrease of total protein content in plants (Figure 4F).

As for soluble fractions, TEA allowed great stimulation of all enzyme activities. In particular, 1 mg C L⁻¹ showed the largest percent of increased activities (Figures 5A–F). Similar effects were observed in HiDOM treated plants (Figure 6). On the contrary, when plants were treated with HoDOM, all enzyme activities and total protein content decreased or were not significantly different from control (Figures 7A–F).

Nitrogen is the major limiting factor in plant growth and productivity (35). Because of this, nitrogen use efficiency and activity of enzymes in catalyzing nitrogen uptake and assimilation are essential for plant adaptation to environmental conditions. In particular, NR leaf activity represents direct information on the efficiency of inorganic nitrogen metabolism in plants. Indeed, both NR and NiR catalyze the reduction of NO₃⁻ to NH₄⁺ (36). Then, NH₄⁺ is rapidly incorporated into organic compounds through GS activity, which catalyzes glutamine formation. For this reason, GS is considered one of the major checkpoints in plant growth and productivity (2). GS works in association with GOGAT, which, in turn, allows the production of glutamate in the GS/GOGAT cycle (37). Glutamate is the amino nitrogen donor for AspAT, which is a key enzyme for both nitrogen and carbon metabolism (38), and promotes formation of

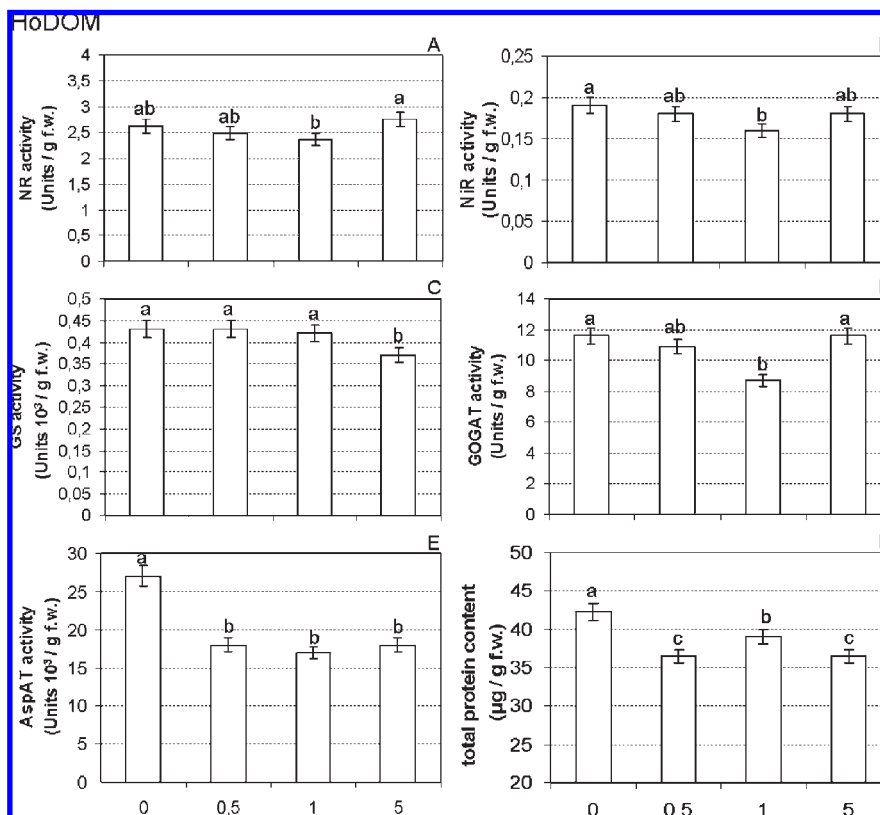


Figure 7. Effect of HoDOM fraction treatment on nitrate reductase (NR) (A), nitrite reductase (NiR) (B), glutamine synthetase (GS) (C), glutamate synthetase NADH-dependent (NADH-GOGAT) (D) and aspartate aminotransferase (AspAT) (E) activities and total protein content (F) of maize leaves. Seedlings were grown for 14 days in a Hoagland modified nutrient solution (0) and treated for the last 2 days with HoDOM (0.5, 1, and 5 mg C L⁻¹). The activities were expressed in unit g⁻¹ fresh weight. The protein concentration was expressed in µg of protein g⁻¹ fresh weight. Values are the means of three independent determinations (±SE). Letters above bars indicates significant differences ($P < 0.05$).

aspartate, an important precursor of several essential amino acids (39).

In our study, the negative effect of compost in hydroponic conditions was observed not only on growth parameters but also on nitrate metabolism. In fact, N exerts a direct effect on plant growth (2), thereby suggesting that physiological effects seem to be related to the low plant N demand. This behavior may be ascribed to specific compounds contained in the organic matrices of compost. In particular, the large concentrations used here may provide plant toxicity possibly due to the content of phenolic compounds. Phenolics, which are known to exert inhibitory effects on a variety of organisms (40–42), might have acted as allelochemicals in our fractions, thus decreasing protein content and enzyme activities of the nitrogen pathway. In fact, allelochemicals are reported to inhibit protein, enzyme activity, nitrogen uptake and metabolism, damage membranes and change physiological functions (43, 44). Furthermore, the decrease of nitrogen assimilation causes reduction of amino acid synthesis, and, consequently, leads to reduced plant growth and development. The modes of action of allelochemicals can be multiple and/or synergistic, thus sometimes making their distinction difficult (45). A similar behavior was observed in plants treated with HoDOM, which exhibits a complex structure that includes tannins, polyphenols and oxidized polyphenols (14).

Conversely, the more hydrophilic and less structurally complex HiDOM and TEA enhanced root weights and changed root hair development, possibly due to their larger water hydration, which may favor diffusion of active compounds from soil solution to maize cells. TEA and HiDOM increased not only growth

parameters but also the enzyme activities for nitrate assimilation. Moreover, these hydrophilic and readily available fractions largely increased the activity of AspAT, which, as a precursor for several essential amino acids (39), controls the synthesis of pyrimidines, purines, and plant growth regulators. Furthermore, the amino acids are also implicated in osmotic regulations and provide a link between nitrogen and carbon metabolic pathways (46). The positive effects of HiDOM and TEA may be attributed to the presence of poly- and oligosaccharides. Such components are liable to ensure the solubility of these compost fractions, thereby conferring a more flexible conformational structure, and promoting a more efficient diffusion of the bioactive humic-like components at cellular membrane level (47). These results are consistent with previous findings where a relation between hydrophilic components of humic substances and their biological activity in plant metabolism has been reported (19).

In conclusion, this work indicates that plant growth and metabolism were inhibited by the most structurally complex and hydrophobic bulk compost and HoDOM samples. On the contrary, the most hydrophilic and less structurally complex HiDOM and TEA fractions significantly enhanced growth parameters and physiological activities in maize seedlings. Therefore, some fractions of composted organic matter possess the potential to act not only as supplier of nutrients to plants but also as stimulator of plant biochemical activities. The stimulating function seems to pertain mostly to the compost fractions which are either sufficiently hydrophilic or less structurally complex to be readily bioavailable to plants. Further result confirmation may stem from a future extension of the experimental period.

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