

Characterization of Humic Fractions in Leachates from Soil Under Organic and Conventional Management and Their Interactions with the Root Zone

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Abstract Humic fractions were shown to be closely involved in gene expression and promotion of different PM H⁺-ATPase isoforms, as well as in lateral root development, indicating an enhanced nutrient absorption capacity of the plant root system. HPLC-SEC confirmed that water-soluble humic substances (WSHS) correspond to a subfraction of the fulvic fraction of humic substances. This was supported by E_{465}/E_{665} ratios higher than 8.5. These ratios generally increased over the growing season in cultivated soils but showed significant differences between conventionally and organically managed bare soils. FTIR data and the analytical quantification of carboxyls confirmed relevant structural changes in bare soil under both organic and conventional farming management. Absorption intensities ratios at 1,590–1,570 cm⁻¹ and 1,440–1,380 cm⁻¹ showed the predominant aliphatic character of these molecules.

Keywords Humic substances • Leachates • Nitrate uptake • Organic farming

Introduction

Water-soluble humic substances (WSHS) are known to stimulate nitrate uptake mechanisms (Pinton et al. 1999), thus suggesting the possible existence of a complex interaction among soil microorganisms, labile soluble C, humic substances, nutrient cycling, and roots (Badalucco and Nannipieri 2007; Locci et al. 2001; Chen et al. 2004). This study focused on the characterization of the chemical nature of WSHS

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collected from a soil under organic and conventional management and their effect on root development. The effect of the different WSHS on nitrate uptake mechanisms of maize roots has also been evaluated, considering furthermore the modulation of the related genes.

Materials and Methods

Water-soluble humic fractions were isolated from leachates collected into lysimeter pans at three different periods (November 2006, April 2007, and June 2007) from bare soils or soils under *Triticum aestivum* (var. capo), managed for more than 10 years following organic or conventional farming. None of the treatments was fertilized nor amended during the experiment. The field experiment was performed through the use of 200 lysimeter pans, 100 for cultivated soils and 100 for bare soils (respectively divided into 50 for organically and 50 for conventionally managed soils). In order to obtain a sufficient quantity of humic substances, 80 L of leachate water was collected at each sampling time. To achieve this volume, each pan was normally irrigated with 1.4–1.7 L of water within 36 h, and collected leachate samples were pooled together. The collected leachate samples were filtered on Whatman WCN 0.2- μm membrane filters and acidified to pH 1–2 with H_2SO_4 before being loaded onto SPE columns (400 mm \times 30 mm) of cross-linked PVP. Each column was washed with double-distilled water, and adsorbed humic substances were eluted from the column with NaOH 0.1 M. The eluates were treated with Amberlite IR-120 (H^+ form) down to pH 3–4, adjusted to neutrality with KOH 0.1 M, and freeze-dried for further analyses.

Molecular weight distribution was determined with a Bio-Rad Bio-Sil SEC 250-5 column (300 mm \times 7.8 mm). Elution was performed with a 75 mM Tris-phosphate buffer at pH 7.5 and column calibration with polystyrene sulfonate standards. Freeze-dried WSHS samples were dissolved into the Tris-phosphate buffer at a concentration of 2 mg mL^{-1} and filtered with Minisart filters (0.20 μm); 20 μL of each sample was injected into the flux of the eluting solution.

FTIR spectra of freeze-dried WSHS (pH 7) were recorded on KBr pellets. About 2–3 mg of oven-dried humic sample and anhydrous KBr powder (both dried for 24 h at 105°C) were mixed together, grinded, and hydraulically pressed into 1-mm thick pellets.

Estimation of the concentration of carboxylic functional groups was performed using a Mettler Toledo titrator DL50 version 2.4. Freeze-dried WSHS samples were dissolved in ultra-deionized deaerated Milli-Q water to obtain a sample concentration of 4 mg mL^{-1} . Solutions were acidified to about pH 2 with Amberlite IR-120, and 4 mL aliquots was titrated under nitrogen by addition of 0.05 mL of NaOH 0.1 M with an equilibration time of 2 min up to a maximum volume of 1.5 mL of the titrant.

Maize (*Zea mays* L., hybrid PR33T56) seeds were germinated over aerated 0.5 mM CaSO_4 solution in a dark growth chamber at 27°C. After 3 days, the

seedlings were transferred into an aerated solution containing 0.5 mM CaSO_4 in a controlled climatic conditions (day/night photoperiod, 16/8; light intensity, $220 \text{ mE m}^{-2} \text{ s}^{-1}$; temperature (day/night) 25/20°C; RH 70–80%). After 2 days, seedlings were treated with the nutrient solution described by Pinton et al. (1999) with or without $5 \text{ mg C}_{\text{org}} \text{ L}^{-1}$ WSHS for 4 h. Net nitrate uptake by root was determined spectrophotometrically as nitrate depletion from the solution containing 0.5 mM CaSO_4 and 0.2 mM NO_3^- . In parallel, expression levels of genes involved in nitrate acquisition were evaluated by RT-PCR as described in Tomasi et al. (2009).

Results and Discussion

In November, at the beginning of the experiment, the concentration of DOM was much larger in conventional (0.73 mg L^{-1}) than in organically managed soil (0.44 mg L^{-1}). The latter also contained a lower proportion of WSHS (34.7% against 53.7%). In June 2007, the organically managed soil still released less DOM: the bare soil released 0.27 mg L^{-1} and the planted 0.36 mg L^{-1} , whereas the conventionally managed soil released 0.49 mg L^{-1} from the bare and 0.45 mg L^{-1} from the planted, respectively. The proportion of WSHS in DOM increased to 78.3% in the leachate collected from the organically managed planted soil but was only 44.3% in the bare soil. In the conventionally managed soil, WSHS reached, respectively, 70.1% in the planted soil and 78.2% and in the bare soil. This indicates an enhanced mineralization of soil organic matter and supports the hypothesis that the availability of labile C promotes the transformation of humified soil organic matter. A further observation that supports this hypothesis is the progressive disappearance of large molecules (i.e., $>5,000 \text{ Da}$ apparent MW), which were appreciably present only in the organically managed soil at the beginning of the experiment. They were probably humic-like materials derived from organic manures, progressively decomposed with time, as the soil did not receive any organic fertilizer or amendment. Apparent MW distributions showed that all WSHS samples were basically composed of low MW fraction of FA (no humic acid fraction could be collected after acidification of leachates): more than 90% of the molecules had an apparent molecular weight of less than $1,000 \text{ Da}$ and a very high E_{465}/E_{665} ratio, typical of low MW fulvic fractions. The time trend of E_{465}/E_{665} (Fig. 1) showed that the WSHS from the two different management systems were very well differentiated at the beginning of the experiment, but although the ratios of the conventionally managed soil remained fairly constant, those of the organically managed soil increased and even more so in the presence of plants. Further information about molecular structures and the related functional groups were provided by FTIR spectra. Even though the main identified functional groups were the same, their relative absorption intensities varied considerably. The stretching vibration of carbohydrate or alcoholic C–O between $1,080$ and $1,040 \text{ cm}^{-1}$ was more pronounced in autumn WSHS and became weaker

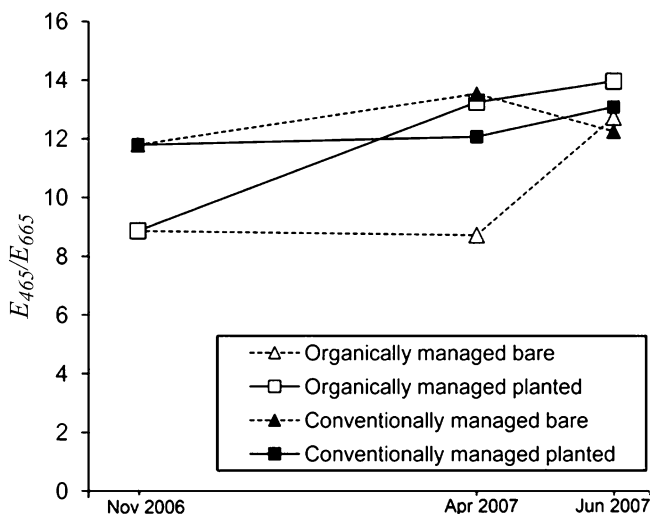


Fig. 1 Trend in E_{465}/E_{665} ratios with time

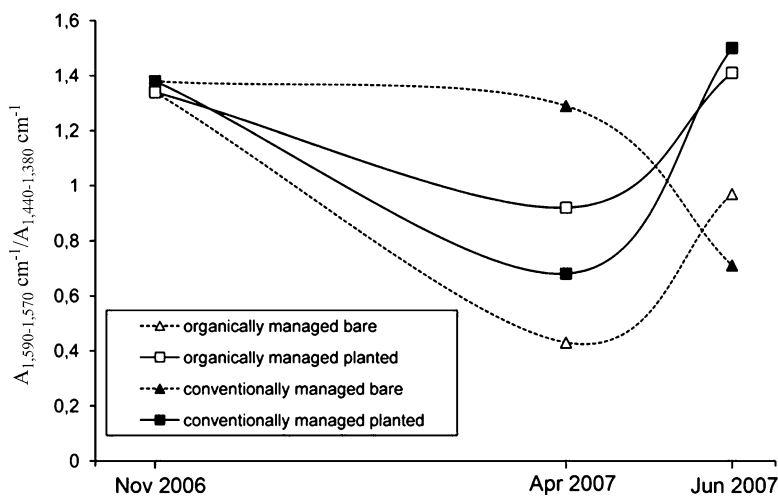


Fig. 2 Trend in $A_{1,590-1,570} \text{ cm}^{-1}/A_{1,440-1,380} \text{ cm}^{-1}$ ratio

with time. Ratios between absorption intensities at $1,590\text{--}1,570 \text{ cm}^{-1}$ and $1,440\text{--}1,380 \text{ cm}^{-1}$ also varied considerably (Fig. 2): a high ratio was found in WSHS collected in November from both soil treatments. The spectrum of WSHS leached from the same soil in April revealed a shoulder around $1,650 \text{ cm}^{-1}$ caused by phenolic -OH groups in the absorbing bands of aromatic compounds, concomitantly to a lower absorption at $3,400$ and $1,060 \text{ cm}^{-1}$ due to the microbial decomposition of carbohydrates moieties. The absorption intensity at $1,570 \text{ cm}^{-1}$ also

decreased, thereby reducing the $A_{1,590-1,570}/A_{1,440-1,380}$ ratio. This ratio should have remained constant if absorption had been caused by carboxylate groups only: its decrease suggests aromatization of the molecular structure followed by further oxidation. WSHS from the organically managed planted soil showed mainly the same trend of structural transformations as the same soil without plants but a lower degree of aromaticity and a higher presence of aliphatic structures at both sampling periods. This is supported by higher E_{465}/E_{665} ratios and low molecular weight subfractions.

At the beginning of the experiment, the total density of carboxyl groups in WSHS of organically managed soil (9.3 mg g^{-1}) was only about half of that of the WSHS from the conventional soil (20.7 mg g^{-1}) but increased with time, reaching at the end of the experiment about the same level in both bare and planted treatments (20.0 and 20.8 mg g^{-1} , respectively). On the contrary, a slightly declining trend was observed in the conventionally managed soil. These trends do not seem related to any of the changes observed for the other parameters.

In order to evaluate the effect of WSHS collected from agricultural soils on nitrate acquisition mechanisms in roots, experiments with a commercial maize hybrid (PR33T56) plants treated for 4 h with or without the WSHS have been conducted. Results showed that WSHS fractions extracted from bare soil leachates collected in autumn and spring, from both organically and conventionally managed soils, were able to enhance the net nitrate uptake rate after 4 h of root contact with these fractions. However, the fractions extracted from the same soils in June had a lower but still evident effect, thus indicating that this effect weakens over the growing season. WSHS fractions extracted from planted soil did not exert any significant influence upon nitrate acquisition process in roots neither in spring nor in summer. The positive effect in nitrate acquisition process of the WSHS samples extracted from bare soil leachates appeared to be closely related to a higher level of transcripts of genes *ZmMHA2* and *Nrt2.1* codifying for proteins involved in the nitrate acquisition mechanisms (data not shown). Furthermore, a higher proliferation of secondary roots and a general lateral root development in presence of WSHS samples were observed, thus confirming their contribution in enhancing the nutrient absorption capacity of the maize plant root system.

Conclusions

The changes of WSHS composition and structure registered during the experiment seemed to lead the WSHS toward more aliphatic and oxidized structures, featuring larger carboxyl content, higher E_{465}/E_{665} ratios, and lower molecular weights. As a likely result of more intense microbial activity, this trend was much more pronounced in the organically managed and in the planted soil. The presence of plants diminished differences between organic and conventional management, supporting the hypothesis that humic substances in the rhizosphere may play a role in a network of complex interlinked regulation processes involving plant nutrient

uptake, microbial activity, and SOM mineralization. However, it was not possible so far to find clear and congruous correspondences between structural changes and observed effects on the mechanisms regulating nitrate uptake in maize plants, and further work is necessary to elucidate these mechanisms at the molecular level.

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