Steam treatment of surface soil: how does it affect water-soluble organic matter, C mineralization, and bacterial community composition?

Dad Roux-Michollet · Yves Dudal ·
Lucile Jocteur-Monrozier · Sonia Czarnes

Received: 11 January 2010 / Revised: 15 May 2010 / Accepted: 25 May 2010 / Published online: 15 June 2010
© The Author(s) 2010. This article is published with open access at Springerlink.com

Abstract Steam soil disinfection is efficiently used in the field for pre-planting pest control. Providing steam to the soil must have consequences, either beneficial or detrimental for the soil functioning. We set up a laboratory experiment to quantify the soil quality dynamics induced by this agricultural practice. In steamed and control soil, we monitored the size, the activity, and the genetic structure of the bacterial community in the top 2 cm soil every second day over a 10-day period after the treatment. We also characterized the bioavailable organic matter using fluorescence and ATR-FTIR spectroscopy. We showed that steaming induced the release of twice as much dissolved organic carbon in steamed soil as in the control soil. This extra carbon was much less fluorescent and contained a higher proportion of aliphatic compounds (alkyl chains, primary alcohols). After an initial drop in the bacterial community, we observed a tenfold increase in the number of bacteria, a flush in carbon mineralization, and genetic structure modification, which

could be related to the newly released carbon. Steam treatment showed strong but quickly reversible impacts on the soil functioning, enabling farmers to sow approximately 1 week after treatment.

Keywords Bacterial community · Soil disinfection · Water-extractable organic matter · Organic farming system

Introduction

Disinfection of agricultural soils has been extensively performed with organic fumigants like methyl bromide for pre-planting pest control (Stromberger et al. 2005). Due to ozone-depleting side effects (Prather et al. 1984; Yagi et al. 1993), such volatile halogen chemicals have been prohibited, and alternative methods are being developed. Soil disinfection treatments are designed to reduce soil-borne crop pests but can also affect directly (i.e., physiological stress) and indirectly (i.e., changes in environmental condition) all soil organisms including those essential for soil functioning.

Among physical treatments, steam soil disinfection is gaining interest in farming systems as an alternative to chemical fumigation (Bond and Grundy 2001). Steaming processes are designed to reach a temperature from 60°C to 80°C at 10 cm depth, depending on the target organisms. The procedure was improved by mathematical modeling for control purposes (Berruto et al. 2004), and steamed surface has extended (Aubertot et al. 2005). Despite its widening use, the effects of steam disinfection on soil functioning are poorly documented. The few published studies focused on weeds and phytopathogens viability to assess the treatment efficiency (van Loenen et al. 2003) and also on diversity, biomass, and activity of soil microbial communi-

D. Roux-Michollet (✉)
Donald Bren School of Environmental Science and Technology,
Institute for Computational Earth System Science,
University of California—Santa Barbara,
3516 Bren Hall,
Santa Barbara, CA 93106, USA
e-mail: droux@bren.ucsb.edu

D. Roux-Michollet · L. Jocteur-Monrozier · S. Czarnes
CNRS-UMR 5557, INRA USC 1193, Ecologie Microbienne,
Université Lyon 1,
43 bd du 11 novembre 1918,
69622 Villeurbanne, France

Y. Dudal
UMR Climat, Sol et Environnement,
Institut National de la Recherche Agronomique,
Domaine St Paul, Site Agroparc,
Avignon 84914, France

ties (Khan et al. 2010; Roux-Michollet et al. 2008; Tanaka et al. 2003). Nevertheless, heated vapor must affect the soil's physical, chemical, and biological properties, thus nutrient cycling.

As a common consequence to biocide treatments, the components of dead organisms might be released and used as substrates by surviving organisms and fast-growing colonizers. Indeed, fumigation methods (Ladd and Amato 1988) as well as autoclaving (Serrasolsas and Khanna 1995) were shown to increase potentially bioavailable C and N by releasing water-soluble compounds. Also, the potential release of soil components in water may affect soluble compounds as well as colloids involved in soil aggregation. Release of soil organic components may supply substrates for microbial growth, although contrasting results showed either immediate use by microorganisms (Serrasolsas and Khanna 1995) or use after a lag phase (Leinweber et al. 1995). Moreover, the water-extractable organic matter (WEOM) quality may differ among soil types and managements (Chantigny 2003).

Soluble organic components were shown to influence microbial biomass, activity, and community structure in different types of soils (Cleveland et al. 2007; Eilers et al. 2010; Montano et al. 2007; Saison et al. 2006). Our objective was to describe steam impact on the substrate quality by characterizing the main organic components extractable and/or dispersible in water immediately after the treatment of surface soil and the further changes in the soil bacterial community. An experiment was designed where repacked soil cores were steamed at the top. Steamed and control soil microcosms were then incubated in controlled conditions for several days. Water-extractable components from the surface layer (0–2 cm), which has been shown to be the most affected by steam treatment (Roux-Michollet et al. 2008), were obtained by a standard procedure, collected after filter sterilization, and analyzed by fluorescence and ATR-FTIR spectroscopy. In the same top layer, the impact of steaming on the soil bacterial community was assessed by (1) enumerating culturable heterotrophic bacteria, (2) measuring the soil respiration rate, and (3) characterizing the genetic structure of the eubacterial community.

Materials and methods

Soil properties and experimental procedure

Surface soil (0–20 cm depth) was collected in April 2006 from a green manure plot (*Secale cereale* and *Vicia sativa*) in an organic farming station (46° North, 5° East, at 325 m a.s.l., Station Expérimentale Rhone-Alpes et Information Légumière, Brindas, France). The soil texture is characterized as loamy sand with 16% clay, 17% silt,

and 67% sand. It contains 1% organic carbon and has a water holding capacity of 31% (w/w). Fresh soil was transported to the laboratory, sieved to 2 mm, homogenized, and progressively moistened to 14% (w/w) gravimetric water content (corresponding to a water potential of −5 kPa and 45% of the soil's water holding capacity). Rewetting was performed at 4°C using a suction sand table.

Compacted cores were obtained according to Fazzolari et al. (1998) by uniaxial confined compression of a mass of calibrated and wet aggregates in a cylindrical PVC mold (7.5 cm diameter and 8 cm height). The mass of aggregates (m) was calculated on dry weight basis from the equation: $m = V \times d$, where V is the mold volume (376 cm³) and d is the mean bulk density (1.1 g cm^{−3}). Compaction was realized within three layers to improve the homogeneity of the bulk density. Steam treatment was simulated with a Vaporetto steaming source (Eco Pro 3100, Polti France) at the top of the repacked soil microcosms. The experimental system provided steam jet at a temperature of 120°C under 0.5 bar pressure, conditions which were similar to those performed with a standard generator in the field. The soil temperature rapidly rose from 17°C to 100°C in the upper layer (0–5 cm) and reached 55±4°C at 8 cm (exposure time of 1'20±23 s, data not shown). After 1 h at room temperature, 24 steamed microcosms (four replicates × six incubation times) and 12 untreated microcosms (four replicates × three incubation times) were incubated at 21°C, for 0 to 10 days, on a suction table (−5 kPa). Only three time points (0, 4, and 10 days) were analyzed for the control treatment as we did not expect major changes during the time-course incubation. At each sampling date, soil was collected from 0 to 2 cm and homogenized in UV-sterilized pots. Three sub-samples were immediately used for organic matter extraction, soil respiration, and bacteria enumeration. Another sub-sample was frozen for further genetic structure analysis.

Extraction, purification, and characterization of soluble organic components

Water extraction was performed by shaking fresh soil in ultrapure water (solid/liquid ratio 1:2, w/v) for 1 h at 28°C and 180 rpm on an oscillating table (Biolabo, Scientific Instrument, France). Then, the solution was centrifuged for 10 min at 13,000 g (Eppendorf Centrifuge 5804R). The supernatant was filtered at 0.45 µm, collected under vacuum in a sterile Vacutainer® tube, and freeze-stored. Extraction was performed in two replicates. One replicate was used for total organic carbon measurement and fluorescence analysis; a second replicate was frozen in liquid nitrogen and then freeze-dried for further ATR-FTIR spectroscopy.

The amount of water-extractable organic carbon (WEOC) was determined with a total organic carbon

analyzer (TOC-5050A, Shimadzu). To remove inorganic C components (carbonates), water extracts were acidified using small volumes of concentrated hydrochloric acid (2 M). Removal of inorganic CO₂ was achieved by CO₂-free air bubbling for 10 min. Organic carbon was measured as CO₂-C evolved after thorough oxidation at 680°C in the presence of platinum as catalyst.

Fluorescence spectroscopy provides an overall assessment of the nature of an organic mixture. Initially developed by Coble et al. (1990) to study seawater-dissolved organic matter (DOM), the use of three-dimensional (3-D) fluorescence signature has later been extended to a wide variety of aqueous organic mixtures, in particular soil DOM (Akagi et al. 2007). Fluorescence spectra were acquired on a Perkin-Elmer LS55 spectrometer equipped with a 150 W pulsed xenon lamp. Samples were placed in a specific four clear-face quartz cell and 3-D excitation–emission matrices (EEMs) were generated at 23±2°C, with excitation and emission slit widths of 10 nm. Each EEM was acquired by collecting fluorescence emission spectra (from 200 to 600 nm) for excitation wavelengths ranging from 200 to 575 nm with a 20 nm interval. The spectrometer calibration was performed using an inner Raman-based procedure. Possible inner-filter effects were checked for by diluting each sample and ensuring linearity between carbon content and fluorescence intensities. The 3-D isointensity maps (Fig. 1) were built using the FLWinlab software (Perkin Elmer). Correspondence of the main noticeable peaks (Table 1) was established in these maps based on data collected from the literature (Alberts and Takacs 2004; Burdige et al. 2004; Saadi et al. 2006; Sierra et al. 2005).

Middle infrared spectroscopy is a method to assess the main biochemical components of complex material. The FTIR absorption bands are used to determine functional groups of soil organic components as well as to characterize hot water solutes (Ellerbrock and Gerke 2004). Attenuated total reflectance Fourier transformed infra-red (ATR-FTIR) was developed to determine the global composition of solids on sections or on thin deposits. It has been applied in the determination of solute in biological media (Dokken et al. 2005) and in biofilm characterization (Schmitt and Flemming 1998). Also, inorganic entities are usually found in water extracts together with organic components and their specific functional groups can be evidenced by FTIR spectroscopy techniques, including ATR-FTIR (Madejova 2003). In our study, the preliminary examination of soil water extracts showed the presence of inorganic clay components in the 0.45 µm-filtered extracts. Therefore, to study the organic components, soil water extracts were centrifuged (5,000g for 15 min at 20°C, Beckman Coulter Allegra 25R) to remove the fine particles dispersed as colloidal entities. After collection, each supernatant free of

most inorganic colloids was freeze-dried for analysis. For each ATR-FTIR run, the dry powder was spread onto the Ge crystal and scanned (Nexus, Thermo Fisher Scientific, Courtabeuf, France) in the range of wavenumbers from 650 to 4,000 cm⁻¹; 128 scans were accumulated with 4 cm⁻¹ resolution. To assess the changes in soil water extracts resulting from steaming, mean spectra were compared on the four main regions defined by Schmitt and Flemming (1998) and usually found on FTIR spectra from organic soil extracts (Olk et al. 2000) and from bacteria EPS (Beech et al. 1999): the polysaccharide region from 1,040 to 1,100 cm⁻¹, the protein region from 1,520 cm⁻¹ (N–H deformation and C=N stretching of amides II) to 1,660 cm⁻¹ (C=O stretching of amides I), the alkyl region from 2,850 to 2,950 cm⁻¹ (aliphatic C–H group stretching), and the O–H region around 3,400 cm⁻¹ (3,350–3,550 cm⁻¹ for O–H stretching in intra- and intermolecular bonds; 3,620–3,650 cm⁻¹ for O–H stretching in most clays). Additionally, peaks assignment for O–H in primary and secondary alcohols (1,045 cm⁻¹ and 1,140–1,150 cm⁻¹, respectively), O–Si (in clays, 1,030–1,045 cm⁻¹ or amorphous silica, 1,115 cm⁻¹), N–O in nitrate (1,355 cm⁻¹), and molecular H₂O (1,630 cm⁻¹) allowed to achieve a global “fingerprint” of the main functional groups of both organic and inorganic compounds found in the studied soil water extracts.

Biological properties

Soil respiration rate was assessed as CO₂ release. Fresh soil was placed in a sterile 150 ml plasma flask. Distilled sterile water was provided to ensure a water content equivalent to 80% of the water holding capacity. Flasks were sealed with rubber stoppers and incubated at 28°C for 7 h. Gas samples were periodically analyzed for CO₂ concentration using a gas chromatograph (Agilent P200 Micro, USA). Soil respiration was expressed as micrograms of CO₂-C released per gram of dry soil per hour.

To analyze the survival and the potential growth of culturable heterotrophic bacteria in fresh soil, we used the most probable number technique as described by Alexander (1982). Soil samples (5 g equivalent oven-dried) were ground and homogenized in 25 ml of NaCl (0.8%). Soil suspensions were then serially diluted (1/5) and inoculated into 12×8-well plates containing 100 µl of mineral salts medium “Nutrient Broth” (2X). For each dilution, eight wells were inoculated (100 µl). The plates were incubated aerobically for 8 days, at 28°C in the dark. The growth of heterotrophic bacteria was shown as turbidity in wells. The number of heterotrophic bacteria was estimated by the Cochran’s method (Cochran 1950).

Total DNA was extracted and purified from 0.5 g of soil using the Fast DNA SPIN Kit (BIO 101 Systems,

Fig. 1 Fluorescence 3-D isointensity map from the excitation–emission spectra of dissolved organic matter in control soil. *A* and *C*, lower molecular weight components (fulvic and humic acids); *H*, higher molecular weight components (lignin-derived); *SR*, low excitation wavelength protein-like; *BT*, high excitation wavelength protein-like

Qbiogene, Carlsbad, CA, USA) and a polyvinylpolypyrrolidone column. The eubacterial genetic structure was monitored by Ribosomal Intergenic Spacer Analysis (RISA) according to Ranjard et al. (2001). Soil DNA was amplified using the primers S-D-Bact-1522-b-S-20 (eubacterial rRNA small subunit) and L-D-Bact-132-a-A-18 (eubacterial rRNA large subunit) (Normand et al. 1996). Amplification was carried out with 50 μ l reaction mixtures containing 5 μ l of 10 $\times$ PCR buffer (100 mM Tris–HCl, pH=9, 500 mM KCl, 15 mM MgCl₂, 1% triton X100, 2 mg ml⁻¹ BSA), 200 μ M of each dNTP (Euromedex, Mundolsheim, France), 0.5 μ M of each primer (Proligo, Paris, France), 1.75 U of Taq polymerase (Qbiogene, Illkirch, France), and 6 ng of soil DNA. Amplification was performed in a thermocycler (T-personal

48, Biometra, Goettingen, Germany) with an initial denaturing step (5 min at 94°C) followed by 25 cycles of 1 min at 94°C, 1 min at 55°C, 1 min at 72°C, and a final extension of 7 min at 72°C. PCR products were resolved on 5% nondenaturing polyacrylamide–Tris–borate–EDTA gels (Euromedex, France) running at a constant temperature (20°C) for 15 h at 65 V. Fingerprint banding patterns (Fig. 2) were analyzed using GelCompar II software (Applied Maths, Belgium).

Statistical analysis

For each sampling date, a one-way analysis of variance was performed to determine the steaming effects on (1) the

Table 1 Correspondence between excitation and emission spectra and organic molecular groups in soil water-extractable organic matter

Assigned structure	Excitation (nm)	Emission (nm)	Symbols
Humic-like material			
Lower molecular weight	240–250	440–450	A
	330–340	430–440	C
Higher molecular weight	380–400	460–470	H
Protein-like material			
Low excitation wavelength	225–235	325–335	SR
High excitation wavelength	270–280	300–310	BT

Fig. 2 RISA profile of bacterial community in steamed soil at day 2 (*S2*), day 4 (*S4*), and day 10 (*S10*) and in control soil at day 10 (*C10*). A molecular weight ladder (100 bp) was loaded to identify the size of DNA fragments ranging from 300 bp to 1500 bp

WEOC, (2) the fluorescence intensity, (3) the microbial activity, and (4) the number of culturable heterotrophic bacteria.

To analyze the genetic fingerprints of the eubacterial community, we created a data matrix corresponding to the relative intensity and the position of the bands detected for each sample. With the PRIMER software (PRIMER-E Ltd, Plymouth, UK), similarity matrices were computed using the Bray–Curtis index. The rank similarity of genetic structures among soil samples was assessed by non-metric multidimensional scaling (MDS). Then, one-way analysis of similarity was performed to compare statistically the composition of the eubacterial community between steamed soils and control soils. Finally, we surveyed the percentage of dissimilarity between the community structures in steamed and control soils to assess the resistance and resilience of the genetic structure after steam soil disinfestation.

Results

WEOC content

Average soluble organic C content in control soil was about 0.09 mg g⁻¹ of dry soil during the whole incubation (Fig. 3). Immediately after the steam treatment, it increased to 0.18 mg g⁻¹ and then to 0.23 mg g⁻¹ during the next 4 days. Also, we observed an increase in the carbon content variability between 2 and 4 days after the treatment. After 10 days, the amount of soluble organic C was still twice as high in the steamed soil as in the control soil.

Fluorescence signature

Changes in fluorescence intensity of the three main peaks (Fig. 1) were monitored during incubation (Fig. 4). The

modification of organic matter progressed in two phases for both the treated and the control soils. In the control soil, the amount of LMW and protein-like components decreased during the first 4 days and then remained at a constant value of 160 and 40 au, respectively. In the steamed soil, the amount of protein-like components decreased right after treatment, whereas lignin-derived components (HMW) increased compared to the control. From day 0 to day 4, the fluorescence intensity increased for the protein-like material, whereas the fluorescence was stable for humic-like material (LMW and HMW). At day 4, the fluorescence intensity of the three groups was higher in steamed than in control soils, as well as the WEOC content (Fig. 3). Finally, between day 4 and day 10, the fluorescence intensity decreased dramatically for the LMW and the protein-like peaks—more smoothly for the lignin-derived components (HMW). This resulted in a much lower intensity of the LMW at day 10 than at the

Fig. 3 Amount of water-extractable organic carbon between 0 and 10 days after treatment for steamed soil (open square) and control soil (filled square). Bars indicate standard errors ($n=4$). Different letters indicate significantly different values ($p=0.05$)

Fig. 4 Fluorescence intensity of soil water extracts showing the time evolution of the three main components. *Square*, LMW fulvic and humic acids (A and C); *triangle*, HMW lignin-derived organic matter (H), and *circle*, protein-like material (BT + SR) for steamed soil (*open symbol*) and control soil (*filled symbol*). Bars indicate standard errors ($n=4$). Different letters indicate significant difference between values ($p=0.05$)

beginning of the incubation. Since the amount of WEOC was slightly increasing between 6 and 10 days following the treatment, changes in nonfluorescent moieties were expected to occur.

ATR-FTIR spectroscopy

Changes in WEOM pattern were revealed by ATR-FTIR spectrum immediately after treatment (Fig. 5a). Residual inorganic components revealed by the absorbance at $3,530\text{ cm}^{-1}$ (O–H stretching in silicate) and $1,115\text{ cm}^{-1}$ (Si–O bending) were not found in the steamed soils. A new peak appeared at $2,940\text{ cm}^{-1}$ which evidenced that the steaming process enhanced the solubilization of alkyl compounds. Concomitantly, a shift of the main peaks of OH stretching in alcohol functional groups was observed. In the control soil, the dominant peak arose at $1,140\text{ cm}^{-1}$, i.e., at the OH stretching frequency of secondary alcohol. Soon after, steaming the main peak was observed near $1,045\text{ cm}^{-1}$ (Fig. 5b), which revealed the increase of primary alcohol functional groups in steamed soil. The distinct peaks at $1,655$ and $1,620\text{ cm}^{-1}$ from protein-like material were embodied, after steaming, in a broad unresolved band at $1,610\text{--}1,630\text{ cm}^{-1}$. The asymmetrical intermolecular OH binding which, in control extracts, peaked sharply at $3,400\text{ cm}^{-1}$ with a shoulder at $3,530\text{ cm}^{-1}$ was broadened symmetrically in the steamed samples, at a slightly lower frequency (near $3,390\text{ cm}^{-1}$). The main peak at $1,355\text{ cm}^{-1}$ was not characteristic of organic functional groups but specific to N–O binding in nitrate. This peak remained nearly unchanged neither by steaming nor by the incubation. During incubation, the aliphatic compounds ($2,940\text{ cm}^{-1}$) and the primary alcohol

($1,045\text{ cm}^{-1}$) tended to decrease when, contrastingly, a significant increase of the absorbance at $1,100\text{ cm}^{-1}$ (polymeric exopolysaccharides) was observed. At day 10, the ATR-FTIR profile of WEOM from incubated steamed samples was closer to the control profile than to the profile of extracts from soil immediately after steaming.

Soil respiration

Carbon mineralization in control soil was nearly constant ($0.01\text{ }\mu\text{g CO}_2\text{-C g}^{-1}\text{ soil h}^{-1}$) along the incubation (Fig. 6). Immediately after steaming, microbial activity was dramatically reduced down to four times less in the treated soil than in the control soil. Two days after the treatment, a flush of CO_2 was observed in the steamed soil up to $0.07\text{ }\mu\text{g of CO}_2\text{-C per gram of soil per hour}$. Then, the respiration rate in steamed soil decreased progressively until it reached the control value between 8 and 10 days after the treatment.

Fig. 5 ATR-FTIR spectra of freeze-dried extracts for steamed soil (*black line*) and control soil (*gray line*) immediately after treatment (**a**) and during incubation at day 0, day 4, and day 10 for steamed soil (**b**). Each spectrum is the mean of four replicates

Fig. 6 Respiration rate during the incubation in steamed soil (open square) and control soil (filled square). Bars indicate standard errors ($n=4$). Different letters indicate significantly difference values ($p=0.05$)

Enumeration of culturable bacteria

The average number of culturable heterotrophic bacteria was 71% lower just after steaming (data not shown). The abundance of bacteria increased during incubation but remained significantly lower in steamed soil (2.5×10^7 bacteria g^{-1} dry soil) than in control soil (2.2×10^8 bacteria g^{-1} dry soil), even 10 days after the treatment.

RISA profiles

Multidimensional scaling analysis illustrated the changes in the overall community composition (Fig. 7). The RISA profiles of the eubacterial community in control soils grouped together, whatever the sampling time, suggesting a minor effect of incubation on the soil bacterial community composition. Immediately after the treatment, the DNA amount was too low to obtain PCR products, which means that no data on the genetic structure of the bacteria surviving the steaming process could be obtained. From day2 to day10, the community structure in the steamed soil significantly differed from the community structure in the control soil (Fig. 7). Dissimilarity percentage ranged from 88% to 91% at day2 and day4 and was close to 84% from day6 to day10. Ribosomal Intergenic Spacer Analysis fingerprinting is based on the length polymorphism of intergenic spacer (IGS) sequences between the small (16S) and the large (23S) subunit rRNA genes. From day2 to day10 after the treatment, the IGS distribution shifted from long-length IGS to short-length IGS compared to the control (Fig. 2).

Discussion

Soil extraction by hot water has been used mainly as an assessment of labile soil organic carbon and resources

available for the activity and the growth of soil micro-organisms. Sparling et al. (1998) reported that boiling soil in water resulted in the extraction of both microbial and non-microbial organic C. Steaming soil can be considered as hot water treatment without water saturation, then disconnecting the effects of heating soil from the removing of lysates by the excess of hot water. In our study, the consequences of heating soil on the fate of organic C were assessed in two ways: (1) CO₂ release during soil post-incubation as in the work of Franzluebbers et al. (2000) and (2) analysis of cold WEOM by spectroscopic techniques: UV–fluorescence and ATR-FTIR.

WEOC content and respiration rate

Steam treatment resulted in a strong and sustainable (>10 days) increase of WEOC. Our experiment using cold water after steaming to extract organic matter would simulate field conditions where rain water is a natural extractant. Such conditions explained that the values obtained for WEOC amount, from the studied loamy sand, were much lower than the WEOC resulting from hot water extraction, as measured by Sparling et al. (1998) in arable sandy loam and loamy sand. Water-extractable organic C content showed nearly constant values during the whole incubation. Contrastingly, the CO₂ released from steamed soils exhibited variation with time: a lag phase was observed immediately after steaming followed by a flush and progressive decrease in organic C mineralization until the value measured in the control soil. The flush of soil respiration was commonly observed as the response of microbial biomass to new organic substrates and has been

Fig. 7 Non-metric multidimensional scaling plots of the genetic structure generated from RISA profiles of the eubacterial community characterized in steamed soil at day2 (cross symbol), day4 (open triangle), day6 (open square), day8 (open inverted triangle), and day10 (open circle), and in control soil at day0 (multiplication symbol), day4 (filled triangle), and day10 (filled circle). The stress value, inferior to 0.1, indicates a very good representation by the MDS map of the information included in the rank similarity matrix

widely described (Falchini et al. 2003; Ganjegunte et al. 2006). The lag phase would evidence that most soil microorganisms could not resist the treatment. We can also assume that the decrease in cultivability was either due to cell lyses or reflected a physiological stress. Bacteria surviving the steaming process have been exposed to osmotic stress and could release intracellular solutes (Halverson et al. 2000), which is consistent with the observed increase in carbohydrates in the soil solution. Consequently, the dead bodies and/or osmolytes would substantiate the following flush in CO₂ release. The amount of CO₂-C released from the incubated soil was a very small proportion of the WEOC, with a maximum of 0.3% of available C during the respiration pulse (0.07 µg CO₂-C h⁻¹), and could not influence the amount of measurable WEOC. This result contrasted with what is generally observed as a positive priming effect, when soluble organic compounds are added to soil (Blagodatskaya and Kuzyakov 2008; Ganjegunte et al. 2006). To determine if mineralization could process on WEOC, either native soil organic matter or dead microbial biomass, we characterized the nature of water-extractable compounds.

Nature of WEOM

Steaming was shown to directly influence the water-soluble components of treated soils on several ways. At day0, WEOM fluorescence revealed the release of previously non-soluble HMW aromatic components into soil solution. Also, FTIR showed the new evidence of alkyl compounds with absorbance at 2,940 cm⁻¹ as well as OH in alcohol I organic compounds (1,045 cm⁻¹). Alkyl functional groups are normally found as part of long chains that steam treatment may have broken off. These free and extremely reactive moieties may have been used as substrates by microorganisms, which is consistent with the progressive disappearance of the alkyl peak from day0 to day10. Contrastingly, the signals attenuation from aromatic amino compounds in UV-fluorescence spectra and the extinction of amides I in FTIR (1,655 cm⁻¹) after the treatment highlighted that proteinaceous entities disappeared. During the incubation, LMW fluorescent components decreased which corresponded to the disappearance of the ATR-FTIR band assigned to phenolic OH in fulvic acids with absorbance at 1,408 cm⁻¹ (Plaza et al. 2003).

The detailed examination of FTIR spectra before purification (data not shown) evidenced the presence of dominant clay minerals with major absorbance at 915, 1,036–1,040, 3,630, and 3,700 cm⁻¹ which were not found in the purified water extracts (Fig. 3a). As a consequence, the changes in absorbance in the polysaccharide region should be mostly due to modifications in carbohydrate components and not to clay weathering.

From these data, steaming would likely result in a hydrolytic process which affects the organic residues, lignin-like material (HMW), and possibly carbohydrate components. The appearance of non-fluorescent alkyl moieties immediately after steaming would evidence soil microstructures disruption held by both inorganic and organic macromolecules. The suggestion of polysaccharide preferentially to lipid structure is supported by the IR spectra. Indeed the strong absorbance of alcohol groups in various molecular environments (large peaks) revealed carbohydrate structures (R-CHOH) from polysaccharides. Alcohol groups are abundant in carbohydrates and quasi-absent from fatty acids and other soil lipids which have been also reported to be involved in soil aggregation at the micro-scale. Analysis of WEOM during the incubation of steamed microcosms showed an increase of non-fluorescent organic components together with an increase in II alcohol functional groups (1,150 cm⁻¹). Those observations reinforced the hypothesis of polysaccharides biosynthesis during microbial resilience after steaming. Exopolysaccharides may protect bacteria from fluctuations in environmental conditions (Roberson and Firestone 1992) and are involved in biofilm formation. In addition, since an increase in WEOC was observed, it can be concluded that steaming enhanced carbohydrate solubilization then organic substrates availability for microbial growth.

Potential use of the steaming process in organic farming

Steam disinfection induced modifications in the soil structure. One side effect of this agricultural practice was the disruption of soil micro-aggregates held by both macromolecular organics and clay mineral colloids, resulting in a better accessibility of organic substrates to surviving bacteria in steamed soil. Free organic particles are less resistant to biodegradation (Chenu and Plante 2006), which could contribute to microbial recovery after steaming. During incubation following steam treatment, the WEOM spectral characteristics showed progressive resilience to the original control soil. Similar observation was obtained for the microbial activity. We have also shown in a previous study (Roux-Michollet et al. 2008) that the abundance of culturable heterotrophic bacteria in the top 10 cm layer was resilient 27 days after the treatment. Finally, colonization dynamics is probably a long process which could explain that community composition was still different in the steamed soil compare with control, 10 days after the treatment, and dominated by *r* strategy populations according to the IGS length distribution among the bacterial phyla (Ranjard et al. 2000). Considering that microbial diversity is related to the environmental conditions and season variability, a longer time may be required for the installation of endogenous microflora. Steam treatment could be evaluated as an audacious and

sustainable chemical-free practice in specific agricultural management like horticulture.

Acknowledgments We are indebted to Dominique Berry who managed the field site (SERAIL, Brindas). We thank Julien Fernandez and Claire Commeaux for assistance during the experiment. We are also grateful to Aline Effosse for her advices in samples freeze-drying, Serge Alamercury for ATR-FTIR spectroscopy analysis (Service centrale d'analyse du CNRS, Solaize), and Ghislain Sevenier for support with the TOC-Analyzer (UMR Climat Sol et Environnement, Avignon). This work was funded by the French National Science Foundation.

Open Access This article is distributed under the terms of the Creative Commons Attribution Noncommercial License which permits any noncommercial use, distribution, and reproduction in any medium, provided the original author(s) and source are credited.

References

- Akagi J, Zsolnay A, Bastida F (2007) Quantity and spectroscopic properties of soil dissolved organic matter (DOM) as a function of soil sample treatments: air-drying and pre-incubation. *Chemosphere* 69:1040–1046
- Alberts JJ, Takacs M (2004) Total luminescence spectra of IHSS standard and reference fulvic acids, humic acids and natural organic matter: comparison of aquatic and terrestrial source terms. *Org Geochem* 35:243–256
- Alexander M (1982) Most probable number method for microbial populations. In: Page AL, Miller RH, Keeney DR (eds) *Methods of soil analysis, part II—chemical and microbiological properties*. Soil Sci Soc Am and Am Soc Agron, Madison, WI, pp 815–820
- Aubertot JN, Barbier JM, Carpentier A, Gril JJ, Guichard L, Lucas P, Savary S, Savini I, Voltz M (2005) Pesticides, agriculture and the environment. Reducing the use of pesticides and limiting their environmental impact. In: Ministère Agriculture Pêche, Ministère Écologie Développement Durable (ed) *Collective Sci Expertise INRA Cemagref*, Fr
- Beech I, Hanjagst L, Kalaji M, Neal AL, Zinkevich V (1999) Chemical and structural characterization of exopolymers produced by *Pseudomonas* sp. NCIMB 2021 in continuous culture. *Microbiol* 145:1491–1497
- Berruto R, Gay P, Piccarolo P, Tortia C (2004) Grey-box models for steam soil disinfestation simulation. *Math Comput Simul* 65:191–200
- Blagodatskaya E, Kuzyakov Y (2008) Mechanisms of real and apparent priming effects and their dependence on soil microbial biomass and community structure: critical review. *Biol Fertil Soils* 45:115–131
- Bond W, Grundy AC (2001) Non-chemical weed management in organic farming systems. *Weed Res* 41:383–405
- Burdige DJ, Kline SW, Chen WH (2004) Fluorescent dissolved organic matter in marine sediment pore waters. *Mar Chem* 89:289–311
- Chantigny MH (2003) Dissolved and water-extractable organic matter in soils: a review on the influence of land use and management practices. *Geoderma* 113:357–380
- Chenu C, Plante AF (2006) Clay-sized organo-mineral complexes in a cultivation chronosequence: revisiting the concept of the 'primary organo-mineral complex'. *Eur J Soil Sci* 57:596–607
- Cleveland C, Nemergut D, Schmidt S, Townsend A (2007) Increases in soil respiration following labile carbon additions linked to rapid shifts in soil microbial community composition. *Biogeochemistry* 82:229–240
- Coble PG, Green SA, Blough NV, Gagosian RB (1990) Characterization of dissolved organic-matter in the Black-Sea by fluorescence spectroscopy. *Nat* 348:432–435
- Cochran WG (1950) Estimation of bacterial densities by means of the "most probable number". *Biometrics* 6:105–106
- Dokken KM, Davis LC, Marinkovic NS (2005) Use of infrared microspectroscopy in plant growth and development. *Appl Spectrosc Rev* 40:301–326
- Eilers KG, Lauber CL, Knight R, Fierer N (2010) Shifts in bacterial community structure associated with inputs of low molecular weight carbon compounds to soil. *Soil Biol Biochem* 42:896–903
- Ellerbrock RH, Gerke HH (2004) Characterizing organic matter of soil aggregate coatings and biopores by Fourier transform infrared spectroscopy. *Eur J Soil Sci* 55:219–228
- Falchini L, Naumova N, Kuikman PJ, Bloem J, Nannipieri P (2003) CO₂ evolution and denaturing gradient gel electrophoresis profiles of bacterial communities in soil following addition of low molecular weight substrates to simulate root exudation. *Soil Biol Biochem* 35:775–782
- Fazzolari E, Guerif J, Nicolardot B, Germon JC (1998) A method for the preparation of repacked soil cores with homogeneous aggregates for studying microbial nitrogen transformations under highly controlled physical conditions. *Eur J Soil Biol* 34:39–45
- Franzuebbers AJ, Haney RL, Honeycutt CW, Schomberg HH, Hons FM (2000) Flush of carbon dioxide following rewetting of dried soil relates to active organic pools. *Soil Sci Soc Am J* 64:613–623
- Ganjegunte G, Condron L, Clinton P, Davis M, Mahieu N (2006) Effects of the addition of forest floor extracts on soil carbon dioxide efflux. *Biol Fertil Soils* 43:199–207
- Halverson LJ, Jones TM, Firestone MK (2000) Release of intracellular solutes by four soil bacteria exposed to dilution stress. *Soil Sci Soc Am J* 64:1630–1637
- Khan K, Müller T, Dyckmans J, Joergensen R (2010) Development of ergosterol, microbial biomass C, N, and P after steaming as a result of sucrose addition, and *Sinapis alba* cultivation. *Biol Fertil Soils* 46:323–331
- Ladd JN, Amato M (1988) Relationships between biomass 14C and soluble organic 14C of a range of fumigated soils. *Soil Biol Biochem* 20:115–116
- Leinweber P, Schulten HR, Korschens M (1995) Hot-water extracted organic matter: chemical composition and temporal variations in a long-term field experiment. *Biol Fertil Soils* 20:17–23
- Madejova J (2003) FTIR techniques in clay mineral studies. *Vib Spectroscopy* 31:1–10
- Montano NM, Garcia-Oliva F, Jaramillo VJ (2007) Dissolved organic carbon affects soil microbial activity and nitrogen dynamics in a Mexican tropical deciduous forest. *Plant Soil* 295:265–277
- Normand P, Ponsonnet C, Nesme X, Neyra M, Simonet P (1996) ITS analysis of prokaryotes. In: Akkermans DL, van Elsas JD, de Bruijn FJ (eds) *Mol Microb Ecology Man*. Kluwer Academic, Netherlands, pp 1–12
- Olk DC, Brunetti G, Senesi N (2000) Decrease in humification of organic matter with intensified lowland rice cropping: a wet chemical and spectroscopic investigation. *Soil Sci Soc Am J* 64:1337–1347
- Plaza C, Senesi N, Polo A, Brunetti G, Garcia-Gil JC, D'Orazio V (2003) Soil fulvic acid properties as a means to assess the use of pig slurry amendment. *Soil Tillage Res* 74:179–190
- Prather MJ, McElroy MB, Wofsy SC (1984) Reductions in ozone at high concentrations of stratospheric halogens. *Nat* 312:227–231
- Ranjard L, Brothier E, Nazaret S (2000) Sequencing bands of ribosomal intergenic spacer analysis fingerprints for characterization and microscale distribution of soil bacterium populations responding to mercury spiking. *Appl Environ Microbiol* 66:5334–5339

- Ranjard L, Poly F, Lata JC, Mougél C, Thioulouse J, Nazaret S (2001) Characterization of bacterial and fungal soil communities by automated ribosomal intergenic spacer analysis fingerprints: biological and methodological variability. *Appl Environ Microbiol* 67:4479–4487
- Roberson EB, Firestone MK (1992) Relationship between desiccation and exopolysaccharide production in a soil *Pseudomonas* sp. *Appl Environ Microbiol* 58:1284–1291
- Roux-Michollet D, Czarnes S, Adam B, Berry D, Commeaux C, Guillaumaud N, Le Roux X, Clays-Josserand A (2008) Effects of steam disinfection on community structure, abundance and activity of heterotrophic, denitrifying and nitrifying bacteria in an organic farming soil. *Soil Biol Biochem* 40:1836–1845
- Saadi I, Borisover M, Armon R, Laor Y (2006) Monitoring of effluent DOM biodegradation using fluorescence, UV and DOC measurements. *Chemosphere* 63:530–539
- Saison C, Degrange V, Oliver R, Millard P, Commeaux C, Montange D, Le Roux X (2006) Alteration and resilience of the soil microbial community following compost amendment: effects of compost level and compost-borne microbial community. *Environ Microbiol* 8:247–257
- Schmitt J, Flemming HC (1998) FTIR-spectroscopy in microbial and material analysis. *Internatl Biodeterioration Biodegrad* 41:1–11
- Serrasolsas I, Khanna PK (1995) Changes in heated and autoclaved forest soils of S.E. Australia. I. Carbon and nitrogen. *Biogeochemistry* 29:3–24
- Sierra MMD, Giovanela M, Parlanti E, Soriano-Sierra EJ (2005) Fluorescence fingerprint of fulvic and humic acids from varied origins as viewed by single-scan and excitation/emission matrix techniques. *Chemosphere* 58:715–733
- Sparling G, Vojvodic-Vukovic M, Schipper LA (1998) Hot-water-soluble C as a simple measure of labile soil organic matter: the relationship with microbial biomass C. *Soil Biol Biochem* 30:1469–1472
- Stromberger ME, Klose S, Ajwa H, Trout T, Fennimore S (2005) Microbial populations and enzyme activities in soils fumigated with methyl bromide alternatives. *Soil Sci Soc Am J* 69:1987–1999
- Tanaka S, Kobayashi T, Iwasaki K, Yamane S, Maeda K, Sakurai K (2003) Properties and metabolic diversity of microbial communities in soils treated with steam sterilization compared with methyl bromide and chloropicrin fumigations. *Soil Sci Plant Nutr* 49:603–610
- van Loenen MCA, Turbett Y, Mullins CE, Feilden NEH, Wilson MJ, Leifert C, Seel WE (2003) Low temperature-short duration steaming of soil kills soil-borne pathogens, nematode pests and weeds. *Eur J Plant Pathol* 109:993–1002
- Yagi K, Williams J, Wang NY, Cicerone RJ (1993) Agricultural soil fumigation as a source of atmospheric methyl bromide. *Proc Natl Acad Sci* 90:8420–8423