Pane et al. Chemical and Biological Technologies in Agriculture (2015) 2:4
DOI 10.1186/s40538-014-0026-9

Chemical and Biological Technologies in Agriculture
a SpringerOpen Journal

RESEARCH Open Access

Effects of on-farm composted tomato residues on soil biological activity and yields in a tomato cropping system

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Abstract

Background: The use of compost may relieve the factors that limit productivity in intensive agricultural systems, such as soil organic matter depletion and soil sickness. Concomitantly, the practice of on-farm composting allows the recycle of cropping green residues into new productive processes.

Results: We produced four vegetable composts by using tomato biomass residues in an on-farm composting plant. The tomato-based composts were assessed for their chemical, microbiological properties, and their effects on soils and plants were evaluated after their application within a tomato cropping system. Compost characteristics affected plant development and productivity through increased nutrient uptake and biostimulation functions. Soil biological activities, including basal respiration, fluorescein diacetate hydrolysis, β-glucosidase, dehydrogenase, alkaline phosphatase, arylsulphatase, and Biolog community levels of physiological profiles, were differently affected by the on-farm tomato-based composts.

Conclusions: Changes in soil activity and community structure due to compost amendments were related to classes of biomolecules such as polysaccharides and lignin-derived compounds, as revealed by nuclear magnetic resonance (NMR) spectra of compost materials. The nutrient content and fertility potential of composts were positively related to the amount of tomato residues present in the feedstock.

Keywords: Carbon structures; C-CPMAS-NMR; Soil microbial activity; Tomato yield; Vegetable compost

Background

On-farm composting is an efficient, cost-effective and environmentally safe biological process for the recycling of residual agricultural biomasses into new cropping production cycles [1]. It is a simple technology consisting of user-friendly small composting plants equipped with tools already available on a farm, where undegraded organic biomasses are transformed and stabilized through an aerobic biooxidation [2]. On-farm composting substantially contributes to solve the problem of disposing agricultural biomasses and vegetable feedstock and concomitantly provides the farmer with a self-supply of quality compost for the improvement of agricultural productivity.

Loss of soil quality is related to soil organic matter (SOM) depletion that is increased by continuous cropping without rotations, frequent soil tillage and large use of both inorganic chemical fertilizers and non-selective pesticides. Intensively exploited soils need an external supply of stabilized organic matter, such as compost, in order to counteract progressive SOM decline. Soil compost amendments contribute to the general soil quality recovery and improvement of plant growing conditions [3] by providing numerous ecosystem services, including replenishment of soil carbon stocks, increase of microbial activity and biodiversity and restoration of plant nutrition and natural soil suppressiveness [4].

In some developed horticultural areas of Southern Italy, significant amounts of agricultural wastes, such as cropping residues, unmarketable products and vegetable processing leftovers, are currently produced. They represent an

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important source of organic matter to be composted and returned to soil. Tomato (*Solanum lycopersicum*) green wastes from greenhouse systems produce about 15 t ha^{-1} y^{-1} of fresh plant residues and are among the most abundant biomasses suitable for transformation in compost.

López-Pérez et al. [5] proposed the direct incorporation of tomato residues into soil as a green biofumigating practice, but it failed in controlling nematode *Meloidogyne incognita* infestation. Risks of plant pathogen dissemination and phytotoxicity hazards are eliminated when an effective sanitation of tomato wastes is achieved through a thermophilic composting process before amendment to soils [6,7]. Although some studies focused on tomato plant composting [8,7], little attention has been so far paid to assess the agronomic effectiveness of the produced compost. By assuming that on-farm composting of tomato plant wastes is the best sustainable practice to improve soil quality, our aim was to investigate (i) the effects of field compost amendments on tomato yields and resulting soil biological characteristics, (ii) the quality of on-farm composts from tomato plant residues in comparison with a commercial organic waste compost, and (iii) the molecular biomarkers which could differentiate tomato-based composts according to different amounts of tomato and other composted additives.

Methods

On-farm composting

Tomato plant residues were used as main compost feedstock, while escarole (*Cichorium endivia*) residues, wood chips and mature compost as starter were also added. The four composting piles had the following compositions: C_1, 17.5% tomato, 15.5% escarole residues, 65% woodchips and 2% mature compost as starter; C_2, 25% tomato residues, 13% escarole residues, 60% woodchips and 2% mature compost as starter; C_3, 37% tomato residues, 11% escarole residues, 50% woodchips and 2% mature compost as starter; and C_4, 50% tomato residues, 48% woodchips and 2% mature compost as starter. All four raw piles were set up with an initial C/N ratio of about 30 in order to hasten the composting switch-on. The mature compost starter was a 2-year-old C_{OW}, purchased at Gesenu (Perugia, Italy). The on-farm composting process was carried out in four parallel static piles of about 6 m^3 in volume, under forced aeration, through an overall 90-day cycle that included a thermophilic and a mesophilic phase, followed by a final curing period. The on-farm composting system was assembled by using currently available tools in common farms. Mechanical aeration was provided by air injection through a net of tubes connected to a blower (0.75 KW) that was periodically activated (5 min every 3 h) with an electronic timer. Pile wetting was achieved through a PVC irrigation system, manually activated on demand (when RH < 50%). Composting temperatures were measured by thermo-sensors placed in the pile core at 15 cm from the pile bottom.

Results

On-farm compost characteristics

Chemical features of feedstock and composts are reported in Tables 1 and 2. Compost samples exhibited a sub-alkaline pH value (>8.0). The levels of electrical conductivity and macronutrients, including N, P and K, increased with the amount of tomato residues used, while, in all cases, the heavy metal contents detected were below risk levels according to Italian laws.

The ^{13}C cross polarization magic angle spinning nuclear magnetic resonance (^{13}C-CPMAS-NMR) spectra of compost materials were characterized by strong signals in the O-alkyl-C (61 to 110 ppm) region, revealing a molecular composition dominated by carbohydrates (Figure 1). In fact, the signals related to O-Alkyl-C components represent most of the organic carbon, accounting for 42.5% up to 56.3% of the total area of the nuclear magnetic resonance (NMR) spectra. The different resonances in the O-alkyl-C region are currently assigned to monomeric units in oligo and polysaccharide chains of plant tissue [9]. The intense signal around 72 ppm corresponds to the overlapping resonances of carbon 2, 3 and 5 in the pyranoside structure in cellulose and some hemicelluloses, whereas the signal at 106 ppm is assigned to the anomeric carbon 1 of the glucose unit in cellulose [10]. The shoulders localized around 62 to 65 and 84 to 88 ppm results from carbon 6 and 4 of monomeric units, respectively. The low-field resonances (higher chemical shift) of each pair indicate the presence of crystalline forms of cellulose, while the high-field ones (lower chemical shift) are assigned to either amorphous

Table 1 Chemical determinations on plant residues used as composting feedstock

	Chemical features											
Residues	**N (%)**	**P (%)**	**K (%)**	**Ca (%)**	**Mg (%)**	**Na (%)**	**Mn (ppm)**	**Cd (ppm)**	**Cr (ppm)**	**Cu (ppm)**	**Pb (ppm)**	**Zn (ppm)**
Tomato	2.1	0.0062	3.04	1.46	0.20	0.57	140.94	0.253	32.33	55.81	1.06	nd
Escarole	3.8	0.0154	3.09	1.01	0.17	0.33	35.71	0.166	4.31	112.75	nd	nd
Woodchip	1.0	0.0004	0.06	0.39	0.06	0.12	7.96	0.143	6.71	5.42	nd	nd

nd, not detected.

Table 2 Main chemical quality characteristics of the composts

Composts	Chemical features													
	pH	EC (mS cm^{-1})	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Na (%)	Mn (ppm)	Cd (ppm)	Cr (ppm)	Cu (ppm)	Pb (ppm)	Zn (ppm)
C_1	8.40	2.69	1.25	0.023	1.46	3.87	1.02	0.20	328.99	0.47	34.80	40.16	4.17	64.62
C_2	8.19	4.12	1.23	0.020	1.21	5.32	1.25	0.15	297.42	0.51	34.84	55.68	4.58	108.50
C_3	8.12	5.09	1.41	0.045	1.99	4.43	1.18	0.22	415.18	0.45	57.96	52.96	4.87	140.40
C_4	8.31	8.92	1.52	0.048	1.92	4.90	1.23	0.15	260.96	0.58	17.99	45.02	3.09	57.06
C_{OW}	8.93	5.07	2.72	0.029	1.18	6.58	0.40	0.27	427.30	0.30	16.05	45.98	28.15	247.40
Legal limits										<1.5	<100	<150	<140	<500

cellulose or hemicellulose structures [11]. The various O-alkyl regions could also include signals related to carbon in the propylic side chain of lignin molecules, whose smaller resonances around 62, 72 and 82 ppm, could be masked by the predominance of polysaccharides. Besides the signals usually assigned to cellulose, the spectra of different composts revealed two additional resonances around 98 and 101 ppm. These signals may be related to the di-O-alkyl-C of, respectively, monomeric units of simple carbohydrates [10] and those of either hemicellulose or pectic polysaccharide chains contained in cell walls of tomato plants, such as α-1,5 arabinan, β-1,4 galactan and α-1,4 galacturonan [12]. The broad peak in the Alkyl-C region (0 to 45 ppm) of the NMR spectra indicated the presence of alkyl chains (-CH_2- groups) derived mainly from various lipid compounds, plant waxes and polyesters. The signal at 56 ppm is associated with either the methoxyl substituent on the aromatic rings of guaiacyl and syringyl units in lignin structures or the C-N bonds in amino acid moieties [9]. Moreover, this O-alkyl region may also include the resonances related to ether and epoxy groups of plant

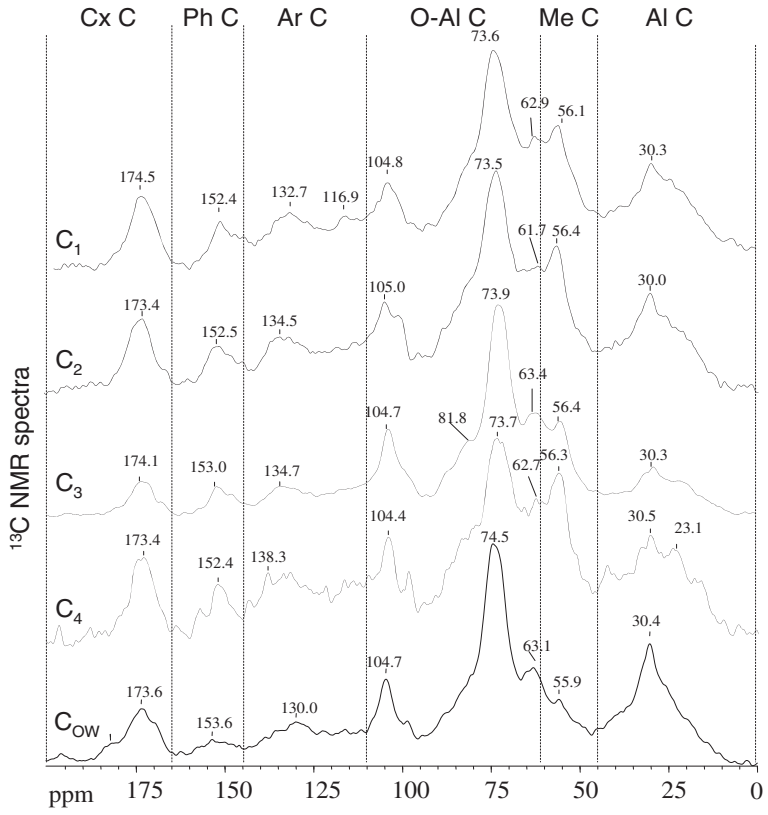

Figure 1 ^{13}C-CPMAS-NMR spectra of compost (C_1 to C_{OW}) samples. Vertical lines delimitate six different spectral regions: aliphatic and aromatic carboxyl C (Cx C, 190 to 166 ppm); oxygen-substituted aromatic C from lignin and non-hydrolyzable tannins, phenolic and O-aryl (Ph C, 165 to 146 ppm); unsubstituted and alkyl-substituted aromatic C, aryl (Ar C, 145 to 111 ppm); anomeric C and di-Oalkyl and oxidized and/or carbohydrate C, O-alkyl (O-Al C, 110 to 61 ppm); methoxyl/N-alkyl (Me C, 60 to 46 ppm); and aliphatic C, alkyl (Al C, 45 to 0 ppm).

Table 3 Relative distribution (%) of signal area over chemical shift regions (ppm) in ^{13}C-CPMAS-NMR spectra of the composts

	Carboxylic-C	Phenolic-C	Aromatic-C	O-Alkyl-C	CH_3O/C-N	Alkyl-C
	195 to 166 ppm	165 to 146 ppm	145 to 111 ppm	110 to 61 ppm	60 to 46 ppm	45 to 0 ppm
C_1	8.56	4.79	13.18	43.41	11.82	8.24
C_2	7.46	4.62	13.12	44.82	12.08	17.90
C_3	5.61	4.15	11.50	52.97	11.39	14.37
C_4	6.25	3.75	14.55	42.41	13.05	19.99
C_{OW}	8.36	3.26	13.08	52.49	9.22	24.84

biopolyesters. In the aromatic/olefinic-C region (111 to 145 ppm), the different resonances around 116 and 130 ppm are related to unsubstituted and C-substituted phenyl carbon pertaining to lignin monomers of guaiacyl and syringyl units [11] as well as to the ring components of plant polyphenols. The signals shown in the specific phenolic aromatic region (146 to 165 ppm) confirmed the presence of O-substituted ring carbon derived from different aromatic structures. In fact, the resonances included in the 148- to 155-ppm range are usually assigned to carbon 3, 4 and 5 in the aromatic ring in lignin components, carbon 3 and 5 being coupled to the corresponding methoxyl substituents. Conversely, the peaks found at 143 and 157 ppm in the NMR spectra of C_4 suggest the significant incorporation of polyphenol derivatives originating from tomato residues [10]. Finally, the broad signal at 173 ppm indicates the contribution of carbonyl groups of aliphatic acids and amino acid moieties in all the compost materials. The ^{13}C-CPMAS-NMR signals exhibited differences among composts (Table 3). The aliphatic alkyl C region (45 to 0 ppm) was most evident in commercial organic-waste compost (C_{OW}), followed by C_1 and C_4 then C_2 and C_3. The CH_3O/C-N region (60 to 46 ppm) was slightly variable among composts. The O-alkyl C region (110 to 61 ppm) was largely developed in C_3, whereas it was less noticeable in the remaining samples. Moreover, the intensity of the region associated with aromatic C (145 to 111 ppm) was large for C_4, decreased in the order passing from C_1 and C_2. Conversely, the spectral regions associated to phenolic C (165 to 146 ppm) was relevant in C_2 and limited in C_{OW}, while that for carboxyl C (195 to 166 ppm) was smaller in C_3 than for the rest of the other composts. Fluorescein diacetate hydrolytic (FDAH) activity resulted as the largest for C_4 and was followed, in the

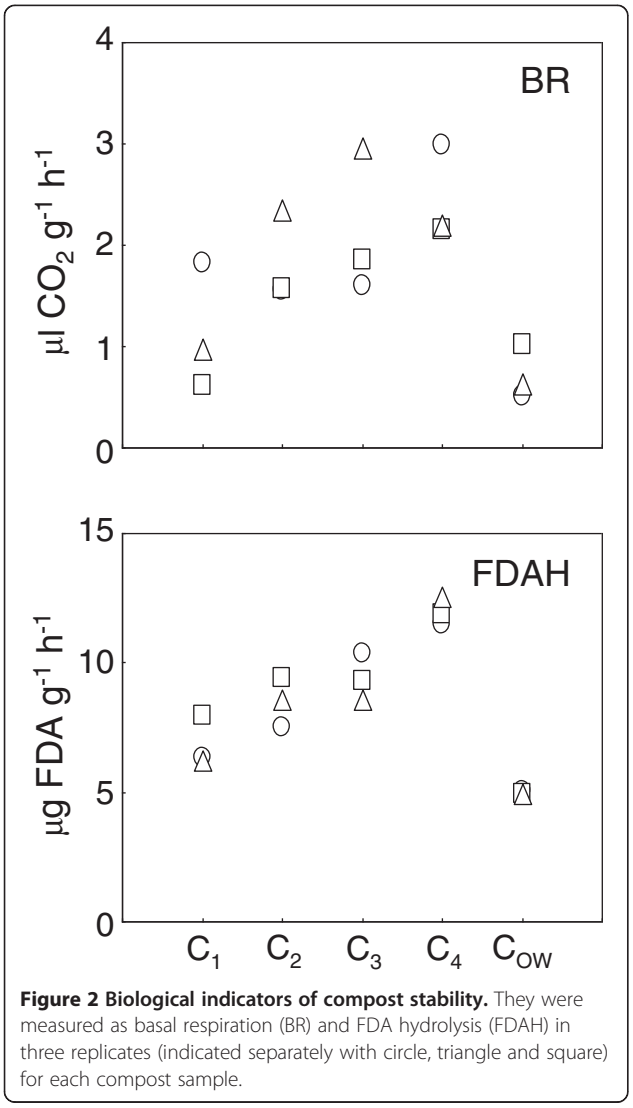

Figure 2 Biological indicators of compost stability. They were measured as basal respiration (BR) and FDA hydrolysis (FDAH) in three replicates (indicated separately with circle, triangle and square) for each compost sample.

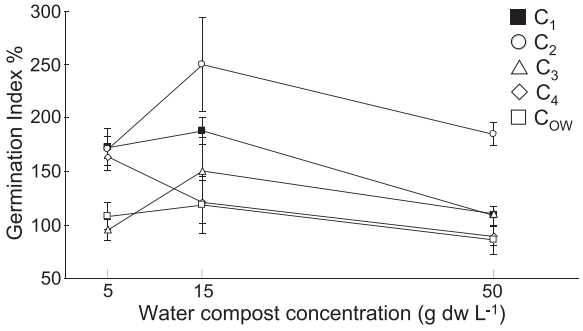

Figure 3 Evaluation of the cress germination index on water extracts of composts. C_1 to C_{OW} samples were assayed at high, medium and low concentrations (50, 16.6 and 5 g l^{-1}, respectively).

Table 4 Effects of soil treatments on tomato cropping response

	Tomato system response						
	Green biomass (Q ha^{-1})	Yield			Quality of berries		
		Total (t ha^{-1})	Marketable (t ha^{-1})	Discard (t ha^{-1})	Weight (g)	pH	Optical residue (°Bx)
C_1	98.05 bc	66.6 de	62.5 c	8.51 a	75.8 a	4.15 a	3.02 a
C_2	117.08 b	83.3 bcd	75.7 bc	10.70 a	84.3 a	4.09 a	3.37 a
C_3	128.13 b	73.6 cde	69.6 bc	8.72 a	78.4 a	4.15 a	2.98 a
C_4	149.38 a	93.1 abc	88.1 ab	13.80 a	86.9 a	4.19 a	3.71 a
C_{OW}	91.81 c	60.6 e	55.1 c	8.35 a	82.0 a	4.03 a	3.03 a
M_{NR}	144.24 a	105.8 a	101.2 a	12.1 a	74.3 a	4.31 a	3.44 a
M_{SR}	148.61 a	100.6 ab	96.7 a	12.2 a	74.9 a	4.20 a	3.56 a
CTRL	109.72 bc	73.0 cde	68.2 bc	12.4 a	81.0 a	4.17 a	2.81 a

Different letters indicate significant differences (ANOVA, Duncan's test, $P \leq 0.05$).

order, by C_3, C_2 and C_1, while C_{OW} showed the smallest value (Figure 2). Similarly, basal respiration (BR) was the greatest for the C_4 compost.

Phytotoxicity of on-farm composts

On-farm compost water extracts proved variable effects on cress germination index percentage (GI%) (Figure 3). In fact, while C_2 showed the lowest toxicity, the one observed for C_{OW} was the largest. Germination was increasingly repressed by on-farm compost extracts passing from C_1 to C_4. In the case of C_1, C_2 and C_3 extracts, the percent cress GI% showed a singular pattern, since it significantly increased at an intermediate concentration, while it dropped back down at the lowest concentration. C_4 and C_{ow} extracts exhibited a dose-dependent behaviour.

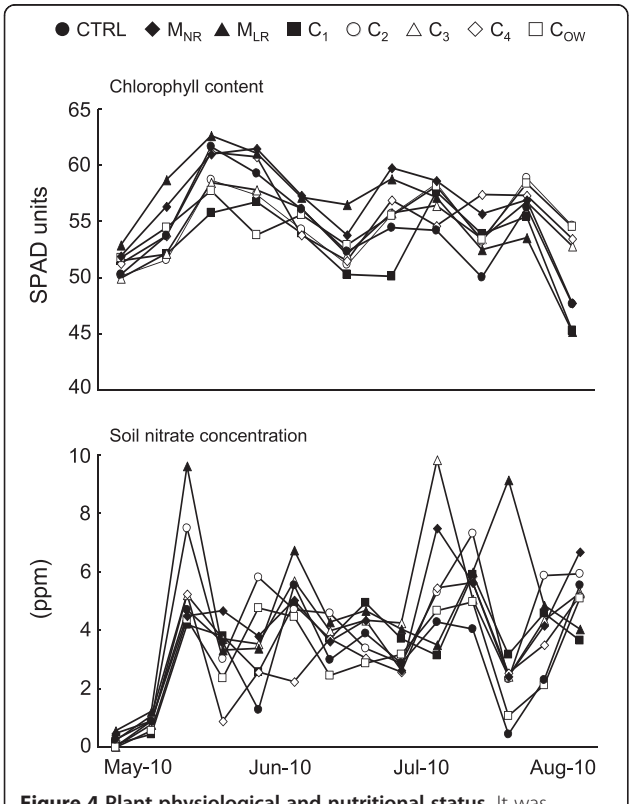

Figure 4 Plant physiological and nutritional status. It was evaluated weakly by the chlorophyll content, assessed by SPAD, and soil nitrate concentration that was available for plant nutrition.

Crop response to soil amendment with on-farm composts

The commercial and total yields of C_4-treated plots were larger by about 20 t ha^{-1} than for control plots. The remaining on-farm composts (C_1, C_2 and C_3) led to increasing yields that did not differ significantly from those of untreated plots (Table 4). On-farm composts did not show any phytotoxic symptoms, whereas C_{OW} that induced the lowest yield caused a slight growth reduction in the early phases of the crop cycle. No significant differences were observed regarding the discarded production. Plant weight was significantly affected by treatments (Table 4). Similarly, berry quality (single weight, pH and optical residue) was not significantly affected by treatments, nor was plant physiologically status, which was generally observed to be at standard levels, as confirmed by chlorophyll content, likely sustained by nitrate availability throughout the tomato cycle (Figure 4). All raw compost eluates showed *in vitro* antibiosis against *Fusarium oxysporum* f. sp. *lycopersici* (data not shown).

Effects of on-farm composts on soil properties

In order to assess the impact of compost amendments on soil properties, a set of biological indicators was used. The BR analyses showed an initial burst of activity due to compost amendments that approached the control over time. Levels of BR were in the following order: C_{OW}, C_4, C_1, C_2 and C_3 (Figure 5). Soil enzymatic activities that were also significantly activated by composts showed a durable effect during the whole incubation time (Figure 5). The largest values of FDAH, β-glucosidase (βGLU) and

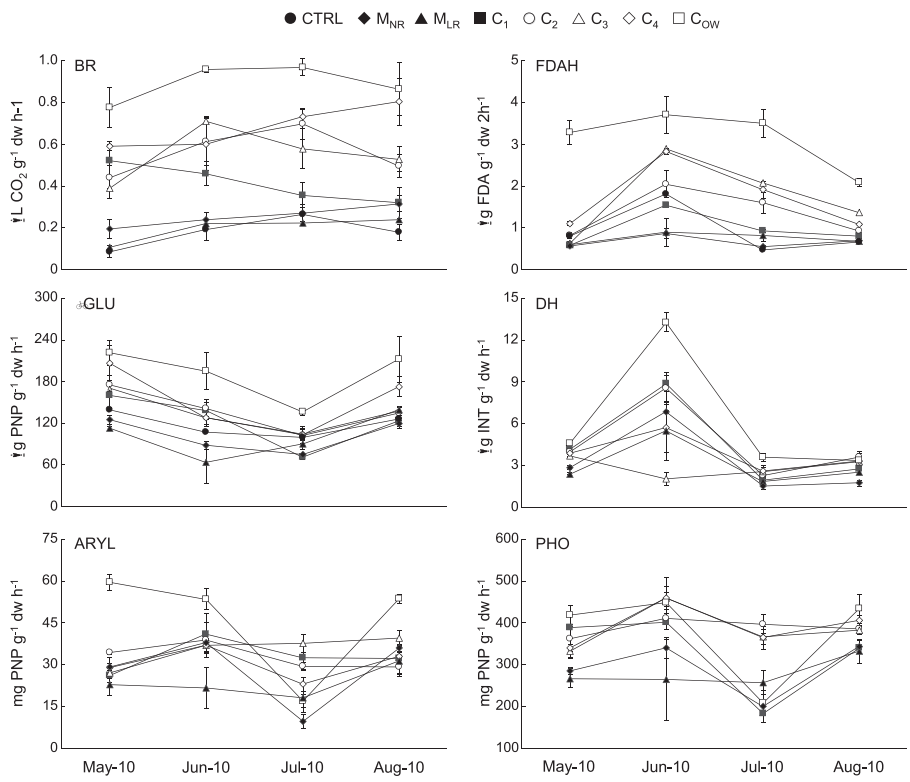

Figure 5 Changes over time in soil BR, FDAH, βGLU, DH, ARYL and PHO. They were assessed monthly in experimental plots after soil treatments.

dehydrogenase (DH) activities were observed in soils amended with the municipal waste compost. Similarly, arylsulphatase (ARYL) and alkaline phosphatase (PHO) activities that resulted in large values for soils treated with tomato-based compost also showed peaks of activity only at the later stage of incubation (Figure 5). βGLU and DH, on the other hand, showed almost constant values over the whole incubation time.

The relationships between carbon distribution in compost and soil enzymatic activities during the incubation period were elucidated by calculating the Pearson's coefficient between these two variables. In fact, coefficient profiles for cumulative regressions were generated: interestingly, it was found that polysaccharides, as well as degradation forms of lignin, produced the most significant correlations (Figure 6). Average well colour development (AWCD) and Shannon index (H′) temporal shifts showed that on-farm composts significantly increased a progressive functional diversity passing from C_1 to C_4 (Figure 7). The soil amended with municipal waste compost showed an intermediate behaviour as compared to that of C_2. However, the activity enhanced by compost treatments slightly, but substantially, regressed at the end of incubation time.

To show differences in microbial community structure, levels of carbon source catabolism were subjected to principal component analysis (PCA). The PC1 explained 61.39% of the variance, while PC2 explained only 12.53% (Figure 7). Along the PC1 axis, the compost-amended plots clustered together and resulted different from the unamended plots, though the C_4 cluster was most distant from the rest. In general, the communities in each plots grouped closely, thus indicating a little influence from sampling time. PC1-variable correlation resulted as significant ($R > 0.60$) and negative for all carbon sources (factor loadings), with the exception of hydroxy benzoic acid, d-malic acid, l-asparagine and phenylethylamine, that were not significantly correlated. Instead, only l-arginine carbon source resulted as significantly correlated ($R = 0.67$) with PC2.

Discussion

Chemical characteristics of agricultural composts and stability

On-farm composting of crop residues is an effective method to produce highly humified organic matter from agricultural green wastes, while they are usefully recycled according to a concept of agricultural sustainability [13]. Moreover, these particular feedstocks significantly influenced compost properties and their ability to condition soil and plant response.

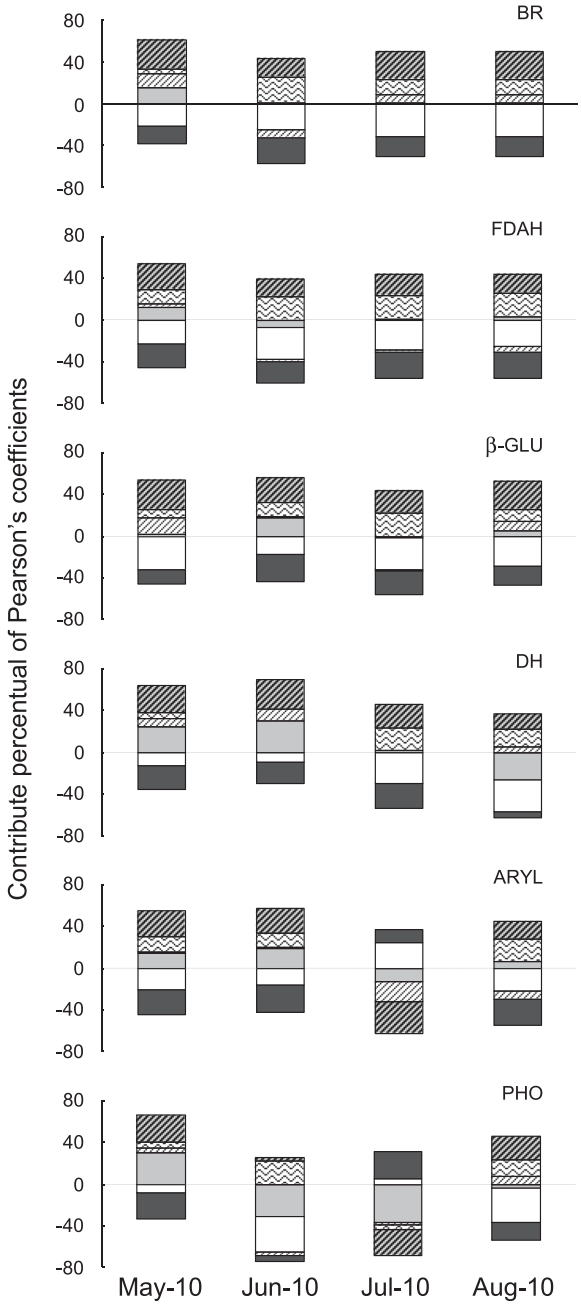

Figure 6 Cumulative regression coefficient profiles produced by cross-correlating soil activities with compost ^{13}C-CPMAS-NMR regions.

Although the ^{13}C-CPMAS-NMR spectra of composted materials indicated an overall similar C distribution, the analysis of specific signals exhibited clear differences in molecular composition. The C_1 and C_2 on-farm composts were characterized not only by cellulosic polysaccharides but also by prominent signals at 30, 56 and 152 ppm, thereby revealing significant amounts of both alkyl components and lignin derivatives. This finding suggests that the inclusion of larger initial rates of stabilizing lignocellulosic materials, represented by wood chips, promoted the incorporation of stable and recalcitrant organic components. Conversely, the NMR spectra of the C_3 compost, showed a lower content of hydrophobic alkyl and aromatic compounds and a corresponding relative increase of more biolabile O-alkyl C components. Among the on-farm composts, evidently different characteristics were found in the C_4 sample, which was made with the initial larger amount of tomato residues. Unlike the previous composts, in addition to the peak at 30 ppm, many distinct signals were shown in the broad alkyl-C region (0 to 45 ppm), thus suggesting the simultaneous presence of different alkyl chains from linear and branched fatty acids and peptidic derivatives [14]. The inconsistency between the sharp intense peaks shown at 56 ppm, as compared to the low abundance of the O-aromatic lignin components in the 148 to 155 interval, also suggested the large contribution of peptidic moieties to the global resonance in the 46 to 60 ppm region, as also indicated by the larger N content found in the C_4 compost. Furthermore, the permanence of biolabile organic compounds was stressed by the peaks positioned at 43 and 98 ppm assigned, respectively, to Cα and Cβ of amino acids [15] and to C1 carbon of monosaccharides components [10]. Lastly, the C distribution found in the NMR spectra of the commercial C_{OW} compost was characterized by the relative predominance of carbohydrates and alkyl-C, combined with the lowest amounts of aromatic and lignin components.

Stability is the compost property, which refers to microbial degradability of organic matter [16]. Changes in biological parameters have been indicated as reasonable and informative markers of compost stability since they shall be linkable with substrate availability for microbial growth [17]. Here, our composted residues showed increasing values of FDAH and BR according to their abundance in phenolic and aromatic-C and the amount of tomato residues used in feedstock. These hydrophobic moieties could be responsible for the maintenance of an unstable carbon reservoir formed by predominating alkyl-C and lignin-deriving compounds which was still subjected to microbial breakdown. These labile carbon pools, possibly, had already been widely removed in the commercial compost.

Effects of on-farm compost on plant growth and productivity

Plant growth sustainability by compost refers to its quality and potential for agricultural applications [18]. Since this property, indicated as compost maturity, is closely linked to the loss of phytotoxicity [19], it can be directly

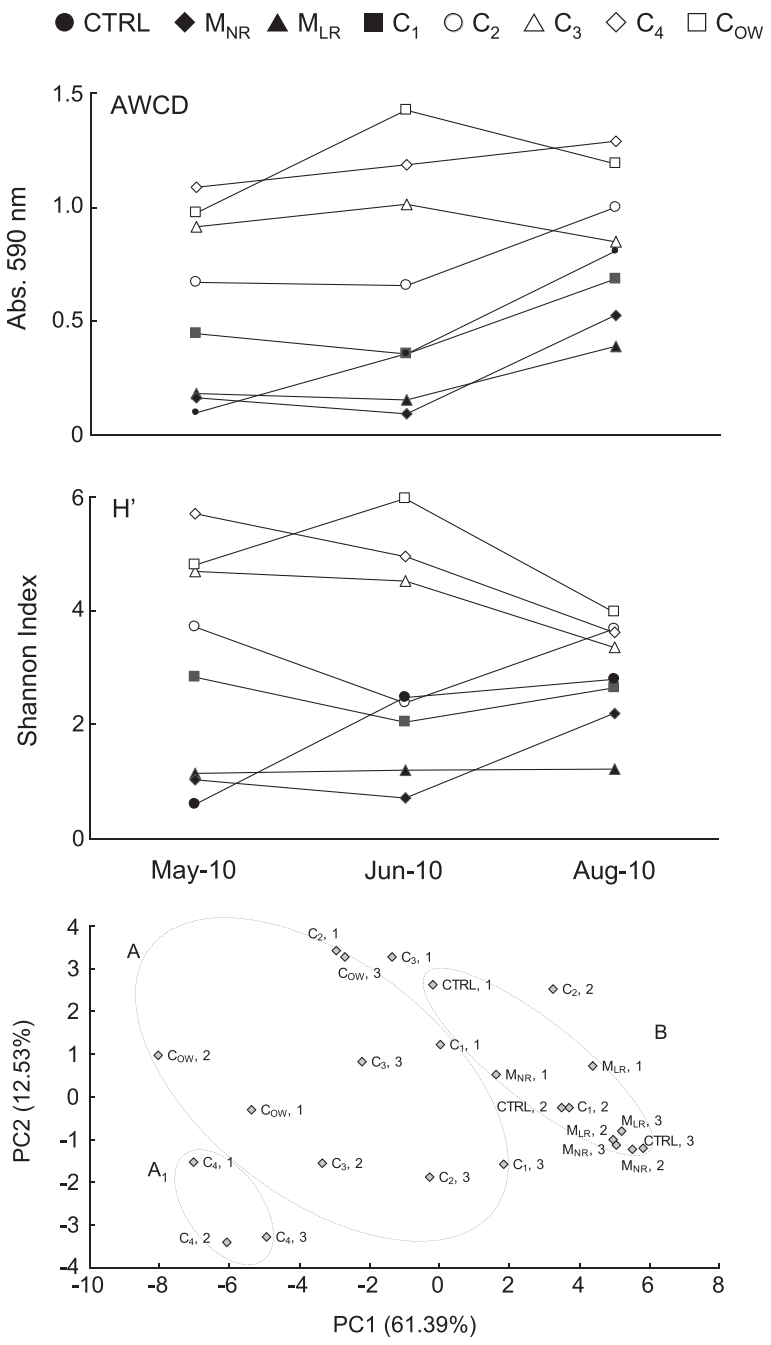

Figure 7 Changes in soil Biolog metabolic activity (AWCD), Shannon Index (H') and biplot PCA distribution of CLPPs. They were assessed in experimental plots after soil treatments at three time points of incubation.

assessed by evaluating the effects of compost eluates on seed germination [20]. In the current study, cress germination assay showed GI% levels exceeding the threshold value of 80%, which is indicative of mature and phytotoxin-free composts [21,22]. In some cases, further diluted compost extracts could have stimulate seed germination. Likely, this could be due to organic molecules dissolved into compost water-extractable fractions, that provide seedling development promotion [22]. However, the detection and the individuation of this kind of molecules from our on-farm composts, needs of further investigations and could be an interesting future perspective of the current research. Growth stimulation effects also may occur thank to the compost humic fraction that, for

example, could be responsible for root proliferation [23,24]. The productive response of the plants to composts may involve firstly nutrient supply and induction of well growth conditions till ripening. Here, tomato yield was observed significantly increased at levels of minerally fertilized plots, under C_4 amendment, as compared to the untreated soil. C_{OW}, instead, although was the most nutrient-richest compost, leads to lowest yields because of the initial detrimental impact on the plants. These findings suggest that the nutritional value alone cannot totally explain the agronomic performances of the composts. But, other factors, such as the absence of phytotoxicity [25], the activation of soil useful microorganisms [26] or disease suppressiveness mechanisms, improvement of soil physical propriety, should be totally considered. Remarkable reduction in crop yield under plant stress due to phytotoxic composts has been widely reported [27]. Furthermore, as development of this work, the extraction and characterization of humic water-extractable fraction in composted samples could contribute to clarify these aspects.

In the current study, the antifungal activity showed by composts against *F. oxysporum* f. sp. *lycopersici* indicated, in addition, their suppressive potential, which was not possible confirm in the field since, under natural conditions, tomato wilt disease did not occurred. Anyhow, a previous study showed that some of these tomato on-farm composts (C_1 and C_4) and C_{OW} significantly suppressed soil-borne diseases caused by *Rhizoctonia solani* and *Sclerotinia minor* and suppressiveness were related to their chemical characteristics and ^{13}C-CPMAS-NMR spectra [28]. Accordingly, Yogev et al. [29] reported the ability of composted tomato plants, to suppress Fusarium wilt on melon.

Effects of on-farm compost on soil biological activities

Compost amendments represent an extraordinary food event for soil microbes that play a crucial role in the turnover of all major plant nutrients. Soil biota, in fact, is involved in the SOM cycle by activating specific enzymatic pathways, through which complex carbon structures are transformed into simple organic and inorganic molecules that can be taken up by plants. External carbon supplies, such as compost additions, cause substantial shifts in SOM chemical composition and soil biological activity profiles [30]. These changes can be followed by monitoring the evolution overtime of soil quality indicators, such as microbiological and biochemical parameters [31]. The respiration rate reflects the instantaneous microbial activation induced by labile-C compounds [30]. In this study, the BR pattern was similar to those described previously by Pane et al. [26] and Cytrin et al. [32] with an initial burst of activity induced by compost, followed by a significant decrease approaching levels of the not-amended soil. In fact, fluorescein degradation due to generalist enzymes, such as proteases, lipases and esterases [33], β-glucosidase and dehydrogenase, exhibited time-shifted trends. The kinetics of these enzymes are closely related to microbial polysaccharide breakdown with the release of low molecular weight organic compounds [31] and organic matter oxidation [34]. Biolog CLPPs showed prolonged changes in microbial structures due to compost supply and were observed over incubation time, as indicated by PCA analysis. More specific enzymatic activities, such as phosphatase, which hydrolyze organic P into phosphates and arylsulphatase, which hydrolyze aromatic sulphate esters into phenols and sulphate [35] showed, instead, different behaviours. Response of soils to amendment may be affected by the molecular quality of composts. The levels of soil activity were found positively related to NMR aromatics and polysaccharides over time, suggesting the involvement of these hydrophobic moieties in the modulation of the labile carbon flux for microbial activation. Phenolic-C passes from negative to positive correlation when sulphatase and phosphatase were strongly induced in soils. A number of previous studies highlighted the role of phenolic SOM in the regulation of soil enzymatic activities [36-38]. Jindo et al. [39] reported the up-regulation of phosphatase and other enzymes in biochar-blended composts, concomitantly to increases in lignin polyphenol oxidation. Accordingly, Grandy et al. [37] and Leinweber et al. [38] found that strong predictive character of oxidation and the depletion rates of plant-derived lignins on the intensity of microbial transformations occurred both in less degraded systems, such as forest ecosystems, as well as in secular cropped lands. Here, on-farm composts were rich in lignin-derived compounds thanks to the large contribution of plant residues. Lignin is a relatively stable constituent of SOM that can support a long-lasting broad-spectrum soil microbial activity [40]. In the present work, solid state ^{13}C-CPMAS-NMR spectroscopy revealed differences in NMR resonance signals characteristic for alkyl C (0 to 45 ppm) and aromatic C (111 to 145 ppm) among composts that may explain the differences in induced microbiological soil activities. The alkyl C spectral region includes the aliphatic macromolecular biolipids that were reported as typical biomarkers in green waste-derived composts [41]. Instead, the aromatic C type includes peaks at 152 ppm (O-substituted C in guaiacyl and syringyl units) and 130 ppm (unsubstituted C in *p*-hydroxy phenyl rings of cinnamic units in both lignin and suberin biopolymers) and indicates that the main components of these composts are just lignocellulosic-derived molecules and hydrophobic alkyl moieties [9]. Therefore, it is possible that these lignin residues can affect indirectly biological soil properties by prolonging carbon availability to microbes over time.

Chemical analyses and ^{13}C-CPMAS-NMR spectroscopy of compost samples

Total *N* was determined according to the Kjeldahl method. The contents of P, Ca, K, Mg, Na, Cd, Cr, Cu, Mn, Pb and Zn were determined, after compost acid digestion with a microwave oven, by ICP-OES (iCAP 6000 Series, Thermo Scientific. Waltham, MA, USA). The water content of the composts was determined after drying at 105°C for 72 h. Compost water-holding capacity was determined by measuring water content held against gravity in a filter-paper-lined funnel. Electrical Conductivity (EC) and pH were determined according to the official methods of the Italian National Society of Soil Science [42].

Molecular distribution of compost organic carbon was evaluated by ^{13}C-CPMAS-NMR spectroscopy. The ^{13}C-CPMAS-NMR spectra were obtained with a Bruker AVANCE™ 300 (Bruker BioSpin GmbH, Rheinstetten, Germany), equipped with a 4-mm wide bore MAS probe, operating at a ^{13}C resonating frequency of 75.475 MHz. Compost samples (100 to 150 mg) were packed in 4-mm zirconia rotors with Kel-F caps and spun at 13 ± 1 kHz. To account for possible inhomogeneity of the Hartmann-Hahn condition at high rotor spin rates, a 1H ramp sequence was applied in CP experiments during a contact time (CT) of 1 ms. The ^{13}C-CPMAS experiments implied a collection of 6,000 scans with 2,266 data points over an acquisition time of 25 ms and a recycle delay of 2.0 s. The Bruker Topspin 1.3 software was used to collect and process the NMR spectra. All free induction decays (FIDs) were transformed by applying a 4 k zero filling and a line broadening of 100 Hz. The areas for different ^{13}C resonances were assigned according to previous reports [4,28,43] into six integrating regions as follows: 0 to 45 ppm (alkyl C), 46 to 60 ppm (methoxyl C), 61 to 110 ppm (O-alkyl C), 111 to 145 ppm (aromatic C or aryl C), 146 to 165 ppm (phenolic C or O-aryl C) and 166 to 195 ppm (carboxylic C). The area of each spectral region was divided by the sum of all spectral areas in order to obtain a relative percentage.

Basal respiration, FDA hydrolysis and phytotoxicity of compost samples

BR and FDAH were measured with a modification of method described by Pane et al. [44]. Basal respiration was from a compost (50-g dry weight) wetted with water up to 80% of its water-holding capacity and placed in a jar (500 ml) with an airtight cap. Released CO_2 was measured using a CO_2 Analyser IRGA SBA-4 OEM (PP Systems, USA).

To evaluate fluorescein diacetate (FDA) hydrolysis, 2.5 g of compost was mixed with 15 ml of 0.2 M potassium phosphate buffered at pH 7.6, followed by the addition of 0.5 ml FDA solution (2 mg ml^{-1}). The mixture was shaken for 2 h in an orbital incubator and the hydrolysis reaction stopped by adding 15 ml $CHCl_3$/CH_3OH (2:1 *v*/*v*). The reaction mixture was centrifuged (700×*g*) and the absorbance of the aqueous supernatant measured at 490 nm.

Composts water extracts (CWEs), prepared by vigorously shaking, were assessed for possible phytotoxicity by measuring germination and root elongation of cress (*Lepidium sativum* L. cv. Comune) under CWE treatments [45], as compared to the control H_2O. Experiments comprised three different CWE concentrations (50, 16.6 and 5 g l^{-1}) replicated 10 times. The number of seeds germinated and root length were recorded after 36 h following germination. GI% that was directly affected by phytotoxicity was then obtained by multiplying the number of germinated seeds by the relative mean root length, expressed as percentage of control accordingly to following formula [20]:

$$\mathrm{GI\%} = \left(\frac{\text{No. of seeds germinated on CWEs}}{\text{No. of seeds germinated on water}}\right) \times \left(\frac{\text{Mean root length on CWEs}}{\text{Mean root length on water}}\right) \times 100$$

Assessment of *in vitro* suppressiveness of compost

In order to evaluate compost suppressiveness, we used the tomato fungal pathogen *F. oxysporum* f. sp. *lycopersici* Sacc. isolated from symptomatic plants. This fungus was maintained on a potato dextrose agar medium and stored in the fungi collection of the CRA-Centro di Ricerca per l'Orticoltura (Pontecagnano, Italy). Raw, autoclaved (122°C for 22 min) and filtered (with 0.22 mm sterilized millipore membrane, following bland centrifugation to precipitate suspended cells) CWEs, further diluted in water 1:10 vol., were used to evaluate the suppressive potential of composts against *F. oxysporum* f. sp. *lycopersici*, according to the well diffusion technique as developed by El-Masry et al. [46] and slightly modified by Pane et al. [47]. Compost pathogen suppression was assessed by measuring the CWE mycelial development inhibition as a percentage of growth reduction compared to the control plates.

Field experimental design and plant parameters

Filed experiments were carried out at the experimental farm of the CRA-Centro di Ricerca per l'Orticoltura, Battipaglia (40°35′02″ N; 14°58′50″ E), Salerno, Italy, on a clay loam soil (8.8-g organic C kg^{-1}, 1.0 g Kjeldahl N kg^{-1}, pH 7.4, 34.6% sand, 36% silt, 29.4% clay, in the top 0- to 0.40-m soil layer) and the experimental design adopted was a complete randomized block with three replicates, each consisting of a plot area of 25 m^2. Eight soil treatments were compared: on-farm composts (C_1, C_2, C_3 and C_4) and municipal organic waste compost (C_{OW}) amendments, mineral normal release (M_{NR}) and

mineral low release (M_{LR}) nitrogen fertilizers; untreated plots (without any fertilizers and amendment) were used as the reference control (CTRL). Composts were applied at a rate of 30 t ha^{-1} dry weight according to previous works [48,26]. M_{NR} and M_{LR} consisted of the application of NPK synthetic fertilizers ($N = 150$ kg ha^{-1}; $P_2O_5 = 60$ kg ha^{-1}; $K_2O = 50$ kg ha^{-1}), in which nitrogen was ammonium nitrate and ENTEC®26 (a fertilizer containing 3,4-dimethylpyrazol phosphate, a nitrification inhibitor). Composts, PK and ENTEC®26 were incorporated into the soil, 1 week before transplanting, by rotovating, at a depth of 10 to 15 cm. Tomato plantlets (cv. Stone) were transplanted (29,000 plants ha^{-1}) in double rows. During the cultivation period, plant physiological status was evaluated by assessing foliar chlorophyll contents with Minolta Chlorophyll Meter SPAD-502 (Konica Minolta Sensing INC., Japan).

At the end of the crop cycle, total and commercial production and relative percentage of discard, as well as single-berry weight, were determined on an area of about 4 m^2 for each plot.

Soil sampling, nitrate determination and microbial activities

From May to August, after soil treatments, soil sampling was carried out by mixing 10 sub-samples that were taken from the top layer (0 to 20 cm) of each plot, sieved (2 mm), selected and stored at 4°C until biological laboratory determination. Soil moisture content was determined by measuring water content after soil drying at 50°C until constant weight. Water-holding capacity (field capacity) was determined by measuring water content held against gravity in a filter-paper-lined funnel.

Nitrate concentration in soil was analysed on weekly collected samples by colorimetric technique using Reflectoquant® strips read by a reflectometer RQflex® 10 (Merck, Darmstadt, Germany).

BR and FDAH were measured as reported above for the direct measure on composts. Soil βGLU and DH activities were determined as reported by Pane et al. [44] using 4-*p*-nitrophenyl-β-D-glucopyranoside (PNP) and 2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyltetrazolium chloride (INT) substrates, respectively. βGLU was determined by adding 0.35 g of soil sample to 2 ml of 0.05 M maleate buffer, pH 5.0. The mixture was left for 5 min at 30°C, and the enzymatic reaction was started by adding 0.5 ml of 0.2 mM PNP. After incubation for 1 h at 37°C, the reaction was stopped by adding 0.5 ml of 0.5 M $CaCl_2$, 2 ml of 0.5 N NaOH and 5 ml of H_2O. After centrifugation at 1,500×*g* for 5 min at 5°C and after filtration of the aqueous phase, the absorbance of filtrates was measured at 398 nm. DH was determined by adding 0.5 g of soil sample to 1 ml of 0.2 M Tris buffer, pH 5.0. The enzymatic reaction was started by adding 0.5 ml of 0.2 mM INT. After incubation by shaking for 48 h at 37°C, the reaction was stopped by adding 10 ml of ethanol 96% *N*,*N*-dimethylformamide (1:1) and incubated in the dark by shaking for 1 h at room temperature. After centrifugation at 5,000×*g* for 5 min at 5°C and after filtration of the aqueous phase, the absorbance of filtrates was measured at 464 nm. Soil PHO and ARYL activities were determined using *p*-nitrophenyl phosphate and *p*-nitrophenyl sulphate as substrate, respectively [49]. A 0.5-g soil sample was added to 2 ml of maleate buffer, pH 6.5. The enzymatic reaction was started by adding 0.5 ml of 0.2 mM substrate. After incubation by shaking for 1 h at 37°C, the reaction was stopped by adding 0.5 ml of $CaCl_2$ and 5 ml H_2O. After centrifugation at 5,000×*g* for 5 min at 5°C and after filtration of the aqueous phase, the absorbance of filtrates was measured at 398 nm. All enzymatic analyses blanks, without addition of a reducing substrate, were also included to correct for background absorbance and the activity was determined against a calibration curve. Absorbance was measured by spectrophotometer model SpectroFlex 6600 (WTW, Oberbayern, Germany). Soil respiration and all enzymatic activities were determined on monthly sampled soils.

Biolog CLPPs were determined on soils sampled, after amendment, at beginning (May), at middle (Jun) and at end (Aug) of cropping cycle, as AWCD and H′ index, as previously developed by Pane et al. [44]. Aliquots of 100 μl of water-extracted soil sample, at a final dilution of 10^{-4} (*w*/*w*), were inoculated into the Eco-microplates. These were incubated at 25°C for 4 days and read, 96 h post inoculum, at 590 nm, using the Bio-Rad Microplate Reader 550 (Bio-Rad, Hercules, CA, USA).

Statistical analysis

Tomato agronomic data were analysed by ANOVA and means were separated by Duncan's test. The relationships among biological activities detected in the amended soils and relative quantities of molecular organic ^{13}C groups of composts were assessed using a regression analysis. Biolog AWCD profiles for single substrates were computed by principal component analysis, performed on OD data of the 31 carbon sources to assess distribution biplot of all community samples.

Conclusions

This study showed the great potential of on-farm technology to produce vegetable composts with peculiar characteristics that are different from commercial composted biosolids. Nutrition and biostimulation effects may be responsible for the increased productive response to agricultural compost amendments in cropping systems. NMR profiling showed that molecular composition of on-farm composts is responsible for microbial degradability in the soil and that phenolic C could play a crucial role in modulating soil biological activities.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
CP, GC and DV participated in composting activities and drafted the manuscript. CP participated to the field trial. DV carried out the microbiological studies. AP and RS carried out the NMR studies and drafted the manuscript. AMP carried out chemical analysis on composts and drafted the manuscript. MZ participated in design and coordination of the study and drafted the manuscript. All authors read and approved the final manuscript.

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Received: 8 May 2014 Accepted: 5 December 2014
Published online: 11 February 2015

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