LIFE Project Number

<LIFE12 ENV/IT/000719>

/ FINAL Report

Covering the project activities from 1/07/2013 to 01/07/2018

Reporting Date

<01/10/2018>

LIFE+ PROJECT NAME or Acronym

<LIFE CARBONFARM>

Project Data

Project location	Italy
Project start date:	<01/07/2013>
Project end date:	<01/07/2018> **Extension date:** <dd/mm/yyyy >
Total Project duration (in months)	<60> months (including **Extension of** <XX> **months**)
Total budget	€ 3,051,265
Total eligible budget	€ 3,036,265
EU contribution:	€ 1,495,027
(%) of total costs	49%
(%) of eligible costs	49.24%

Beneficiary Data

Name Beneficiary	CERMANU
Contact person	Mr Riccardo Spaccini
Postal address	Via Università , n°100, IT, 80055,Portici
Visit address	Via Università, n° 100, IT, 80055Portici
Telephone	39-081-2539 + direct n°176
Fax:	
E-mail	riccardo.spaccini@unina.it
Project Website	www.carbonfarm.eu

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2. Executive Summary (maximum 5 pages)

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2.1 Project guidelines and objectives

The LIFE CarbOnFarm project address the basic requirements concerning the sustainable use of agricultural soils coupled with the economic and environmental valorisation of agricultural biomasses. The Life CarbOnFarm has hence combined complementary objectives focused on the application of sustainable methods of SOM managements. The field activities were tailored on the non-livestock farms of agricultural land of Mediterranean area characterized by limited access to exogenous OM sources, the progressive decline of SOM content and the steady degradation of soil system.

In order to match the challenging multitasks approach of LIFE CarbOnFarm project, the activities involve six public and one private beneficiary: the NMR Research Centre on Agriculture, Food and Innovative materials of University of Napoli (CERMANU); the Agricultural Dept of University Torino (AGROSELVIT-DISAFA); the Dept for Environmental and Cultural studies of Mediterranena regios of University of Basilicata (UNIBAS). a research centre of the Minister of Agriculture for the studies of horticultural and orchard cropping systems (CREA-OF); the Agricultural sectors of Regione Basilicata (ALSIA) and Regione Campania (REGCAMP); the farmer's cooperative PRIMA LUCE.

The consortium of Beneficiaries combines different skills and experiences, thus exploiting the synergy effect produced by the integration of scientific advances of research-based activity (AGROSELVIT, CERMANU, CREA-ORT, UNIBAS), the actual and topical commercial farming requirements (PRIMA LUCE) and the communication and dissemination expertise of Regional Institutions (ALSIA, REGCAMP).

The starting requirement of the project was the acquisition of local availabilities of organic biomasses from agricultural activities, for the attainment of high-quality composts used for soil amendments. The demonstrative actions were based on soil addition with high quality compost obtained by the recycling of local agricultural residues. In farm sites of Campania Region the compost is obtained by on farm composting facilities. In farm sites of Piemonte the compost is supplied by external composting plant, using the available organic biomasses represented by solid fraction from anaerobic digestion of cattle slurry. The demonstrative action is associated with the application of eco-friendly innovative technology in agro-ecosystems, focused on the SOC sequestration with the soil addition of biomimetic catalyst that is expected to strengthen the bio-stability of SOM.

The first achievement is represented by the realization of on-farm composting prototype at the project site of Beneficiary PRIMA LUCE. This action put into practice the combination of sustainable SOM managements with the productive and economical valorisation of residual biomasses from the local farming systems.

The set-up of project activities involved the activation of five farm sites (two in Piemonte and three in Campania) with the inclusion of three commercial farms for a reliable evaluation of economical sustainability of proposed strategies in different cropping systems

The actual correspondence with project objectives was ascertained by the monitoring actions that allowed the acquisition of analytical data concerning compost composition, SOC quantity and quality, GHG emission from cultivated soils, soil stability, crop productivity as well as the environmental, energetic and economical sustainability of the applied methodologies. These parameters may act as valuable feedback indicators used for internal timing adjustment of proposed practices and thereby becoming also a side objective to the development of practical tools to support the decisional and planning processes. Beside the first planned goal based on the activation of effective on farm composting facility, measurable deliverables and outputs were hence represented by the increase of SOC and the improvement of abiotic and biotic SOM quality, the decrease of energetic inputs associated with the maintenance of crop productivity, and the restrain of GHG emission from managed soils.

The dissemination of project background and results was aimed to perform an effective communication on the utilization of recycled biomasses as useful combination of environmental protection with crop productivity, emphasizing their valorisation as important local bio-resources in terms of C sequestration and maintenance of soil functions.

2.2 Project Actions

The project framework is composed by three Implementation actions, six Monitoring Actions and by Dissemination Actions split in eight applicative oriented activities

➢ **Implementation actions**

- *Action B.1. Set up of a composting plant and transfer of 'on farm' composting technologies. Main targets achieved Action completed*

This action was dedicated to the attainment of the on-farm composting prototype and to the subsequent production of green compost. The required transfer of know-how to is completed and the composting process is totally managed by the personnel of the Beneficiary PRIMA LUCE. The composting plan represent the first large facility for the on-farm compost production. It is composed by twelve (12) simultaneous composting lines with a total productive surface of 4000m2. Although the average initial productive capacity of mature compost was lower in respect to the planned yields foreseen in the proposal, the adopted corrective measures based on the improvement of crop residues with high cellulose/ligneous fibre contents (e.g. artychoke, tomato) allowed a reasonable increase on the attained mature compost (3000 ton/year). All the expected deliverables were included in the previous reports

The activation of LIFE on-farm composting prototype has activated local economic investments from commercial cooperative farms with the realization of on-farm composting facilities. All the expected deliverables were included in the previous reports and uploaded in the downloading section of the project websites

- *Action B.2 Set up of laboratory facilities for the production of biomimetic catalyst. Main targets achieved: action completed*

This innovative action was focused on the synthesis and application eco-friendly bio-mimetic catalysts aimed to the in-situ stabilization of soil organic carbon. The activity was partially modified in respect to the original objective of the total in-house synthesis of the biomimetic catalysts. The basic molecule was acquired by external sources, while a collaboration with the Chemical Department of University of Rome allowed to maintain the partial goal for the in-house modification of starting compound for the synthesis of the bio-mimetic catalyst for field application. The planned amounts were obtained and used for field applications in Action B.3.

- *Action B.3. Set up of project sites. Main targets achieved: activity in progress*.

This action represents the additional pivot of the project objectives. It involves the annual field operations (soil treatments, plot surfaces, cropping systems) associated with the application of SOM management strategies, carried out in the following five farming sites

<u>Piemonte</u>

1) University farm Tetto Frati: cropping system maize; 7 soil treatments; Control: no N addition; Conventional: traditional soil management with mineral fertilizers; SOM

managements: commercial local compost from solid digestate (2 doses); fresh solid digestate (2 doses); biomimetic catalyst.
2) Commercial farm Grandi: cropping system - open field horticultural crops; 6 soil treatments; Control: no N addition; Conventional: traditional soil management with mineral fertilizers; SOM managements: commercial local compost from solid digestate (2 doses); fresh solid digestate (2 doses).

Campania

3) University farm of Castel Volturno: cropping system - maize; 4 soil treatments; Conventional: traditional soil management with mineral fertilizers; SOM managements: local on-farm compost from cow and buffalo manure (2 doses), biomimetic catalyst.
4) Commercial farm Prima Luce: cropping system - open field horticultural crops, 4 soil treatments; Control: no N addition; Conventional: traditional organo-mineral fertilization; SOM managements: LIFE on farm compost (2 doses)
5) Commercial Farm Mellone: cropping system - orchards (peach and kiwy), 5 soil treatments; Conventional: no compost – mineral fertilizer; SOM management: 2 types of LIFE on farm compost: winter (*heavy*) and summer (*light*) composts (2 doses) Project activity continue at Mellone farm to complete the crop cycles foreseen in the proposal

- **Monitoring actions**

- *Action C.1 Monitoring of composting processes and characterization of compost quality Main targets achieved; action completed*

This action regarded the evaluation of the processing variables of on-farm composting process and the analytical characterization of compost quality and composition The following parameters were evaluated for 14 on-farm green compost types produced in the LIFE prototype, the on-farn manure based compost used in Castel Volturno and the solid digestate and derived compost used in Piemonte: pH, EC, heavy metals, TOC and total N content, 13C-OC isotopic content, enzymatic activity (FDA), microbial analyses, phytotoxicity/biostimulation, molecular characterization by thermochemolysis-GC-MS and solid state NMR. An additional analysis not foreseen in the original proposal is the application of metagenomic approach to get an insight on the microbial composition of compost materials to correlate with the suppressive properties against plant pathogens.
All the expected deliverables were included in the previous reports and uploaded in the downloading section of the project websites

- *Action C.2 Monitoring of the agronomical, phytopathological and practical sustainability of proposed strategies Main targets achieved; action completed*

This action includes the evaluation of the effect of SOM managements at different project sites. The following parameters have been analysed: soil aggregate stability, SOC and total N content, initial isotopic 13C-OC content, soil humic substances; SOM characterization by TAHM-GC-MS, microbial analyses. According with the expected targets, the results indicate a prospective increase of TOC content in compost amended plots of all project sites and a variation of SOM composition with the inclusion of compost derived molecules in both bulk SOM and humic substances (lignin derivatives, cutin and suberin components). A beneficial effect was found in the biomass composition of amended plots with an overall increase of the main representative classes of microbial biomass including the mycorrizhal fungi. In the second half of project course also the soil treatment with biomimetic catalyst showed a positive response on the increase of TOC content.
The intermediate results were included in the previous reports and uploaded in the downloading section of the project websites. The final deliverable on SOM analyses is attached to the current report in the technical annexes

• *Action C.3 Monitoring of the agronomical, phytopathological and practical sustainability of proposed strategies Main targets achieved; action completed*

This action refers to the agronomic results obtained from the SOM management practices as compared to the conventional local farming methods, for different involved project sites and cropping systems:

1) Tetto Frati: 4 crop cycles: maize yield, physiological status, P and N content; 2) Grandi: 4 crop cycles (lettuce, cabbage broccoli): crop yields, physiological status, P and N content;

3) Castel Volturno: 4 crop cycles: maize yield, physiological status, P and N content; 4) Prima Luce: 4 crop cycles (endive-scarole, pumpkin, broccoli, beans). Crop yields, physiological status, dry matter content 5) Mellone: 3 crop cycles (peach, kiwi): yields, nutrient uptakes and quality indicators (N, P, dry matter content, firmness, colour, solids soluble content, tritable acidity, total nitrogen, total polyphenols, antioxidant activity; photosynthetic status). For this project site an unexpected agronomic problem resulted in a loss of one crop cycle on peach orchard. The acquisition of agronomic data will hence proceed in order to complete the planned crop cycles

The best performances of applied SOM management were obtained for both horticultural crops (larger yields) and fruit orchards (quality improvements). A decrease of total dry yield was recorded in the first two year of SOM management, as compared to conventional fertilization, in maize crops for both University sites, although with comparable amounts of nutrient content. The final result however, indicate a progressive recover of maize yields in compost amended plots thus approaching the project target of the maintenance of soil productivity. Project activity continue at Mellone farm to complete the analyses foreseen in the proposal

The intermediate deliverables were included in the previous reports and uploaded in the downloading section of the project websites. The final deliverable is attached to the current report in the technical annexes

• *Action C.4 Monitoring of greenhouse gases emissions Main targets achieved; action completed.*

The measurements of GHG emissions have been performed at the following project sites:

Castel Volturno 4 year of field measurements, Tetto Frati 3 year of field measurements and laboratory measurements of GHG emission from different organic matrices used in SOM managements, Mellone 2 year of field measurements. An important achievement of this action was the development of a new prototype for the automatic acquisition of field GHG data with a remote control; the system is applied at Mellone project site. Project activity continue at Tetto Catel Volturno, Tetto Frati, Mellone farms to complete the analyses foreseen in the proposal

The intermediate results were included in the previous reports and uploaded in the downloading section of the project websites. The final deliverable is attached to the current report in the technical annexes

• *Action C.5 Monitoring the environmental and economical sustainability of proposed strategies Main targets achieved; Action completed*

This activity was focused on the evaluation of the sustainable feature of proposed methodologies by the Life Cycle Assessment. The LCA evaluation of LIFE on farm composting plant, on farm composting process and the LCA analyses of applied SOM management strategies have been completed. The intermediate deliverables were included in the previous reports and uploaded in the downloading section of the project websites. The final deliverable is attached to the current report in the technical annexes

• *Action C.6 Monitoring the acquired awareness about available techniques for soil organic carbon stabilization and accumulation and bioresources valorisation Main targets achieved; action completed.*

Two typologies of questionnaires have been produced on the assessment of current awareness of farmers on ground hypotheses, working themes and strategies, tested and developed within LIFE CarbOnFarm. Currently about 950 questionnaires have been completed and analysed. The resume of the interviews will be available in the download page of the project website

✓ **Dissemination actions**

- *Action D1 Project website*- the project website is active from December 2013 with the update of project activities, results, dissemination products and communication initiatives;
- Action D.2- This action was focused on the preparation of basic dissemination materials

- 11 initial Notice boards with project backgrounds and objectives: one for each Beneficiary plus 4 (2 in Italian 2 in English) for the participation at Conferences and Meetings; 4 additional Notice boards with the main project results have been attained
- initial brochure with project description; intermediate leaflet and technical report, with the description of project results; the final expected deliverables represented by the final leaflet and technical report were not produced following the acknowledgement of the low communication utility and effectiveness of these materials, acquired during the dissemination events

About 100 meetings and events were organized for different stakeholder categories within the following actions: Action D.3: Workshops, dissemination events = 65; Action D.4 info days for students = 33 Action D.5 Project conferences = 3, with the participation of about 5000 persons

These activities included the organization of a international Summer school and of Training Course on composting process dedicated to Technician and Agronomists

Deliverables associated with meetings and workshops: *Manual on nn-farm composting*; *Manual and toolkit for self-soil evaluation*; 2 videos on on-farm composting prototype and composting process; 3 videos on Mid term and Final Conferences (Potenza, Torino and Napoli); Videos on on farm composting course

.- D8 LIFE Networking: about 10 connections have been activated with other LIFE projects

2.3 Report summary

The present report includes the following information:

➢ Administrative part (chapter 4):

- organigram and tasks of Coordinating and Associated beneficiaries
- description of management system and minor administrative variations

➢ Technical part (chapter 5)

- Implementation actions: description of technical progress and variations in respect to the planned commitments of the activated Implementation actions

List of Abbreviations:

13C/31P-CPMAS NMR (solid state Cross Polarization Magic Angle Spinning Nuclear Magnetic Resonance on 13C and 31P nuclei); FDA: hydrolase activity with fluorescein diacetate method; EMI maps: Induced Electro Magnetism; ESAP software: Electromagnetic Sampling Analysis; GHG: green house gases; N: nitrogen; PLFA: phospholipid- fatty acids; PORF iron-porphyrin/ biomimetic catalyst; S/TOC soil/total organic carbon; SOM soil organic matter; TAHM-GC-MS: off-line thermally assisted hydrolysis and methylation pyrolysis GasChromatography Mass Spectrometry

3. Introduction (1 page)

- Environmental problem/issue addressed

The project intended to address the targets outlined within the Soil Thematic Strategy framework, concerning the sustainable use of agricultural soils, through the restoration and preservation of soil functionalities, counteract soil degradation and enhance the economic and environmental role of soil resource in the agroecosystems. A reference keystone is represented by the widespread decline of Soil organic matter in cultivated lands, which in turns promote as unavoidable collateral effect the progressive loss of soil fertility. The requirements of adequate SOM management for the restoration of soil fertility is even more emphasized in the agricultural areas of Mediterranean regions where 74% of cultivated lands containing less than 2% of organic carbon (3.4% organic matter) in the topsoil (0-30cm).

- Outline the hypothesis to be demonstrated / verified by the project

In this context the main working hypothesis of LIFE CarbOnFarm is the combination of complementary objectives: recover of organic biomasses from residues and by-products of local agricultural activities, conversion in valuable organic fertilizers as those represented by mature compost and the amendment of cultivated soils. These methodologies acted as valuable pathways to valorise the organic biomasses, thus reducing the loss of C ant the expenses related to the disposal, and to reverse the decline of SOC content with long term beneficial effect on soil fertility thereby maintaining the crop productivity, and avoiding a decrease of farmer's income

- Description of the technical / methodological solution

Based on the local productive organizations and regional distribution and availability of organic biomasses, the adopted solution were planned as follow: in Campania region attainment of demonstrative on-farm composting prototype, within a cooperative farm association, for the recycling and reuse of organic biomasses in valuable green compost; in Piemonte region the compost was obtained by the recycle of the solid residue (digestate) deriving from the conversion plants of livestock slurries in biogas. The compost materials were hence used for SOM management within 5 farm sites with representative local cropping systems: maize, horticultural crops, orchards

- Expected results and environmental benefits

The achieved targets are represented by the realization of a demonstrative large facility for the on-farm composting process; effective recycle of agricultural residues into high quality compost; valuable application of SOM managements with an increase of SOM content and quality; maintenance of farm productivity; demonstration of environmental and economic sustainability of proposed strategies, effective transfer on local territory of the proposed approaches

- Expected longer term results

In respect to the possible contribution and update of policy and legislation, a direct consequence of communication of project approaches to local stakeholders, is the reception in the recent Provisions of Regione Basilicata of the validity of on-farm composting chain for the effective recycle and valorisation of agricultural and urban organic wastes. For the long-term relapses on the economic aspect of applied strategies, an achieved target is the already performed replication of additional on-farm composting plants by other regional farmer's organization and the activated collaboration with two main representatives of association of Biological and Biodinamic producers: HUMUS network and ConProBIO. A possible implication of proposed link between the SOC and climate change in agroecosystems, activated in the LIFE CarbOnFarm, is faced by the recent indications provided by FAO organization in the meeting on *SOC: the hidden potential*, and on the indications issued by the LIFE meetings held in Brussels and Madrid, in 2017 and 2018, on the current challenge represented by sustainable land management strategies and climate change

4. Administrative part (maximum 3 pages)

4.1 Description of the management system

The start of project activities was focused on the preparatory Action A1, which included the starting definition of operative conditions and requirements for the realization of on-farm composting prototype (Action B1), the modification of Action B.2 and the organization of field activities (Action B3). The initial planning regarded also the monitoring checks associated with composting facility and field managements (Action C1-C4), with the definition of working protocols, project and local responsibilities. In this period each Beneficiary has individuated the respective responsible for the Technical and Administrative management, as well as the assignment of specific roles for the local commitment in Monitoring and Dissemination tasks. A second step, starting from the 2014, was represented by the operative set up of project sites (Action B3) at four farming systems (Grandi, Tetto Frati, Castel Volturno, Prima Luce). As foreseen in the proposal, the set-up of the fifth farming site, Mellone, started at the end of the 2014. Notwithstanding with the modification of the commitments related with the synthesis of bio-mimetic catalyst (Action B2), the activity started in line with the field management. After the first organization period (9-12 months) the operations related to the Implementation and Monitoring Actions were carried out following the initial planning at the different project sites. A steady local evaluation, at each project site, of the correspondence or deviation from the scheduled programme, was performed in the I and IV trimesters of annual activities. The periodic reporting and update of project progress was based on monthly video-conferences and on the Project Committee Meetings. With respect to the project phase related to Communication activities, excepted the Initial Conference and the starting operative dissemination tool (Notice boards), the main dissemination events and products were performed since the second trimester of 2014, following the achievement of the first project objective represented by the on-farm composting prototype.

• Presentation of the coordinating beneficiary, associated beneficiaries and project organisation
Please find in Annex 8.5 and 8.6 the Gannt scheme of project activities and the Project management chart

Coordinating beneficiary CERMANU

Project responsible for Actions: A1, B2, B3, C2, C4, D7, D8, E1; Local responsibilities for Actions C1, C3, D3, D4, D5; Supporting activities for Actions C5, C6, D1

❖ Mr. Riccardo Spaccini, Associate Professor– Project Manager: responsible for planning and coordination of technical activities and fulfilment of administrative obligations and for the fulfilment of project goals. Crossing point for coordination of main dissemination products (e.g. Website, Conference, Manual etc.),

❖ Mr. Alessandro Piccolo, Full Professor, Director of CERMANU: - Technical manager for CERMANU: responsible for the fulfilment of project activities and for the accomplishment of tasks under the responsibility of CERMANU. Direct responsible for CERMANU of the Dissemination activities in Campania Region in collaboration with REGCAMP

❖ Mrs. Filomena Sannino, Senior Researcher – Responsible for Action B.2 related to the acquisition and utilization of biomimetic catalyst. Involved in the organization and realization of dissemination activities of CERMANU

❖ Mr. Enzo Di Meo , Qualified technician; responsible for the activities included in Action B.3 *Set-up of project sites*, for the project site of University of Napoli Castel Volturno. Involved in the dissemination activities of CERMANU

❖ Mrs. Valentina Gagliotti, technician, administrative officer; responsible, in coordination with the Project manager, for the administrative tasks

❖ Mrs Vincenza Cozzolino: temporary staff hired for the project. Responsible for Actions C1, C2, C3; supporting activity for Actions B3, C4

Associated Beneficiaries

ALSIA: Project responsible for Action D3, D5. Local responsibilities Actions: D2, D6, E1; Supporting activities Actions D1, D4, D7. Permanent staff: Mr. R. Sileo (Official Manager) Role in the project. Technical manager, Responsible Action D.3; Mr. G. Ippolito (Functionary) Role in the project responsible action D5; Mrs. M. Lombardi (Functionary) Role in the project: Responsible for Action C.6 and *Manual and kit for self- Soil Evaluation*; Mr. A. De Rosa (Technician) Role in the project Administrative officer. The additional personnel is represented by Technicians involved in the organization of activities of Action D.3, D4, D5 and associated Deliverables (*Manual on on-farm composting*; *Manual and kit for self- Soil Evaluation)*

AGROSELVIT: Project responsible Actions C5, C6; Local responsibilities Actions: A1, B3, C1, C3, C4, D3, D4, E1; Supporting activities Actions D1, D5, D7, D8. Permanent staff: Mr. C. Grignani (Full professor), Role in the Project: Technical Manager; Mrs. L. Zavattaro (Technician), Role in the project: responsible Action C.6; Mr. M. Gilardo (Technician), Role in the project: responsible Actions B.3, C3; Mr. W.Gaino (Technician), Role in the project: responsible Actions B.3, C3. Temporary staff: Mrs. C. Bertora (Post.doc fellow), Role in the project: responsible Action C4; Mr. Stefano Gaudino (Post.doc fellow), Role in the project: responsible Action C5

CREA-OF: Project responsible Actions C.3; Local responsibilities Actions: A1, B.3, C1, C2, D3, D4, E1; Supporting activities Actions D1, D5, D8. Permanent staff: Mr. M. Zaccardelli (Senior reseracher), Role in the project: Technical manager; Mrs. A. Napolitano (Senior researcher), Role in the project: Responsible Actions C1, C3; Mrs. P. Ioveno (Researcher), Role in the project: Responsible Action C2; Mr. F. Raimo, C. Pane (Researchers), Role in the project: Responsible Action B.3, Supporting Action C.3; Mrs. G. Alfano, Mr. G. Di Stefano, Mr. A. Petangelo (Technicians), Role in the project: Supporting Actions C1, C2, C3. Mrs M. Di Stefano (Technician), Role in the project: Administrative officer

PRIMA LUCE: Project responsible Actions B1; Local responsibilities Actions: A1, B3, E1; Supporting activities Actions D3, D4, D5. Pemanent staff: Mr. D. Esposito (Manager), Role in the project: Technical manager; Mrs. P. Gagliardino (Technician), Role in the project: Administrative officer. The additional personnel included in the Financial report is represented by Specialized Farm Workers for the activities related with the management of On farm Composting process and compost distribution (Actions B1, B3)

REGCAMP: Project responsible Actions D1, D2, D6; Local responsibilities Actions: A1, D3, D4, D5, E1; Supporting activities Actions D7, D8. Pemanent staff: Mr. A. D'Antonio (Managerial staff), Role in the project: Technical manager, Responsible of Administrative tasks; Mr. G. Marseglia (Functionary), Role in the project: Responsible for Action D.1; Mr. F. Ferrer (Technician), Role in the project: Graphical and editorial unit, responsible Action D2

UNIBAS: Project responsible Actions C1, D4; Local responsibilities Actions: A1, B1, B3, C2, C3, C4, D3, E1; Supporting activities Actions D7, C5, C6, D1, D5, D6, D8. Permanent staff: Mr. G Celano (Associate professor), Role in the project: Technical manager (2013-2016; Mr. Cristos Xiloyannis (Full Professor), Role in the project: Responsible for Dissemination activities; Mr. Vitale Nuzzo (Associate professor), Role in the project: Responsible for Action C1, Technical manager from 2017; Mr. G. Montanaro (Technician/Researcher), Role in the project: Responsible for Action B.3; Mr. B. Onorati (Technician), Role in the project:

Responsible for Action C2; Mr. S. Manfreda (Associate Professor), Role in the project: Responsible for Action C3; Mrs. A. Sole (Full Professor), Role in the project: Responsible for Action C4; Mr. B. Dichio (Associate professor), Role in the project: Responsible for Action C5; Mr G. Pentasuglia (Technician), Role in the project: Administrative officer

- Description of changes due to amendments to the Grant Agreement.

Two main modifications have been introduced with the amendment of the Grant Agreement, regarding the replacement, of the original Associated beneficiaries Regione Basilicata (REGBAS) and Terra Orti,, with the respective internal components represented by ALSIA and Prima Luce, in the order. The Amendment to Grant Agreement has been approved by the Commission with the communication sent on 28/04/2014 (Ares 2014 1326270), thereby becoming fully active from the 1st December 2013. This modification did not produce any variation in the allocated budget.

The Partnership Agreements signed with the Associated Beneficiaries involved in the original proposal were submitted with the Inception Report on 03/03/2014. The Partnership Agreements signed with the new incoming Beneficiaries ALSIA and Prima Luce, after the approval of Amendment to Grant Agreement, were submitted with the Progress Report on 16/03/2015

4.2 Evaluation of the management system

All the associated beneficiaries carried out a fruitful collaboration in the management of technical and administrative tasks. Each beneficiary has appointed a technical manager and administrative officer which collaborated with the coordinating beneficiary. A change in the Technical Management of Beneficiary UNIBAS was introduced since 2017 with the replacement of Prof Celano, enrolled by University of Salerno, with Prof Vitale Nuzzo. An administrative modification has regarded the associated beneficiaries CREA-ORT that has modified the official name in CREA-OF since the 2nd half of 2017

A monthly contact of Project manager with the assigned representative of Monitoring team, has been activated since the start of project activities, for the steady report and update on project progress mainly based on short resume of current activities Six annual Monitoring visits were performed with the representative of Monitoring team

5. Technical part (maximum 50 pages)

5.1. Technical progress, per task

In respect to the request of clarification and the indication on Technical Issues, included in the Commission Communication (Ares(2018)4574194 - 06/09/2018), the corresponding explanations were included in the **Annex 8.3 Answer to the EC-s issues** and detailed in the specific actions, as outlined hereafter:
Point 1- Action B3; **Point 2** - Action C.2; **Points 3, 4, 5** - Action C.4; **Point 6** - Action D.2; **Point 7 –** Action D.3; **Point 8 –** Annex 7.3.3 Other Dissemination Annex: Resume of Dissemination events: **Points 9, 10, 11 –** Action D.5

5.1.1 **Action A1** *Technical planning*

Foreseen start date: 1 July 2013	Foreseen end date: 20 August 2013
Actual start date: 1 July 2013	Actual end date: 31 December 2013

Milestones
Technical planning Foreseen date 20/8/2013 Actual date: 31/12/2014
Deliverables
1) Detailed working protocols for C.1, C.2 and C.3 actions
Foreseen date 30/09/2013; Actual Date: 31/12/2014 Annexed to the Inception Report
2) List of selected composting plant for farm sites located in Piemonte
Foreseen date 30/09/2013 Actual date: 31/12/2014 Annexed to the Inception Report
3) Technical design of on-farm composting facility
Foreseen date 30/09/2013; Actual date 30/4/ 2014 Annexed to the 1st Progress Report

In compliance with the proposed schedule, a technical planning was activated at the project start as Preparatory action (Action A.1). This action was focused on the planning and organization of project activities:

- planning the technical requirements (timing and organization) to build up the on-farm composting facility (B.1)
- planning the technical requirements for the production of the biomimetic catalyst (B.2)
- organization of field activities (B.3)
- individuation and selection of composting plan, selection of the organic biomasses that will be used for compost production for the farming sites located in Piemonte Region
- coordination of the monitoring activities and protocol definition for the monitoring of the impact of the project actions (C.1, C.2, C.3., C.4, C5, C6)

The extension of the Technical planning beyond the planned end date, was related to the settlement of management and operative technical issues The main problems were related to the administrative/organisational problems to the role of the two Beneficiaries OP Terra Orti and Regione Basilicata, and to the organization of the working activities related to the synthesis of biomimetic cataaltst (Action B.2). The participation of the farmers organization named Terra Orti was related to the realization of the prototype for on-farm composting systems (Action B.1) The inclusion in the proposal of this organization was based on the expected impact of the project actions, related to compost application, on the local agricultural territory. However, for the administrative point of view, all the required authorizations, from local public authorities, could only be assigned to individual agricultural companies, which are the legally owner of the lands. In order to overcome this problem and to avoid any modification to the financial framework and to the project budget and to maintain both project strategies and objectives, the management board of LIFE CarbOnFarm conceived the possibilities to modify the partnership in the Grant Agreement, with the replacement of Terra Orti organisation with a single associated of the Terra Orti organisation, PRIMA LUCE as Beneficiary of LIFE CarbOnFarm project. With respect to the Public beneficiary Regione Basilicata, its role in the project was heavily reduced during the revising process of the proposal. In fact the Regione Basilicata was involved in the original Action C.5 "Estimation of carbon sequestered in soils" that was completely removed by the Commission during the revising process. The role of the Public Beneficiary Regione Basilicata was therefore essentially related to communication and dissemination activities. To this respect the Regione Basilicata has an operative agency (Lucana Agency for Innovation and Development in Agriculture -ALSIA) specifically dedicated to the dissemination and innovation in agricultural sector. The involvement of ALSIA agency in the project will improve the operational and functional management of project activities and the efficiency of the communication and dissemination actions. However, since the ALSIA agency is a Public company with a proper distinct legal status it could not serve as subcontractors in External assistance for the Regione Basilicata. The direct inclusion of ALSIA agency during the revising process was not possible since the company was without a legal representative and was under the Regional control. Following administrative settlement of ALSIA, the project Committee conceived the possibility to involve directly involved in the project with a modification of the partnership of the Grant Agreement. The technical drawback was related to the Action B.2 “Set up of laboratory facilities for the production of biomimetic catalyst”. This action was focused on the synthesis and the attainment of target amounts of the porphyrin-based catalyst for the application of innovative SOM management in the subsequent implementation Action B.3. The unforeseen delay in the implementation of the new laboratory of the Dept. of Agriculture of University of Napoli, has prevented the realization of the pilot plan for the scale up of the synthesis of the biomimetic catalyst. Therefore, the alternative plan, already included in the original proposal, was activated during the technical planning as indicated in the specific contingency plan.

5.1.2 **Action B.1** *Set up of a composting plant and transfer of 'on farm' composting technologies.*

Proposal start date: 1 July 2013	Proposal end date: 31 December 2013
Actual start date: 31 December 2013	Actual end date: 31 May 2014

Milestones

1) Composting plant construction Foreseen date: 31/12/2013; Actual date 31/5/2014
2) Set up of the controlled composting procedure Foreseen date 10/04/2014 Actual date 31/7/2014
3) Production of different types of compost and their quality characterization Foreseen date 15/04/2014 Actual Date: 30/10/2014
4) The production of a high-quality compost in the 95% of the composting cycles Foreseen date 30/04/2018 Actual date 31/12/2016

Deliverables

1) Economical evaluation of the composting procedure Foreseen date 31/07/2014 Actual date: 31/12 2014 Annexed to the 1st Progress Report
2) Draft of standard composting procedure Foreseen date 30/09/2014 Actual date 31/12/2014 Annexed to the 1st Progress Report
3) Report on B1 action Foreseen date 30/01/2015 Actual date 30/01/2015 Annexed to the 1st Progress Report

Beneficiary Responsible PRIMA LUCE

Responsibilities in case several beneficiaries are implicated: UNIBAS was involved in the technical support for the devising and planning of compost facility, and in the initial transfer of know-how for the set-up of correct composting procedure

The scheduled milestones related with this implementation action may be split as follow:

- the set-up of composting plant has been actually completed within the III semester 2014 with the attainment of the LIFE of farm composting facility and the transfer of the know-how for the composting procedure;
- the use of prototype for the achievement of on farm compost is currently in progress and will continue after the end of LIFE project

This activity was postponed in respect with the planned initial set up for the delay associated with the amendment to Grant Agreement related to the replacement of initial Beneficiary Terra Orti with the current Beneficiary Prima Luce. The main problems encountered where related to the administrative problems for the realization of the first large prototype of on-farm composting facility, which exceeded the minimal requirements usually requested for the managements of organic residues in a small farm. Therefore, a collaboration was activated by the involved Beneficiaries, PRIMA LUCE and UNIBAS, supported by the coordinating Beneficiary, with the local administrations for the definition and acquisition of different administrative permissions, given the lack of specific regulations and clear institutional referents. The maximum efforts were hence sustained by the project management and by the Beneficiaries PRIMA LUCE and UNIBAS to overcome all the initial constraints and drawbacks. With a delay of about 5 months with the scheduled programme, the implementation Action B.1 was completed within II the trimester 2014, with the achievement of the LIFE on-farm composting plan at the Project site of Associated beneficiary Prima Luce. All the technical data related to the composting plant have been already submitted and uploaded in the project website. The composting plant has a total area of about 4000m^2, with 2400m^2 under coverage.

the transfer from UNIBAS of composting technologies and know-how, to the management of beneficiary PRMA LUCE has been completed. The beneficiary Prima Luce is currently the main responsible for the attainment of compost and the management of the composting plan. The Action B.1 is hence focused on the attainment of on farm compost for field tests plots located in the horticultural and orchard systems. The actual composting activities is based on multiple (12) simultaneous lines of compost production, with two main types of composts based on either vernal/summer or winter horticultural crops, and woody structural materials, using the cultural residues from agricultural activities of both, beneficiary Prima Luce and on the contribution of the other farms belonging to the farmer association. Particularly, since the yield in compost appears to be strongly influenced by the type of input material and especially by its dry matter content, two different types of compost were obtained: "heavy compost" and "light compost". The first type was obtained with the use of nutritional matrixes with a high dry matter content (i.e. tomato plants, pepper, walnut husk, artichoke, etc.) and, therefore, characterized by an high yield into compost. Instead, the "light compost" (the most common product at “Prima Luce” composting plant) was obtained from matrices with a low dry matter content (i.e. Lettuce, arugula, fennel, etc.) which typically determine low yields of the final product. Therefore, with regard to the composting process the main operative problems are mainly related to the huge amounts of fresh organic biomasses and residues which would require a corresponding complement with ligneous-woody fractions, not always available in the associated farms of Terra Orti, for the timing required for the 12 composting lines activated. The periodical shortage of raw woody fraction is partially faced with the recycling of coarse ligneous fraction with the final refining and screening procedure of mature composts. This lack of structuring fraction has as a direct consequence a decrease on the yield in the dry weight compost produced and the relative increase on the specific costs for tn unit of final dry composts. The actual costs breakdown for 20 years of composting activity (including all fixed and operative expenses) yield a result of 168 €/tn for light compost and 98 €/tn for heavy compost. These amounts refer to dry materials and include also the costs for transport of biomass to composting facility and the expenses related to field distribution. The starting working hypothesis was based on a produced annual amount of about 5000 ton of fresh mature compost, with an average of 10 tons ha^{-1} for soil amendment. The amount of mature compost was calculated, in the proposal, from the final 50 % yield from the following estimated amounts of available crop residues

Crop residues and plant biomasses for on farm compost production

Residue tipology	% Water content	% C Dry Weight	% N Dry Weight	C/N	Residue amount (tons/year - Fresh Weight)
Fennel	92,0	55,1	6,0	9,2	1700
Cauliflower and cabbage	90,0	55,2	2,8	19,4	250
Salad	91,0	55,2	4,1	13,4	260
Tomato	40,5	46,6	1,8	25,3	5000
Pepper/eggplant	41,0	42,3	3,2	13,4	2500
Cantalupe melon/pumpkin	87,5	42,0	3,0	14,0	500
Watermelon	88,0	42,0	3,0	14,0	150
Total (Substrates with nutritional function)	**56,5**	**45,5**	**2,4**	**19,0**	**10360**
Structuring material (pruning material + Sorghum)	**60,0**	**48,0**	**1,0**	**48,0**	**1772**
Total (substrate mixture)	**57,0**	**45,8**	**2,2**	**20,8**	**12132**

In respect to these estimated availabilities, lower amounts were currently processed with respect to high dry weight residues represented by artichoke, tomato, pepper, eggplants funnels thereby reducing the annual amount of fresh mature compost (3000 tn/year). As outlined hereafter in the dissemination activities the achievement of the composting plant has produced a “flywheel” response with respect to the innovation related with the recycle of organic residues in the on-farm compost, thereby engaging the partners (ALSIA, UNIBAS) to develop a large range of dissemination activities. This has promoted an effective transfer and replication op prosed approach and activity with the request of other farmer association to have a technical support for the development of similar composting plants. For the operative point of view, as outlined in the Financial Chapter 6 of the present report, in comparison with the planned budget, a larger amount of personnel was requested in the activities related to, both, the set-up of composting plant and especially for the planning, organization, activation associated with the transfer of know how (beneficiary UNIBAS), and with the daily working management of composting process (beneficiary PRIMA LUCE). The production of on farm compost is becoming a key central aspect for the operative farming activities of beneficiary PRIMA LUCE, which main productive target is based on biological products. Therefore, the operation of composting plant and the attainment of green compost is expected to continue without any problems beyond the time limit (5 years) after the end of LIFE project, foreseen in the Common Provisions and in the Grant Agreement for the attainment of the composting prototype.

5.1.2 **Action B.2** *Set up of laboratory facilities for the production of biomimetic catalyst.*

Foreseen start date: 1 November 2013	Proposal end date: 31 March 2017
Actual start date: 1 November 2013	Actual end date: 31 March 2017

Milestones

1) onset of laboratory facilities for synthesis of biomimetic catalyst (Iron-porphyrin)
   Foreseen date 01/11/2014 Actual date:1/11/2014
2) Attainment of steady production of biomimetic catalyst
   Foreseen date 15/04/2014 Actual date: 28/2/2015

Beneficiary responsible CERMANU

This Action regards the attainment of the eco-friendly compound for the innovative technological practice of in-situ SOC stabilization, based on field application with bio-mimetic catalyst (iron-porphyrin). This molecule is expected to support the formation of chemical covalent bonds between the SOM components (mainly lignin and polyphenol derivatives) thereby strengthening the biostability organic inputs and slackening the SOC decomposition. The implementation of Action B.2 has been partially modified with respect to the planned objective to develop an *in-house* synthesis of the biomimetic catalyst (iron/manganese-porphyrin). This contingency was already envisaged among the possible constrains in the Grant Agreement and the documents related with technical modifications have been already submitted in the previous reports (Inception and Progress report).

The technical change has proceeded as follows:

–an initial attempt was done to evaluate the possibility to carry out an outsourcing of the in-house synthesis of the catalyst; in this respect an agreement was established with the SienaBiotech company. Despite the technical feasibility to establish the synthesis of the catalyst, the diseconomies of the process at small technical scale should have resulted in an increase of the planned budget

–the activity has been hence structured with the acquisition of the basic molecule (porphyrin) from international suppliers, followed by the “catalytic activation” based on metal insertion and addition of sulfonate side groups, in order to increase the water solubility of the catalyst required for the field application and for the improvement of reacting activity. This partial in-house synthesis achieved trough a collaboration agreement with the Dept. of Chemical Science and Technology of II University of Rome-Tor Vergata. This activity allowed to keep both technical objective and planned budget. The agreement with the Chemical Dept. of University of Tor Vergata, has partially maintained the operative in-house modification of chemical structure of the catalyst: this is a key point for the synthesis of an eco-friendly biomimetic catalysts with the attainment of final water-soluble product which allow an easy application to the cropped fields with normal available systems and equipment used for the soil management without any required specific technology, thus avoiding additional costs for soil treatment

Both the above cited initiatives were already included in the proposal as possible alternative solutions for the constraints and risks related to the synthesis of biomimetic catalyst.

The modification did not produce a variation in the total budget, with a change of allocated amounts betin the financial categories Consumables and External assistance (please refer to the comments provided in the financial section 6.1)

In order to maintain the innovative aspects on SOC sequestration based on the field application of biomimetic catalysts, and to reduce the risk of delays in the planned activities, the first amount of biomimetic catalyst for the initial set up of project sites in 2014 was provided by the stocks of CERMANU. The CERMANU has in fact available stocks of iron and manganese porphyrins derived from the well documented long term (15 years) experience in the utilization of metal porphyrin for SOC sequestration, with the development of previous research project and the acquisition of national and international patents on these applications.

Following the agreement with the University of Tor Vergata, the stocks of activated iron porphyrin were delivered to CERMANU for the field applications from the second year of field activities (March 2015). Starting from the quantity of basic molecule (600 g of basic porphyrin) about 840 g of final catalyst were obtained with the activation step. The inclusion of iron or manganese and the addition of sulfonate groups produce in fact an increase of 40% in the final molecular weight.

The annual addition for field application for the two project sites of University of Torino and Napoli (Tetto Frati and Castel-Volturno) is: plot $48m^2$*0.5g $/m^2$*4 replicates*2 sites= 192 grams

The innovative action was included among the activities to continue beyond the end of LIFE project for the farm site of University of Napoli. Notwithstanding the unfavourable economic feedbacks responses already obtained, as indicated in the proposal, this activity will be extended after the LIFE project in the project site of University of Napoli, given the scientific and practical interest and the increasing concern of international policies on SOC sequestration practices.

5.1.3 Action B.3 *Set up of project sites*

Beneficiary responsible CERMANU

Responsibilities in case several beneficiaries are implicated:

- Piemonte (beneficiary responsible AGROSELVIT-DISAFA):
  project sites Tetto Frati, Grandi
- Campania:
  - project site Castel-Volturno (beneficiary responsible CERMANU);
  - project site Prima Luce (beneficiaries responsible PRIMA LUCE, CREA-ORT);
  - project site Mellone (beneficiary responsible UNIBAS);

Foreseen start date: 31 March 2014	Foreseen end date: 1 June 2018
Actual start date: 31 March 2014	Actual end date: 1 June 2018

Milestones

1) Selection of Composting plant for farm sites located in Piemonte
   Foreseen date 20/08/2013 Actual date 31/12/2014
2) Set up of project sites Foreseen date 31/03/2014 Actual date 31/3/2018

Deliverables

No Deliverables were expected for the Action B.3. Specific reports of fields activities (Report on Action B.3) were Annexed to Inception Report, 1st Progress Report andd Mid Term Report

This Action involves the annual field activities related to the application of SOM managements, based on either compost application and soil treatment with biomimetic catalyst. Five farming sites have been activated with different cropping systems and soil managements, as detailed in the subsequent scheme.

No deliverables were expected for Action B3. However, as request by the Commission, specific reports were included in the Inception, 1st Progress and Mid term report with the detailed overview of field activities carried out in each farm site

With respect to the issues raised by the **Commission in the last communication (Ares(2018)4574194 - 06/09/2018)**, for Action B3 (**Technical issues point 1**), we confirm the extension of the field activities to II trimester of 2019 for kiwi and peach orchards at Mellone site, to complete the 4 cropping cycles as stated in the Grant Agreement.

For the project site of CastelVolturno, the Committee of CERMANU in accordance with the Agricultural Department of the University of Napoli, decided to convert the filed plots of LIFE CarbOnFarm in a long-term trial experiment, with the annual application of proposed management

The set-up of project sites of Universities of Napoli (Castel Volturno) and Torino (Tetto Frati) and at the commercial farm Grandi (Grugliasco) started in the II semester 2014 (April/May). The field activities involving the utilization of on-farm compost produced by the LIFE composting plant, started at Prima Luce with the set-up of field plots for horticultural crops in the III trimester 2014. The project activities for two orchard system (kiwi and peach) in the project site of farm Mellone, started in the IV semester 2014. An agronomic problem on the peach orchard produced the removal of selected plants for peach cultivation; therefore, an additional field has been set up by the personnel of UNIBAS to continue the application of SOM managements. As previously outlined, this unforeseen delay will be matched with the extension of the filed trials at Mellone farm

Field schemes:

TETTO FRATI: Cropping system = maize. Soil managements = **0N:** Control with no Nitrogen addition. **TRA**: Conventional management based on mineral fertilization. **CMPB and CMPA**: soil addition with local compost from solid digestate at low and high doses (1000 and 2000 kg of C ha^{-1}) without addition of mineral Nitrogen and Phosphorus. **SSMPB and SSMPA:** soil addition with local fresh solid digestate at low and high doses (1000 and 2000 kg of C ha^{-1}) without no addition of mineral Nitrogen and Phosphorus. **PORF:** application of biomimetic catalyst (0.5 g/m2= 5kg ha^{-1}) and traditional agronomic technique (mineral fertilization). Plot size 60 m2 with 4 field replicates

GRANDI: Cropping system = open field horticultural crops with annual cycle of lettuce and brassicaceae. Soil managements = **0N:** Control with no Nitrogen addition. **TRA**: Conventional management based on mineral fertilization. **CMPB and CMPA**: soil addition with local compost from solid digestate at low and high doses (1000 and 2000 kg of C ha^{-1}) without addition of mineral Nitrogen and Phosphorus. **SSMPB and SSMPA:** soil addition with local fresh solid digestate at low and high doses (1000 and 2000 kg of C ha^{-1}) without no addition of mineral Nitrogen and Phosphorus. Plot size 24m2 with 4 field replicates

CASTEL VOLTURNO: Cropping system = maize. Soil managements = **TRA**: Conventional management based on mineral fertilization. **CMPB and CMPA**: soil addition with on farm manure-based compost at low and high dose (10, 20 t ha^{-1}); **PORF:** application of biomimetic catalyst (0.5 g/m2=5kg ha^{-1}) and traditional agronomic technique (mineral fertilization). Plot size 48m2 with 4 field replicates

PRIMA LUCE: Cropping system= open field horticultural escarole (october 2014-march 2015); pumpkin (may 2015-august 2015); broccoli (October 2015-april 2016); bean (june-october 2016)
Soil management = A: Control no fertilization; B: traditional organo-mineral fertilizer; C on-farm compost 10 tons ha^{-1}; D on-farm compost 20 tons ha^{-1} Plot size 280m2, 3 field replicates

MELLONE: Cropping system: kiwi and peach. Soil management= 0 control with mineral fertilization. A “summer” and B “winter” two types of on-farm compost addition depending on the crop residues used in composting process at two dose, *1* 10 tons ha^{-1}, *2* 20 tons ha^{-1}
Plot size 900 m^2 with 3 field replicates (total 18 plots for each system)

5.1.4. **Action C.1** *Monitoring of composting processes and characterization of compost quality*

Beneficiary responsible UNIBAS

Responsibilities in case several beneficiaries are implicated:
Biological Analyses: CREA ORT
Molecular characterization: CERMANU

Proposal start date 31 March 2014	Foreseen end date: 30 April 2018
Actual start date 31 trimester 2014	Actual end date : 30 May 2016

Deliverables:

1) Intermediate Report on C1 Action Foreseen date 30/04/2015 Actual date 30/4/2015 Annexed to the Mid term Report
2) Closing report on Action C.1 Foreseen date 30/4/2018 Actual date 30/04/2018 (Annex 1 of this Report)

The first monitoring action was focused on the check of composting process and the characterization of compost qualities. The main project objectives were based on the feasibility of composting process as viable way to recycle the organic biomasses into valuable material for effective SOM management. Therefore, following the realization of the on-fam composting facility, dedicated efforts were focused on the evaluation of composting process and compost quality. The related monitoring activities were finalised in advance in respect to the planned schedule. At the intermediate project stage about 15 typologies of compost were analysed including the solid digestate and compost from bio-digestate used in field activities in Piemonte; on-farm compost from cow and buffalo manure used at Castel Volturno and 12 on farm green composts from the LIFE composting plant of Prima Luce produced with the most representative residues, by-products and biomasses available in the local territory., The following analyses have been performed:

- chemical analyses: pH, electrical conductivity, heavy metals, elemental analyses (C, N, H)
- biological analyses: microbial activity (hydrolase activity FDA), total amount od bacteria and fungi with particular focus on potentially harmful bacteria, such as *Enterobacteria, Clostridium spp., Escherichia coli, Salmonella, total and faecal Coliforms and Streptococcus spp;* phytotoxicity/biostimulation on Lepidium sativum
- molecular characterization: solid state 13C CPMAS NMR; thermochemolysis GC-MS
-13C-OC isotopic content

All the analysed composts were characterized by a scarce or null amount of heavy metals, with a variation of total C and N contents depending on the different origins of raw biomasses used for the composting processes. The biological assays showed a significant microbial activity in all biomasses as measured by total respiration and FDA methodologies. As expected from previous experiences and analyses the composts produced from agricultural biomasses are characterized by a suppressive activity against two “soil-borne” phytopathogenic fungi, *Rhizoctonia solani* and *Sclerotinia minor* coupled with biostimulation activity on *Lepidium*, although with some variability among analyzed composts depending on the different origin. The results further strengthen the well acknowledged working hypothesis that an important role in the biological activity is played by the phenolic components related to the lignin derivatives; however also the different combination of hydrophobic fraction and bio-labile components (carbohydrates, peptide derivatives) is regarded as important regulator and modulators on the interaction between the molecular composition and the bio-stimulation and hormone like activity displayed by the humified fraction of composted biomasses towards both soil microbial activity and plant physiology.

The molecular characterization revealed the incorporation in final mature compost of more stable components mainly related to the more aliphatic and aromatic molecules of plant origin represented by the derivatives of cutin, suberin, waxes and lignified tissues. The NMR data revealed in fact a carbon distribution characterized by the large level of the Hydrophobic Index and of Lignin Ratio. These two dimensionless indexes are commonly used in the specific literature to evaluate, respectively, the biochemical stability of organic materials and the presence of lignin components. The thermochemoysis followed by Gas Chromatographic Mass Spectroscopy confirmed these indications. The most abundant alkyl compounds shown in the pyrograms were represented by the long chain linear fatty acids and alcohols, typical components of plant waxes. Noticeable amounts were also found for the hydroxy and alkyl-dioc acids which are the main recalcitrant constituents of plant protective barriers cutin and suberin. The microbial inputs were revealed by the detection of branched fatty acids, used as biomarker of PLFA form the membrane of the different classes of soil microfauna.

The released aromatic materials indicated the presence of the three common constituents of lignin tissues: para-Hydroxy-phenil, Guaiacyl and Syringil units: The lack of a clear predominance was related to the combination for the starting materials of different plant species in the attainment of mature green composts. The yields of lignin components increased in mature compost as compared with original biomasses, for the selective preservation of more stable molecules. Besides the large yield of different derivatives, the specific structural index associated to biochemical stability, revealed the classical lower values commonly combined with the stable organic materials (**Annex 1 Closing report on action C.1**)

5.1.5 **Action C.2** *Monitoring of soil organic carbon stabilization and the improvement of physical and biological soil fertility*

Beneficiary responsible: CERMANU

Analyses at project site Prima Luce: CREA ORT
Analyse at project site Mellone: UNIBAS

Foreseen start date: 31 March 2014	Foreseen end date: 30 April 2018
Actual start date: 31 March 2014	Actual end date: 30 May 2018

Deliverables:
1) Report for C.2 action: first year Foreseen date 30/01/2015 Actual date 30/01/2015 Annexed to the 1st Progress report; 2) Report for C.2 action: second year Foreseen date 1/02/2016 Actual date 30/03/2016 Annexed to the Mid term Report; 3) Report for C.2 action: third year Foreseen date 30/01/2017 Actual date 30/03/2017 Annexed to the 2d Progress report; 4) Closing report on Action C.2 Foreseen date 30/4/2018 Actual date 3 /05/2018 (Annex 2 in this Report)

This action was focused on the evaluation of the applied soil management on the quantity and quality of soil organic matter components. The following monitoring activities have been performed in the different project sites:

Piemonte: Tetto Frati, Grugliasco
Soil Aggregate stability; TOC content (bulk soils and soil aggregate fractions); total N (bulk soils and soil aggregate fractions); SOM characterization: Initial and final humic substances (13C CPMAS NMR); bulk SOM by Termochemolysis GC-MS; PLFA; 13Cisotopic analyses (on soil plots added with compost and solid digestate)

Campania:
- Castel Volturno

Soil Aggregate stability; TOC content (bulk soils and soil aggregate fractions); total N (bulk soils and soil aggregate fractions); SOM characterization: Initial and final humic substances (13C CPMAS NMR); bulk SOM by Termochemolysis GC-MS; PLFA; initial 13C labelled SOM (on soil plots added with on farm manure compost)

- Prima Luce

Soil Aggregate stability; TOC content (bulk soils and soil aggregate fractions); total N (bulk soils and soil aggregate fractions); SOM characterization: Initial and final humic substances (13C CPMAS NMR); bulk SOM by Termochemolysis GC-MS; Biological analyses (CREA-ORT): dehydrogenase, hydrolase, β-glucosidase, β-galactosidase, glucosaminidase, invertase, phosphomonoestarase, arylsulphatase, urease, nitrate reductase and protease. EMI maps (UNIBAS)

- Mellone

TOC, total N; analyses of isotopic 13C content of soil plots amended with sorghum-based labelled compost.

In respect to the clarification requested by the **Commission in the last communication (Ares(2018)4574194 - 06/09/2018)**, for Action C2 (Technical issues point 2): the soil sampling at Mellone site was effected in November 2017 for the mid-term evaluation; the results are included in the present report (Annex 2 Closing report on Action C.2). The sampling for the final point will be carried out in November 2018 and the results are expected for March 2019

The evaluation of structural properties revealed no significative differences between soil treatments, in the project sites of CastelVolturno and Prima Luce, with respect to the distribution of soil aggregates and the overall soil aggregate stability. These soils were characterized by a typical structural stability, inherited by the heavy textural characteristic with a large amount of finer particles pertaining to clay fraction. The application of different organic materials did not produce a measurable variation in respect to the high standard initial level of the structural properties as determined by the stability index Mean Weight Diameter in water (MWDw). Conversely, for the light textured soils of Grugliasco and Tetto Frati, although the aggregation processes were mainly affected by the soil textural compositions, a slight prospective increase in the yield of stable macro-aggregates (ϕ > 0.25 mm) was observed for the field treatments based on soil addition with compost and solid digestate. In line with the acknowledged hypothesis on aggregate hierarchy, this effect seems related with the increase in the SOC concentration on micro aggregates and the progressive steady stable incorporation of finest soil fractions into stable larger aggregate size particles. This variation was summarised by the recorded improvement of the MWDw index of about 15-20%, in respect to traditional managed plots. The main Indicators proposed to evaluate the effect of SOM managements are represented by the TOC content and the characterization of SOM quality A positive response was shown by the analyses of TOC content, for all field treatments with organic matter addition. The average increases ranged from 1 to 4‰ of soil organic carbon content, which correspond to about 3 to 17 tons of OC per hectare. These amounts stand also for a preservation of OM that varied from the 70 to 80% of added organic material, depending on type and dose of organic amendment and soil type. A more delayed response was observed for TOC content in soil treatment with biomimetic catalyst. Notwithstanding with the no significative differences found in the first two years of filed activities, in the final project period an average increase of about 1.1 gOC kg^{-1} and of 2.0 gOC kg^{-1}, for 0.30 and 0.15 m sampling depths respectively, were in fact revealed by amended plots in respect to respective control treatments. These data correspond to about 4 tons OC ha^{-1}. For the evaluation of SOC dynamics, the initial analysis on 13C isotopic SOM content, were performed on soil samples added with fresh and composted organic biomasses of Tetto Frati, Grugliasco, Castel Volturno and Mellone sites immediately after the first addition of organic materials. The isotopic enrichment found in either bulk soils and soil aggregates after soil amendments, in respect to starting situations, fairly correspond to the expected variation produced by the added OC amounts, thereby confirming the effectiveness of 13C-OC analyses to discriminate among native SOM pools and exogenous OC inputs. The additional results of 13C-OC isotopic content showed that the observed increase of TOC content is not exclusively related with the added organic materials. About the 10 to 20% of additional OC may be associated with the preservation of original organic materials, either inherited form soil organic pool or deriving from the new inputs provided by crop residues. The SOM characterization by thermochemolysis GC-MS revealed a prospective shift in the molecular composition in all project sites, in field plots amended with different organic materials. The main variations are related to the increase of more hydrophobic recalcitrant biopolyseters compounds (such as cutin and suberin derivatives) and lignin derivatives. These variations have to be associated with the inclusion into SOM pools of composted organic materials deriving from the recycling of agricultural biomasses. The analyses of soil humic materials performed by 13C-CPMASNMR spectroscopy indicated an incorporation of molecules derived from organic inputs. The observed OC distribution of NMR spectra and the associated increase of dimensionless structural indexes, alkyl, hydrophobic and lignin ratios, suggested a contribution of the molecular constituents found in the compost materials thereby outlining the selective preservation, in soil humu compartments, of aliphatic and aromatic derivatives pertaining ti the humified fraction added with mature composts

The specific investigation of fatty acids derived from the membrane cellular components of soil microorganisms (PLFA) allow to estimate with an easy and reproducible protocol the composition and the variation of soil microbiota. The final data confirmed the overall increase for total yields of various microbial communities (bacteria, fungi, mycorrhize) for the field plots based on soil amendment with organic materials in the project sites of Tetto Frati, Grugliasco and Castel-Volturno. No differences in the composition of microbial communities were found among soil treatments, with no evident correspondence between the type of organic inputs, microbial classes and soil type. In order to extend the evaluation of the different SOM managements applied at Tetto Frati and Grandi, additional analyses not previously foreseen were accrues out in the last reporting period; the various enzymatic activities recorded on the last year of field trials (phosphatase, hydrogenase, aryl-sulfatase, B-glucosidase, FDA-hydrolase) indicated an overall stimulation of microbial activities in OM managed plots. In the project site of Prima Luce the soil biological analyses did not revealed sharp changes in soil microbial community catabolic characteristics, resulting in a different utilization of carbon sources. Only the catabolic activity of the plots under the highest dose of compost, was affected by organic amendments and able to metabolize carbon substrates in different way. (**Annex 2 Closing report on action C.2**)

5.1.6 Action C.3 *Monitoring of the agronomical, phytopathological and practical sustainability of proposed strategies*

Beneficiary Responsible CREA-ORT

Responsibilities in case several beneficiaries are implicated:
Project sites Tetto Frati: AGROSELVIT
Project site Castel Volturno: CERMANU
Project site Mellone: UNIBAS

Proposal start date: 31 March 2014	Foreseen end date: 30 April 2018
Actual start date: 31 March 2014	Actual end date: 30 May 2018

Deliverables:
1) Report for C3 action: first year Foreseen date 30/01/2015 Actual date 30/01/2015 Annexed to the 1st Progress report; 2) Report for C.3 action: second year Foreseen date 1/02/2016 Actual date 30/03/2016 Annexed to the Mid term Report; 3) Report for C.3 action: third year Foreseen date 30/01/2017 Actual date 30/03/2017 Annexed to the 2d Progress report; 4) Closing report on Action C.3 Foreseen date 30/4/2018 Actual date 30/05/2018 (Annex 3 in this Report)

In accordance with the continuation of field activities the evaluation of agronomic results will be continued in the two University farms of Tetto Frati and Castel Volturno. In project site of Mellone will be evaluated the results of the last crop cycle.

This action involves the determination of the agronomic response on the applied soil managements on representatives local cropping systems; crop yields, photosynthetic status, nutrient uptakes were evaluated at different project sites as indicators of the specific project objective.

The following data were analysed in the five farming sites:

- Tetto Frati (4 crop cycles) System: maize; Analyses: yield, total N and P content, photosynthetic status
- Grugliasco (4 crop cycles) System: open field horticultural crops (annual succession lettuce, Brassicaceae). Analysis: yields, total N and P content, photosynthetic status
- Castel-Volturno (4 crop cycles) System: maize. Analyses yield, N and P content, photosynthetic status
- Prima Luce (4 crop cycles) System: open field horticultural crops (endive-scarole, pumpkin, broccoli, bean). Analyses; yields, commercial size, dry matter
- Mellone (3 crop cycles) System: orchards (peach and kiwi). Analyses: yield, commercial size, dry matter content, firmness, colour, solids soluble content, titrable acidity, total nitrogen, total polyphenols, antioxidant activity

The main target was to obtain an overall maintenance of the crop productivities in OM managed fields as compared to the conventional methodologies based on mineral and organo-mineral fertilization practices. A positive final response was obtained for crop yields and quality after subsequent addition of organic inputs, although with some differences among cropping systems. The best performances were obtained for horticultural crops in the project sites of Grandi and Prima Luce, with a general larger yield of fresh products and comparable nutrient contents. Moreover, an additional feedback, was related to the consistence of the horticultural products. This parameter associated with the maintenance the nutrient value, is an important commercial characteristic that determine the “shelf-life” of the product which extension represent an important target for the marketing of fresh vegetables to external countries.

For the maize crops, cultivated in the University farms of Torino (Tetto Frati) and Napoli (CastelVolturno), steady lower maize yields were found, for the first three crop cycles, in amended plots with organic matter addition with respects to the traditional mineral fertilization, ranging from 10 to 20%, depending on organic inputs and field site. However, in the last year of project activities a reverse trend was observed at both project sites, with a recover of grain yield for soil treatments with larger amounts of applied mature composts and solid digestate, that approached the results of mineral fertilized plots. Furthermore, for each cropping cycle and for both field sites, no significative differences were shown in respect to the nutrient N and P concentration between treatments, thus suggesting that more than the effective soil content, a time lag in the availability of macronutrients in respect to plant requirements in characteristic specific physiological phases of maize cycle may have affected the total yields.

As general comment it has to be considered that 4 years of application of new soil management, from the research point of view, represent a short transition period to determine a stable conversion of soil equilibrium into new steady state. The soil addition of organic inputs as unique application of nutrients may have produced a differential response between cropping systems. In Grandi and Prima Luce, the usual combination as traditional methods, of mineral and organic fertilizers may have resulted in a microbial community able to use and mineralize the organic inputs in adequate amounts for the short cropping cycles. Conversely the SOM managements applied in conventional system in maize crop, exclusively based on mineral fertilization resulted in a periodic annual shortage of available nutrients. The last results suggested that the steady application of higher level of organic materials may have favoured a shift towards a new equilibrium in the cycling of N and P pools, based on the increase of organic forms which undergo to a slow mineralization and release in the viable amount and timing for the maize uptakes. For the peach and kiwi orchards, the soil addition with compost did not produce noticeable differences in respect to conventional plots. In this case the ten-year farming approach of Mellone farm, based on green manuring may have allowed a faster adaptation of microbial community to the added organic materials withootu a transition state. Although a slight larger yield of peach and kiwi fruit for selected plants have been noticed, no significative statistical differences in total fruit yields per hectare are currently found between traditional and compost amended plots. Conversely the addition with compost promoted a steady increase of measured quality parameters: dry matter, total N, sugars, polyphenols, titrable acidity, antioxidant activity Although, as previously outlined, after short time application of new SOM management, it is not yet ascertainable to claim a direct correlation of observed effects with the modified composition of SOM quality, highlighted in the monitoring action C2 the results of Mellone farm suggested a positive biostimulant effect od added compost, with no significative effect related to winter or summer compost.

(**Annex 3 Closing report on action C.3**)

5.1.7 Action C.4 *Monitoring of greenhouse gases emissions*

Beneficiary Responsible CERMANU

Responsibilities in case several beneficiaries are implicated:
Project sites Tetto Frati: AGROSELVIT
Project site Mellone: UNIBAS

Foreseen start date: 30 June 2014	Foreseen end date: 30 April 2018
Actual start date: 30 June 2014	Actual end date: 30 May 2018

Deliverables:
1) Report for C.4 action: first year Foreseen date 30/01/2015 Actual date 30/01/2015 Annexed to the 1st Progress report; 2) Report for C.4 action: second year Foreseen date 1/02/2016 Actual date 30/03/2016 Annexed to the Mid term Report; 3) Report for C.4 action: third year Foreseen date 30/01/2017 Actual date 30/03/2017 Annexed to the 2d Progress report; 4) Closing report on Action C.4 Foreseen date 30/4/2018 Actual date 30/05/2018 (Annex 4 in this Report)

In respect to the Indication included at **3, 4 5 of Technical Issues of Commission Communication (Ares(2018)4574194 - 06/09/2018**), the activities at Tetto Frati and Mellone project site will continue till September 2019; at CastelVolturno the system will be dedicated to the GHG monitoring activities of the long term filed experiments (see Action B.3); an updated version of the manual for the "*Automatic remote sensing software and system for field GHG sampling and analyses*" (**Annex 4b**), was uploaded in the download page of the project website. This monitoring activity refers to the measurements of field GHG emissions as complementary tool to estimate the effect of SOM management on SOC stability, at the project sites of Tetto Frati, Castel Volturno and Mellone. These act as representative farming systems of both annual herbaceous crops, characterized by the periodical succession of cultivation and bare soil, and of orchard system with the multiannual presence of plant cover. In this respect a modification has been introduced to the original proposal, with the replacing of Prima Luce with the orchard at Mellone farm for the GHG analyses, in order to deal with a more comprehensive dataset which will thus include different cropping systems and soil managements. This action was started in June 2014 for the project site of Castel Volturno and in 2015 in Tetto frati. The delay in the data collection at Mellone site was related to the longer time needed for the design of an automatic electronic system for the GHG collection and analyses as indicated in the proposal (*wireless automatic accumulation system equipped with 8 respiration chambers made by the UNIBAS*). In order to match the initial target of an adequate multi-year array of GHG emissions the analyses were performed with available semi-automatic system as those used for the project sites of Tetto Frati and Castel-Volturno. The monitoring of gas fluxes within maize system, at both farms, include the cropping cycle, from June to October, and uncultivated period from November to May, with some break between December and February during to the unfavourable weather conditions that produce a decrease of soil respiration level below the detection limit of the system and would requires daily maintenance of the system. The measurements in Tetto Frati are carried out with mobile equipment used for 6 weeks for each trimestral campaign, while in Castel Volturno there is a dedicated fixed system. The collection of GHG fluxes is based on ten automated closed static chambers, placed in field test plots, connected to a Photoacustic Field Gas Monitor. Two collecting chambers are placed in the intercrop rows as control. Currently the new system developed by UNIBAS is being used at the project site of Mellone. It is composed by 8 independent wireless analytical chambers, with automatic collection and transmission of GHG fluxes with a remote sensing "wi-fi" control and equipped with a solar battery. Given the large extension of filed plots 4 chambers are used in turn for each field replicate, with the periodic displacement on the different treatments, performing 8 daily measurements for each chamber. An overall higher level of GHG emission was recorder in the maize cycle, due to the contribution of root systems and higher microbial activity. No main global differences were found, for all the analysed periods, in soil treatments with low dose of organic inputs and biomimetic catalyst in respect to traditional plots, while a slight larger CO2 emission was revealed after the second year of SOM managements for the field plots with the large dose of compost addition in CastelVolturno and in the plots with large dose of fresh solid digestate in Tetto Frati, rounding up to about +100-200 kg ha^{-1} C-CO_2 (corresponding to 0.03-0.05 kg OC $tons^{-1}$). A larger CO2 emission was found in the project site of Mellone farm, for the permanent orchard system as compared to the maize fields, thereby strengthening the validity to include in the C4 action different cropping systems. The large extension of root systems and the presence of cover grass in the inter-layers between plant rows may play a role in the CO2 emission of orchard soils. No significant differences were found among treatments which global values on annual bases could be rounded up to 1.2, 1.3, 1.4, 1.2, 1.4 kgC /m2/year for the Control, A10, A20, B10, B20 treatments respectively. In addition to the field GHG estimation a laboratory experiment was performed by AGROSELVIT-DISAFA with the setup of combined activity related to the GHG emission from the organic matrices used in project sites. The laboratory incubation was carried out in closed chambers with four organic materials (compost and solid digestate, used at Tetto Frati and Grandi sites, on farm manure compost used at Castel Volturno and on farm green compost used at Prima Luce) with two model soils of different textural composition and GC measurements of GHG. The GHG analyses (N2O, CO2 and CH4) have been performed from June to October 2015 with about twenty sampling time measurements. The data highlight the decrease of GHG emission of all composts in respect to either the mineral fertilizers (e.g. urea) and to fresh solid digestate, thereby supporting the advantage of organic matter stabilization through the composting process.

(**Annex 4 Closing report on action C.4**)

5.1.8. Action C.5 *Monitoring the environmental and economical sustainability of proposed strategies*

Beneficiary Responsible AGROSELVIT
Responsibilities in case several beneficiaries are implicated: UNIBAS

Foreseen start date: 31 March2014	Foreseen end date: 30 April 2018
Actual start date: 30 June 2014	Actual end date: 30 May 2018

Deliverables

1) Intermediate report on C.5 action Foreseen date 01/02/2016 Actual date 1/02/2016 Annexed to the Mid Term Report
2) Closing report on Action C5 Foreseen date 30/04/2018 Actual date 30/05/2018 (Annex 5 in this Report)

This action regards the comprehensive estimate of the effective sustainability of proposed approaches, with respect to the compost process and the field activities. The Life Cycle Assessment method analyse the environmental and energetic impacts of the different activities, combining all the fluxes of energy and matter forming each production chain, in order to evaluate the incidence of the singe step and detect if possible the critical phases of the system.

The assessment of the on-farm composting prototype, including both facility and the composting process, started in June 2014. The results of energetic and economic impacts have been included with the Mid Term report and are currently available on the project website

Following the set-up of tests plots, a comprehensive protocol for the inclusion of the different inputs related to the different cropping systems was elaborated through feedback process and sharing between partners.In order to assess the environmental and economic sustainability software GaBi 6 was purchased, with a shared protocol for data collection

Two PE INTERNATIONAL GaBi Databases were purchased. They are the largest internally consistent LCA databases on the market today and contain over 7,000 ready-to-use Life Cycle Inventory profiles. The first is “GaBi Professional” database: it is the standard database provided with the GaBi software. The GaBi professional database is regularly updated and is derived from industry sources, scientific knowledge, technical literature, and internal patent information creating a solid foundation for assessing your materials, products, services and processes. The second is more agricultural oriented and it is called “Renewable raw materials” database.

Renewable raw materials contain 140 processes: fertilizers, pesticides, tractors, agricultural equipment, industrial intermediate products, and different crops (corn, wheat, hemp, flax, rape seat, soybean, etc.). The scenarios and impact assessments were modelled and computed by GaBi 6 software by using ILCD (International Reference Life Cycle Data System), EDIP (Environmental Design of Industrial Products) method of impact assessment. Impact assessment category selected are: Global Warming Potential (IPCC), Acidification Potential (EDIP), Freshwater Eutrophication Potential (RECIPE) and Terrestrial Eutrophication Potential (RECIPE). In order to assess and compare the sustainability of different crops production three Function Units were selected. The impacts are reported per harvested production (product oriented), per cultivated land (surface oriented) and finally per crop revenues, in order to add the impact of different crops cultivated on the same land, during the same year.

The analyses of cropping systems highlighted a best energetic efficiency and lower environmental impacts of the organic amendments as compared to mineral fertilization for the horticultural crops, while on maize systems the environmental effectiveness of organic inputs showed a drop, consistently with the lower productive efficiency. However, the organic amendments resulted the best choice when the analyses include effect on SOM content

(**Annex 5 Closing report on action C.5**)

5.1.9. Action C.6 *Monitoring the acquired awareness about available techniques for soil organic carbon stabilization and accumulation and bioresources valorisation*

Beneficiary Responsible AGROSELVIT
Responsibilities in case several beneficiaries are implicated:ALSIA

Foreseen start date: 30 March 2015	Foreseen end date: 30 April 2018
Actual start date: 1 December 2014	Actual end date: 30 May 2018

Deliverable:
1) Intermediate report on C.6 action Foreseen date 01/07/2016 Actual date 1/02/2016 Annexes to the Mid term Report
2) Closing report on Action C.6 Foreseen date 30/04/2018 Actual date 30/05/2018 (Annex 6 in this report)

For The activities related to Action C.6, specific questionnaires dedicated to farmers were conceived, by beneficiaries AGROSELVIT-DISAFA and ALSIA, focused on the current knowledge about soil fertility, organic farming, type of organic inputs, GHG emissions, implication of SOM management of the overall farm productivity. The formats are based on the awareness about the effects of sustainable practices adopted on soil organic matter management and in particular the problems, constrains and effects about the use of available organic biomasses in agriculture. The format is also available on the main page of project website

Besides the initial 240 filled documents made available for the Mid-term report, additional 750 interviews were obtained following the specific activity performed by the Beneficiary AGROSELVIT-DISAFA, in order to maintain the proposed objective of 1000 questionnaires. The elaboration of the questionnaires will be uploaded in the download page of the project website with the previous ones.

The overall depiction delineates a basic awareness on the importance of soil organic matter for the maintenance of the chemical, biological and physical soil fertility in the long-term time span. However, a minority of the selected farmers have a clear indication on the OM content on the own cropped soils. There is a wide spread confidence on the utility of the application of organic inputs; however, there are main perceived limits of hindrance represented by the scarce information on local availabilities, quality of organic biomasses and related provisioning costs.

(**Annex 6 Closing report on action C.6**)

. 5.2 Dissemination actions

5.2.1 Objectives

The goal of dissemination actions was to promote an effective transfer of background, objectives and results of the project to the wider audience of professional categories and target stakeholders in the local territories.

As indicated in the proposal the achievement of on-farm composting prototype at the project site of the Beneficiary PRIMA LUCE, represented a central pillar to display a suitable array of demonstration activities to highlight the virtuous cycle based on the combination of environmental, agronomic and economic objectives. Furthermore, beside the basic objectives of Implementation actions, the utilization of various farming sites with the application of composts from different local organic biomasses, was conceived also to improve the awareness of farmer and technicians on the feasible productive valorisation of agricultural residues as important local bio-resources.

About 100 dissemination events have been organized by the beneficiaries with the involvement of about 5000 participants (Farmers, Farmer organizations, Technicians, Agronomy associations, Students, Representatives of Regional and University Institutions)

The main Dissemination events have been summarised in the **Annex 7.3.3 Other dissemination annexes Resume of dissemination events** (**point 8 of Commission Communication Ares(2018)4574194 - 06/09/2018**)

As outlined in the section of Long term benefits, about 20 additional farms were involved in the project activities related to the on-farm composting, biomass recycling and SOM management, with an effective transfer of project methodologies and the realization of 6 additional composting facilities

(Projects submitting final reports after 1 January 2014 must use this format.)

Additional dissemination activities were represented by the presentation of LIFE CarbOnfarm to the following external meetings and conferences:

Participation to meetings and conferences

Event	Location/Date	Participation
Green Carbon Conference: Making Sustainable Agriculture Real	Brussels (BELGIUM) 1-3 April 2014	R. Spaccini (CERMANU) Poster presentation (2)
Meeting "Rifiuti organici come risorsa"	Matera (ITALY) 3/10/2015	Oral presentations G. CELANO (UNIBAS) R. Sileo (ALSIA)
Meeting ORT-MED Orticoltura in Ambiente Mediterraneo	Pontecagnano (SA - ITALY) 8/10/2015	R. Spaccini (CERMANU) and C. Pane (CREA-OF) Oral presentation
International Italian-Chinese workshop "*Innovative technologies for carbon sequestration* "	Bejing PRC from 8 to 11 March 2016	A. Piccolo CERMANU Presentation of LIFE CarbOnFarm
Global Soil Organic Carbon Conference FAO	Rome (ITALY) 21-23 March 2017	R. Spaccini (CERMANU) Oral and poster presentation
Workshop on Climate action in Agriculture and Forestry – "Climate smart land-use policy: best practices and innovation from LIFE and Horizon 2020 projects".	European Commission, Brussels (BELGIUM) 1 June 2017	R. Spaccini (CERMANU) and S. GaudiNo (AGROSELVIT-DISAFA)
XI Meeting of International Humic Substances Society (IHSS) Italian chapter - e	Siracusa (ITALY) 6-8 June 2017	A. Piccolo and R. Spaccini (CERMANU) Oral presentations
XXXV Meeting SICA (Italian Society of Agricultural Chemistry)	Udine – (ITALY) 11-13 September 2017	R. Spaccini (CERMANU) Oral presentation
Climate Change Adaptation in Agriculture and Forestry in the Mediterranean Region	Madrid (SPAIN), 13 – 14 March 2018	R. Spaccini (CERMANU)
XII Convegno Nazionale Biodiversità "Biodiversità, Ambiente, Salute"	Università di Teramo (ITALY) 15-18 June 2018	M. Zaccardelli (CREA-OF) Oral Presentation
19th International Conference of IHSS (International Humic Substances Society)	Varna (BULGARIA) 16-21 Septmber 2018	A. Piccolo (CERMANU) Oral Presentation

5.2.2 Dissemination: overview per activity

For each activity and output (as for the technical progress – cf. above):

- Provide a description in quantifiable terms, and indicate who was responsible
- Compare with the planned activity – was the objective reached? What reactions and feedback was obtained?

5.2.2 Action D1. *Project website*

Beneficiary responsible REGCAMP

Responsibilities in case several beneficiaries are implicated:
AGROSELVIT-DISAFA, ALSIA, CERMANU, CREA-ORT, PRIMA LUCE, UNIBAS

Foreseenstart date: 6 December 2013	Foreseen end date 1/07/2018
Actual start date: 1 December 2013	Actual end date 1/07/2018

Milestones

Website publication Foreseen dare 06/12/2013 Actual date 1/12/2013

The website (www.carbonfarm.eu) is currently active; it contains all the deliverables, documents produced in the LIFE CarbOnFarm project. After the first half of project activities, a user-access detection system has been activated on January 2016; the current estimation of global accesses on project website round up to 4000 visualizations of different pages and products. The website will continue to be used to update on the initiatives and products associated with LIFE CarbOnFarm

5.2.3 Action D.2 *LIFE+ notice boards, project leaflets, informative technical reports*

Beneficiary responsible REGCAMP

Foreseen start date: 31 October 2013	Foreseen end date: 1 July 2018
Actual start date: 31 December 2013	Actual end date: 1 July 2018

Deliverables

1) Notice boards Foreseen date 03/02/2014 Actual date 1/02/2014 The pictures of Notice boards were delivered with the Inception Report

No specific deliverables were associated in the proposal to the additional products of Action D.2 The expected products were: 3 project leaflets (Initial, Intermediate and Final) and 2 technical reports (1^{st} and 2^{nd} Technical reports). As outlined hereafter only two Leaflets and the 1^{st} Technical report have benne realized and submitted with the Mid term report

This action was associated to the realization of basic dissemination products represent by Notice boards, leaflets and technical reports

In respect to the planned deliverable indicated un the proposal, a larger number of Notice boards have been realized, 1000 copies of first Brochure and 2nd Leaflet, 400 copies of the 1st Technical report, while the last Leaflet and the 2nd Technical report have not been produced (**point 6 of Commission Communication Ares(2018)4574194 - 06/09/2018**).

The Notice boards were 11 Initial (1 for each beneficiary plus 2 in Italian and English version for Dissemination events) with overall description of project grounds and targets, followed by additional 6 with the main achievements used in the dissemination events.

With respect to the final Leaflet, after the first half of project course, based on the feedbacks associated with the dissemination events, no specific positive response was received by the distribution of the 2nd Leaflet and of 1st Informative Technical report. The Project Committee Meetings recognized the lower utility of these technical printed summaries as compared with the direct presentation and discussion of project results during the dissemination events; it was hence decided to not proceed with the production of the 3rd Leaflet and of the Proceedings (see Action D.5), and to focus the efforts and to support the other products, represented by the **On-farm composting manual** and by the **Kit and Manual for Self-soil evaluation** (please refer to next Action D.3)

Two videos on on-farm composting prototype and process were produced to be used in the dissemination events and made available on the project website and on the project *you-tube* platform:

- a specific product focused on the on-farm composting facility of Prima Luce realized by REGCAMP in both Italian (https://www.youtube.com/watch?v=SGwXZ4Kwcrg) and English (https://www.youtube.com/watch?v=5CxjmGae9rw) versions
- a more general overview of the role, effects and environmental and productive benefits of biomass recycling and on-farm composting process, realized by ALSIA (https://www.youtube.com/watch?v=AJNbVYcw0Vo)

The two videos, realized by REGCAMP and ALSIA, have been conceived with different duration to be applied as effective dissemination and communication in workshop events (short clip) and training activities (extended clip) for farmers and students. The electronic versions of the videos are included in the **Annex 7.3.3** **<u>Other dissemination annexes.</u>** **Videos on on-farm composting**

5.2.4 Action D.3 *Press Conferences and workshop/demonstration days*

Beneficiary responsible ALSIA
Responsibilities in case several beneficiaries are implicated:
AGROSELVIT, CERMANU, CREA-ORT, REGCAMP, UNIBAS

Foreseen start date: 31 March 2014	Foreseen end date: 30 June 2017
Actual start date:31 March 2014	Actual end date: 6 June 2018

Milestones

1) Press conference Foreseen date 03/02/2014 Actual date 24/02/2014
A larger number of events were organized in respect to expected milestones; please refer to the explanation of the action activities

Deliverables

1) Brochure for workshops and demonstrative days Foreseen date 01/05/2015 Actual date 1/05/2015 Annexed to the Mid Term Report
2) Compost "on farm" manual Foreseen date 01/06/2015 Actual date 31/12/2015 Annexed to the Mid term Report
3) Handbook on self-soil evaluation Foreseen date 30/06/2017 Actual date 31/12/2017 Annex 7.3.3 Other dissemination annex in this report

This action is related to the organization of dissemination events aimed to the communication and transfer of project topics on the local territories. As indicated in the proposal the main targets were represented by farmers, farmer's associations, agronomists, technicians and manager of regional institutions. With respect to the planned activities indicated in the proposal (minimum of 9 project workshop/demonstration days) a larger number of initiatives have been sustained (**please refer to Annex 7.3.3 Other dissemination annexes *Resume of Dissemination Events***). This positive response was mainly related to the attainment of the first project objective represented by the LIFE *on farm* composting plan at the project site of PRIMA LUCE (Action B.1), and by the project activities related to the valorisation of local recycled agricultural biomasses in agro-ecosystem to restore soil fertility. These actions and concepts have been proposed on the starting dissemination events thereby further improving the feedback interest and the attention of the stakeholders represented by the farmers, farmers association, technician and the responsible of local administrative institutions. The attempt to capitalize the dissemination effectiveness of this "flywheel" option, has hence slightly modified the planned activities, focusing the project efforts in the Basilicata and Campania regions to maximize the positive effects related to the on-farm composting plant.

The effectiveness of these initiatives has produced additional results on the reproducibility and transfer on the local territories of proposed approaches. In fact, about twenty (20) other commercial farm and farm organizations joined the LIFE CarbOnFarm project and started a collaboration with the Beneficiaries to develop a suitable strategy for the application of sustainable approaches for the agro-ecosystem managements (**Annex 7.3.3 Other dissemination annexes:** ***Application letters for Farm and Association collaboration with LIFE CarbOnFarm***). Moreover, six (6) farm organizations following the visit of the LIFE on-Farm composting plan at the site of PRIMA LUCE and the comprehensive discussion with the Beneficiaries and the farmers involved in demonstrative application of on-farm compost, have realized additional on-farm composting plants (**please refer to the point 3 of the section** ***Analysis of long-term benefit***s).

As already outlined in previous reports an underestimated constraint was represented by the asymmetrical geographical distribution of beneficiaries; while the inclusion of two the agricultural departments of Regional institution of Basilicata and Campania has strongly supported, promoted and hastened the dissemination activities, the lack of such corresponding partner in Piemonte, with the AGROSELVIT as unique territorial partner, has also partially slackened the local planned interventions.. However, the planned three events foreseen in the proposal have been carried out in Piemonte Region.

The Deliverables associated with this action, **Manual on on-farm composting** and the **Handbook for self-soil evaluation**, have been realized:

1) The *Manual on On-farm composting* (2000 printed copies and on-line version available on the project website (**Annex 7.3.3 Other dissemination annexes: Manual on on-farm compost**)
2) Manual for self-soil evaluation: 2000 printed copies and on-line version available on the project website (**Annex 7.3.3 Other dissemination annexes: Kit and Handbook on self-soil evaluation**). This manual is also associated to a kit of simple instruments and tools for the practical application of the methodologies explained in the manual. About 100 kits were produced and distributed to the technicians of agricultural departments of the Regional institutions of territories involved in the projects activities. The large part of the proposed methods was based on the operative tools promoted by the organizations and main agriculture institutional organizations (e.g. FAO, USDA etc) The kit and the manual were conceived to allow an easy reproducibility by the technician and by the farmers of the proposed methodologies.

In the last reporting period specific training courses on on-farm composting, dedicated to the Agronomists and Regional technicians were organized by REGCAMP in collaboration with the University of Salerno at the premise of Regione Campania in Salerno and at the on-frm composting facility of Prima Luce (29, 31 January and 2 February 2019). These workshops were focused on the planning of on/farm composting facility and characteristics and use of on farm composts.
Dedicated videos have been realized and uploaded on the youtube channel of the project website

5.2.5 Action D.4 *Info-days (to students)*

Beneficiary responsible UNIBAS

Responsibilities in case several beneficiaries are implicated:
AGROSELVIT, CERMANU

Foreseen start date:30 June 2014	Foreseen end date: 19 May 2017
Actual start date: 15 June 2014	Actual end date. 20 April 2018

Milestones

A larger number of events were organized in respect to expected milestones; please refer to the explanation of the action activities

Based on the participation of three University departments, a specific action was dedicated to the dissemination of project backgrounds, objectives and practices to young stakeholders, represented by the students of middle and higher schools, university and PhD fellows. About 35 events were organized by Beneficiaries ALSIA, AGROSELVIT-DISAFA, CERMANU, UNIBAS, involving also visits at the different field sites and at the on-fam composting facility (**Annex 7.3.3 Other dissemination annexes: Resume of Dissemination events).**
As already communicated in the Second Progress report, a modification was introduced in this action with the organization of an international Summer school, dedicated to young researchers and focused on the topic of the role of natural organic matter and biological agriculture (**point 11 of Commission Communication Ares (2017)4122016 - 22/08/2017**). Based on the positive feedbacks received in the first half of the project course by the University beneficiary (AGROSELVIT-DISAFA, CEMANU, UNIBAS) it was considered useful by the Project Committee, to focus the attention of the young researchers

to some of the project topics, which represent the new frontiers of scientific acquisition and also important targets of European and world wide policies for the development of sustainable SOM and SOC managements. The Summer school included the participation of international speakers from different countries (Australia, Brasil, Germany, Ireland) and from public universities and private company of Italy, and the representatives of other LIFE and European projects. About 50 PhD student, and young researchers, form different European countries attended the workshops. All the materials have been submitted with the 2nd Progress report and is available on the project website.

5.2.6 Action D.5 *Mid-term and final conference*

Beneficiary responsible ALSIA
Responsibilities in case several beneficiaries are implicated:
AGROSELVIT, CERMANU, CREA-ORT, REGCAMP, UNIBAS

Foreseen start date: 30 March 2016	Foreseen end date: 1 June 2018
Actual start date: 26 May 2016	Actual end date: 15 June 2018

Milestones
1) Mid-term conference Foreseen date 30/03/2016 Actual date 26-27/05/2016
2) Final project conference Foreseen date 01/06/2018 Actual date 15/06/2018

Deliverables
1) Mid-term national conference proceedings Foreseen date 30/05/2016 **Not realized**
2) Final project conference proceedings Foreseen date 30/06/2018 **Not realized**

The planned activities were completed with the organization of the Final conference held the June 15th at the Department of Agriculture of University of Napoli. Besides the representatives and personnel of each Beneficiary, the conference involved as active speakers also the Director of the Agriculture Department and a representative of the Direction of Agriculture section of Regional Institution. In addition to this event, the third workshop planned in Piemonte was organized on May 25th as a local Final conference on the overall themes related to the practical, legislative and economic aspects and perspectives of the Organic fertilization. The workshop involved the representatives of Regional institution, Universities of Torino, Napoli and Piacenza, and of private companies and of the national association for the authorization of biological products (AIAB). Both conferences were attended by farmers, farmers associations, technician, agronomist, students. The main concerns of the audience were the improvement of the awareness on the role of SOM and SOM management for the development of sustainable agriculture.

Particular attention was focused on the following topics: economic issues related with the recycling of organic biomasses; the close relation between the adoption of adequate organic farming approaches and the development of biological agriculture; the central role of steady information and communication between different actors as those activated in LIFE CarbOnFarm (farmer associations, Regional Institutions, technicians, research and scientific institutions); the knowledge of the legislative and economic support of the Institutional policies at local, national and European level

In this respect, at both meetings, it was outlined that the last Regional Programme for the Development of Agricultural Sectors of both Piemonte and Campania regions (PSR 2014-2020), include specific measures for the support of specific activities related with the recycling of organic biomasses aimed to the SOM management:

PSR Regione Piemonte - sub-programme 10.1.3 total budget 22,000,000 Euro which include additional score for the inclusion of farm activities within a LIFE project;

PSR 2014-2020 Regione Campania sub programmes 10.1.1, 10.1.2 10.1.3: for soil amendment with organic biomasses, the SOC sequestration and the decrease of GHG emission; sub programme 4.1.1 for the investment aimed to reduce the use of mineral fertilizers, counteract the soil degradation and the realization of facility aimed to the recycle of organic biomasses.

Although it is not possible to claim a direct or univocal correlation of these Programmes with LIFE CarbOnFarm activities, it is ascertained that the proposed approaches were in line and anticipated the actual policies carried out to improve the SOM management in the agroecosystems of Italian territories. An acknowledgement was provided by the representative of Regione Campania, in the Final Conference, on the forerunner role of the LIFE CarbOnFarm project in the development of specific measures related to the importance of sustainable approaches for the use of recycled organic biomass and the carbon storage in agricultural soils, currently included in regional plan for Agricultural development: PSR Regione Campania 2014-2020, Misura 10.1.2 (**Press release of Regione Campania on Final conference of LIFE CarbOnFarm project** http://www.agricoltura.regione.campania.it/eventi/convegno-15-06-18.html**)**.

The Deliverables associated with this action (Proceedings of Mid term and Final Conferences) were not produced (**point 9 Commission Communication Ares (2018)4574194 - 06/09/2018).** Despite the initial indication provided in the original proposal, the subsequent acquired experiences from other National and European projects, have provided evidences that, regardless to the scientific values of the proceedings, these documents usually became, after the specific event, a fruitless material in the library and archives. Therefore, the project Committee decided to transfer the budget to other activities, mainly represented by the organization of the Summer school (Action D.4). The dissemination materials produced in both Mid Term and final Conference and on the Workshop on Organic fertilization was uploaded in the download page of project website as well as in the university websites of Professor involved in the project activities

The presentations and pictures of the events were uploaded in the Download and News pages of the project website; the videos of Torino Workshop and Final Conference were uploaded in the youtube channel of project website (**point 10 Technical issues of Commission Communication Ares (2018)4574194 - 06/09/2018**)

With respect to the indication included in the Second Progress Report regarding the replication of the Final Conference in Brussels (**point 11 Technical issues of Commission Communication Ares (2018)4574194 - 06/09/2018**), no positive feedbacks were received by main involved beneficiaries (CERMANU, ALSIA, AGROSELVIT-DISAGA, REGCAMP) in respect to an effective participation of stakeholders and actors to this eventual event. Additional fruitless attempts to organize a common European workshop with other LIFE projects were made either during the Summer school, and during the participation of the project coordinator at LIFE meetings in Brussels and Madrid

5.2.7 Action D.8 *Networking activities with other LIFE+ projects*

Foreseen start date: 30 March 2015	Foreseen end date: 30 June 2018
Actual start date: 30 March 2014	Actual end date: 30 June 2018

Beneficiary responsible CERMANU
Responsibilities in case several beneficiaries are implicated:
AGROSELVIT, UNIBAS

A connection has been established with the following LIFE projects with the mutual uploading of corresponding links on the project website

LIFE11 ENV/GR/000942- LIFE OLIVE-CLIMA; LIFE12 ENV/IT/000578 - LIFE HELPSOIL; LIFE+GREENWOOLF - LIFE12 ENV/IT/000439; LIFE EKOHEMPKON-LIFE 11 ENV/PL 445; LIFE AGROSTRAT LIFE11 ENV/GR/000951), LIFE15

ENV/IT/000396 - LIFE BIOREST; LIFE15 ENV/IT/000585 - LIFE DOP; LIFE15 ENV/IT/000392 - LIFE VITISOM

A close collaboration has been activated by the beneficiary AGROSELVIT with the LIFE Greenwoolf based on the existing connection among project activities and experiences

The two Life projects share the interest of giving value to agricultural wastes, upgrading them into new valuable resources for the soil. While Carbonfarm is working on farm residues from crop wastes and manure, the Greenwoolf project is working only on a specific residue of the sheep rearing: the wool.A problem of the sheep livestock farming is that Italian breeds wool (but also wool from many European breeds) does not anymore have a commercial value. Therefore the production of wool is a cost for the any specialized sheeo farm and an ecological problem as well as wool is often disposed in landfills. The Greenwoolf team is proposing and calibrating a process for treating raw wool and transforming it into fertilizers. The Carbonfarm helps the project through its own agronomic skills. The industrial project is producing a number of potential fertilizer products, differing in terms of dry matter content, of availability of mineral nutrients and of organic matter quality. The collaboration with Carbonfarm has created the conditions through which these products are now tested in terms of chemical quality and of fertilizer effects on the plant. The screening of all possible industrial processes will then use these agronomic results as a new set of criteria to choose the best strategy to produce the most efficient machine to be eventually commercialized. A collaborative participation to project workshops was performed LIFE Helpsoil with the participation of Beneficiry Agroselvit to a combined demonstrative day on June 9th 2016. The representatives of LIFE projects LIFE15 ENV/IT/000396 - LIFE BIOREST; LIFE15 ENV/IT/000585 - LIFE DOP; LIFE15 ENV/IT/000392 - LIFE VITISOM have been inviated and particiaped to the Summer Scholl organized by CERMNAU with the presentation of respective project backgrounds, results and dissemination materials

• 5.3 Evaluation of Project Implementation

In this section you should evaluate the following aspects of the project:

• Methodology applied: discuss the success and failures of the methodology applied, results of actions conducted and the cost-efficiency of actions

As outlined in the Mid-term report, overall positive feedbacks have been received from involved stakeholders (farmer, farmer's association, technician and responsible of Regional Institutions, student, teachers), already at the first half of project activities, by the application of demonstrative activity focused on SOM management based on the utilization of recycled biomasses from local agricultural activities. There responses were confirmed in the second part of project course, where an effort was made by the consortium to stabilize the attention of local stakeholders on the main focus points and driving topics of applied methodologies

The attainment of the LIFE on-farm composting plant strongly highlighted the feasibility of the ground hypothesis, by promoting an effective demonstration on the possibility to recycle large amount of agricultural organic residues into stable compost with agronomical, environmental and economical valorisation of organic wastes. After the initial problems related to the necessary time lag required for a valuable transfer of know-how and the acquisition of experience from the personnel of PRIMA LUCE, the on-farm composting proceed independently with an adequate self-governing of the entire process: from the estimation of available resources, the evaluation of final product and the adequate balance of the required amounts and timing for the distribution by the associate farmers in the demonstrative fields. The progressive refining of the process allowed to increase the initial amounts of final composts with an optimization of cost efficiency.

A positive response was achieved by the other project target related to the increase of SOC content associated with the SOM management with recycled biomasses. The utilization of different field sites with a representative array of cropping systems, was able to show an overall maintenance of soil productivity in respect to the traditional management based on mineral fertilization. These agronomic applications have sharply supported the dissemination activities and drawn the interest of local farmers and technicians to the adoption of project approaches at different productive scale.

The results of the project methodologies were clearly related with the applied monitoring activities. An important innovation is represented by the transfer from the scientific background of beneficiaries on the evaluation of compost quality with biological essays (suppressivity and biostimulation) and molecular characterization which represent an innovation with respect to the classical analyses currently included in the legislations based on general and broad macro chemical features (e.g. C and N and C/N ratio etc.). The information related to the detailed molecular analyses may in fact greatly promote and steer the utilization of different type of composts.

Important aspects of monitoring activities are based on the field application of GHG measurements, which is situated to our knowledge among the first attempts to organize a multi-year temporal arrays of such a dataset from agricultural soils. The development and application of reliable systems of GHG collection and analyses on open fields represent an important challenge and, although it has already undergone to some delays with respect to planned commitments, it represents a cost-effective activity which may deliver an important set of useful data to evaluate the environmental impact of SOM managements. A specific innovation was represented by the development by the Beneficiary UNIBAS of a new system of GHG analyses. The equipment was conceived to work in open filed in automatic way with a remote control of GHG sampling and evaluation with a real time communication and check od the acquired data carryng a power supply based on solar energy battery.

Also, a recovery of initial objective may be claimed for the SOM management associated to the innovative activity based on SOC sequestration with the application of biomimetic catalyst. In the second half of project course a positive trend was observed in the increase of SOC content in the specific soil treatments. The current unavoidable requirement to refer for the acquisition of the basic porphyrin to the specific restrained market of industrial catalytic application of international chemical companies, affect the cost-efficiency and represent a hindrance to the practical updating of this activity as current agronomic practice. Although at this level the production and use of such technology remain a low cost-efficiency approach, an increasing consensus was ascertained in the scientific community with the presentation of the data acquired in LIFE CarbOnFarm, which conform the previous results obtained in National Mescosagr project.

- Compare the <u>results</u> achieved against the objectives: clearly assess whether the objectives were met and describe the successes and lessons learned. This could be presented in a table, which compares through quantitative and qualitative information the actions implemented in the frame of the project with the objectives in the revised proposal:

Task	Achieved	Evaluation
B.1 on farm Composting prototype	Attainment of large scale on-farm compost prototype (4000 m2 - 12 composting lines)	The on-farm composting plant and the operative technical conditions of composting process were in line with planned commitments
B.2 biomimetic catalyst	Reorganization of initial objective with partial maintenance of the in-house synthesis of active catalyst	The amount of biomemitic catalyst will allow to attain the planned field applications; the actual cost-efficiency may represent a limiting factor for the adoption of large sale agronomic practice
B.3 set up of project sites	Five project sites have completed the expected field SOM managements	Effective application of planned SOM managements in different representative cropping systems: the agronomic problems in Mellone farm have produced a time shift of filed activities in peach orchard, that will continue for the next two years to complete the expects cycle of SOM management
Monitoring actions	6 Monitoring actions completed	Positive responses on the applied methodologies for Compost characterization, SOM evaluation and agronomical impact A large array of data has been obtained regarding: chemical, molecular and biological characterization of compost quality; quantity, quality and dynamics of SOC; positive effect on cropping system with a maintenance of crop yields and of crop quality Evaluation of field GHG emission with a development of innovative prototype for the remote acquisition of GHG data
Dissemination action	6 dissemination activities	Overall significative positive feedbacks and responses on the effectiveness of project approaches;

• Indicate which project results have been immediately visible and which results will only become apparent after a certain time period.

As indicated in the Mid-term report, the main results related with proposed SOM managements were already attained in the first half of project course, and further confirmed in the final period.

The most evident and immediate result is related to the attainment of the on-farm composting prototype and the effective valorisation of biomasses and by-products into high quality compost. Combined with this first achievement, a feasible application of SOM managements has raised the attention of farmers and stakeholders for the evident agronomical and environmental advantages related to the on-farm composting processes

As reported in the Soil Thematic Strategy "*soil is an extremely complex, variable and living medium and a non-renewable resource which performs many vital functions: food and other biomass production, storage, filtration and transformation of many substances including water, carbon, and nitrogen*. The complexity of this multivariable medium, also in agro-ecosystems, in characterized by a strong resilience capacity which response to modifications produce a transition state for the attainment of new equilibrium among the interacting parameters. As indicated in the proposal the adoption of SOM managements introduced in LIFE CarbOnFarm, are thus expected to produce results in a medium- and long-term time span. However, the indication received after four years of soil management confirm the positive effect of SOM management on both SOC content and soil biodiversity. Fast agronomic responses were derived from the field application of composts for the horticultural crops and orchards, which revealed prospective initial benefits on the either crop yields and qualities from the applied composts. These immediate results of commercial farms are also related to the previous steady managements and techniques of organic and biological farming systems; these systems thereby already underwent to a transition state and approaching a new steady state equilibrium, thus allowing the soil systems to take a fast benefit from the applied SOM managements. An initial limit in productivity was observed for the maize crop in the University farms for soil amendment with compost in respect to mineral fertilization. However, we have to consider that the in case of compost addition the Nitrogen is applied in a unique initial solution, just to evaluate the long term effect of SOM management. This approach is expected to produce a mismatch with the plant requirements during the initial crop cycles. Following the subsequent application of organic materials in this case the progressive adaptation of soil ecosystem to the organic inputs has promoted an increasing trend of maize yield in amended plots, that is approaching the normal level attained with mineral fertilization.

• If relevant, clearly indicate how a project amendment led to the results achieved and what would have been different if the amendment had not been agreed upon.

For Action B.2 the supervening impossibility in the wished attempt to perform an in-house synthesis of modified porphyrin in the university laboratory, has modified the action implementation. The foreseen change in the activity allowed to keep both technical objective and planned budget. The agreement with the Chemical Dept. of University of Tor Vergata, has partially maintained the operative in-house modification of chemical structure of the catalyst: this is a key point for the synthesis of an eco-friendly biomimetic catalysts and for the attainment of final water-soluble product which allow an easy application to the cropped fields with the current available systems and equipment used for the soil management without any required specific technology, thus avoiding additional costs for soil treatment.

• Indicate <u>effectiveness of the dissemination</u> and comment on any major drawbacks

The dissemination activities have produced a raising practical and scientific interest from the different stakeholder (farmers, technicians, agronomists, regional Institutions students) on the SOM management practices based on compost production from agricultural biomasses and on the possible benefits deriving from the application of high quality composts as current SOM management. An important “hotspot” for the diffusion of ground hypotheses and working practices was represented by the attainment of LIFE on-farm composting plant which has materialized the effectiveness of project strategies thereby accelerating and supporting the communication and dissemination. A fast and valuable transfer of proposed methodologies was achieved already in the first half of project activities with the acquisition of know-how and actual economic investment and realization of additional facilities for the recycle of agricultural biomasses by commercial farm-organization and companies

The major starting drawbacks were related to the geographical asymmetry in the effectiveness of dissemination activities. The above discussed impacts have centralized the initial project efforts in the territory of Campania and Basilicata. Besides this the positive response of communication events in Campania may have further taken advantage also from the actual attention and high concern devoted to the environmental problems by the public opinion. Conversely in Piemonte the presence of a unique beneficiary has slackened the initial organization of communication events outsides the university sphere. On the other hand, in the second part of the project an additional effort was promoted by the local Beneficiary (AGROSELVIT-DISAFA) to maintain the basic objective to carry out the planned dissemination events.

5.4 Analysis of long-term benefits

In this section please discuss the following:

1. Environmental benefits
   a. Direct / quantitative environmental benefits:

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The on-farm composting allows a feasible recycle and valorisation of organic wastes with a decrease of disposal with environmental, agronomic and economic benefits for the farming systems and local territories. The soil addition of stabilized humified composts result in a long-term steady increase of SOC level, with connected effects on the overalls physical and chemical soil fertility. The stabilization and improvement of SOC retention may contribute to lower the CO2 emission from agricultural soils The estimated increase attained with compost addition may round up to about 1.5 to 4g OC kg^{-1} that correspond to about 6 to 17 tons of OC ha-1. An important side effect is related on the biological activities of cultivated soils, associated with either the suppressive features towards soil pathogens and the bioactive stimulation of plant physiology exerted by the hormone-like humic molecular components. The addition of organic materials may in fact increase the residual and slower releasing fraction of organic N, provide a lower fixation of phosphates and improve the available pool, allowing also the development of beneficial microorganisms such as the arbuscular mycorrhizal fungi. Moreover, the improvement of biological fertility may counteract the development of plant disease. This beneficial effect may be resumed in a decrease on the consume of energetic inputs and associated costs related to mineral fertilizer (- 20 to -35%) and pesticides (-30%).

b. Relevance for environmentally significant issues or policy areas

The project approaches including on farm composting and SOM managements monitoring of soil GHG emission, SOC sequestration, compost quality, compost suppressivity etc, may contribute to match the policy issues outlined in the LIFE guidelines for the thematic Soil:

"*developing and implementing land use practices and techniques, particularly in farming and forestry production processes, which protect and improve the status of soil in terms of* *structure, organic matter, biodiversity, etc. This might include supporting farmers and foresters through pilot agro-environmental schemes, different types of environmental friendly agricultural management and technologies, reduction of residues coming from agriculture"*

as well as to strengthen the indications already issued in the LIFE and Soil protection document:

(*http://ec.europa.eu/environment/life/publications/lifepublications/lifefocus/documents/soil_protection.pdf* - see chapters *Helping agriculture improve carbon storage, Supporting soil monitoring techniques, Reducing degradation of agricultural soils).*

The proposed approaches, aimed to develop effective value chains connecting the transformation of organic wastes into high-quality soil fertilizers, comply with the comprehensive indication outlined in the action plan for EU circular economy (COM 2015 614) "*Recycled nutrients are [..] important category of secondary raw materials, for which the development of quality standards is necessary. They are present in organic waste material [..] and can be returned to soils as fertilisers[..] This will involve new measures to facilitate the wide recognition of organic and waste-based fertilisers".*

For an industrial point of view the project activities, results and further development of LIFE CarbOnFarm comply with the plan of Bio Based Industries framework (https://www.bbi-europe.eu/about/about-bbi) focused on the utilization of recycled biomasses for the development of bio-fertilizers and bio-pesticides for the sustainable management of agricultural productive chain.

In addition to the indications provided by the Bio-based Industries Consortium and European Commission, as defined in the Strategic Innovation and Research Agenda (https://www.bbi-europe.eu/sites/default/files/sira-2017.pdf), these topics well fit with the guidelines included in the recent documents issued by the European Commission, which foster the traditional and innovative use of recycled biomasses to support the potential role of agriculture within the planned actions related with the mitigation of climate changes

(https://ec.europa.eu/clima/sites/clima/files/eccp/second/docs/finalreport_agricsoils_en.pdf)

(https://ec.europa.eu/clima/sites/clima/files/forests/lulucf/docs/synthesis_report_en.pdf),

(http://ec.europa.eu/environment/soil/review_en.htm)

and able to support the "*policy instruments for increasing GHG mitigation efforts in the LULUCF and agriculture sectors*"

(https://ec.europa.eu/clima/sites/clima/files/forests/lulucf/docs/synthesis_report_en.pdf)
The utilization of compost and related demonstrative practices with application of derived bio-molecules, may support the development, or strengthen, the existing proposed regulation on bio-molecules as effective plant promoter: "*plant biostimulant means a product stimulating plant nutrition processes with the aim of improving one or more of the following characteristics of the plant: nutrient use efficiency, tolerance to abiotic stress, crop quality traits"* (COM 2016 157).

The use of recycled biomasses in agroecosystems is in line with the recent indications of EC (COM (2017) 33 final) that strongly support the reutilization of organic biomasses "*to recycling of bio-waste in organic-based fertilisers*" thereby "*turning waste management problems into economic opportunities*" thus improving environmental resilience and circular economy of agricultural sector.

The application of organic fertilizers is believed to represent an effective tool to help the carbon storage, strengthen soil biodiversity and maintain crop productivity, in cultivated lands (FAO 2017 *SOC: the hidden potential*). In fact, the decrease of soil organic matter (SOM) in agroforestry sector is considered a pressing topic in EU countries, and a target objective of Soil Thematic Strategy and related policies.

In this context, the composting process represent a valuable tool for an effective recycle of bio-waste into high quality organic based fertilizer. While the use of manure as traditional soil amendment, for the average of EU-27, is assumed to halve in the 2030, the potential contribution to humified OC content has been estimated to duplicate, for both urban-waste and green composts, attaining for the 2030 the average European level of 0.4 Mg ha-1 (*SOM management across the EU –Technical Report -2011-051*).

A supplementary environmental aspect faced by the recycle of biowastes into organic fertilizer concern the contribution to the green-house gases (GHG) emission. While the shift from landfill disposal to composting may did not produce a clear measurable direct positive balance of GHG emission, the reutilization of stabilized EOM in soils will results in an improvement of C and N storage. The GHG losses from soils were mainly underestimated; in fact, the slow time release and extensive nature, coupled with the lack of reliable analytical evaluations, resulted in larger variability of estimated fluxes. However, the historical global OC losses from soils in the past century, may account for 136±50 Pg, while the current annual fluxes are rounded up to 1.1 Pg yr-1 (IPCC 2013).

The restoration of OC level in croplands and forest soils is regarded as an updated and topical challenge for the sustainable development of agro-ecosystems. As outlined in recent LIFE meetings on Climate Change adaptation held in 2017 and 2018 in Brussels and Madrid, respectively, an increasing effort is dedicated to support the adoption of adequate and suitable SOM managements aimed to revert the trend of SOC decline and enhance the potential of cultivated soils to become an effective sink of organic carbon. Official institutional reports and sectorial studies pointed out that the agricultural soils in either temperate or tropical environments may potentially store thousands of Mt C year-1 thus turning in an effective sink in the global OC cycle

A direct effect on policy regulations was obtained by the project activities carried out in Basilicata region. Following the first indications received after the Mid-term conference by the representative of Environment and Agriculture sectors of Regione Basilicata, a recent provision approved by The Regional Council state the importance of local composting activity thereby promoting with a public financial support the activity related with the creation of diffuse small and medium size composting facilities for the reutilization of agricultural and domestic organic wastes (Delibera Regionale n° 882 31 Agosto 2018 - **Annex 7.3.3 Other dissemination annex: Delibera Regione Basilicata).** An additional effect of the involvement of the Regional Institution in the project activities is the current inclusion of specific measures in the Rural Development programme (PSR 2014-2020) of Regione Campania (sub programmes 10.1.1, 10.1.2 10.1.3) for soil amendment with organic biomasses, the SOC sequestration and the decrease of GHG emission; sub programme 4.1.1 for the investment aimed to reduce the use of mineral fertilizers, counteract the soil degradation and the realization of facility aimed to the recycle of organic biomasses.

2. Long-term benefits and sustainability

   a. Long-term / qualitative environmental benefits

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A sustainable environmental benefit is associated to the valorization of organic biomasses from local agricultural activities, as effective alternative to the disposal of such materials otherwise classified from the National and Regional legislations as “special waste”. Additional direct benefits are related to the conversion into stable composts and the use for SOM managements; in fact the application of fresh biomasses, already foreseen as possible alternative in the national provisions, besides being subjected to specific restriction, may have negative impact for either the pathogenic aspects and for the rapid mineralization of organic materials with the risks of “priming effect” and increase of GHG emission.

As carried out in project activities in Piemonte region, the composting process may be associated with the recycle systems based on the utilization of cattle slurries for energetic purpose, thereby solving the economic problem represented by the final solid digestate. A similar approach has been applied also in Campania region with the development of composting facility to the final by-product of olive oil chain represented by the olive mill waste waters. IN this context the collaboration of LIFE CarbOnFarm with the Cooperativa Nuovo Cilento has promoted the realization of on-farm composting plan that currently eliminate the liquid wastes of the associate producers thereby converting this potential pollutant into valuable final mature compost.

In this context, as previously outlined, an additional environmental benefit for the local territories is represented by the realization of local composting facilities that overcome the problems related with the large yield of organic waste. Following a careful evaluation of the methodologies proposed by LIFE CarbOnFarm this approach is currently adopted by Regione Basilicata, involving the specific knowledge developed by ALSIA beneficiary, with an extension outside the agricultural sphere and the inclusion in the circuit of on farm composting of biomasses collected from the local territory such as the organic fraction of urban waste of villages and country towns, which have not a reliable possibility to confer the biomasses to the large industrial composting plant.

b. Long-term / qualitative economic benefits (e.g. long-term cost savings and/or business opportunities with new technology etc., regional development, cost reductions or revenues in other sectors)

The valorisation of organic biomasses in compost for soil management has been recognized as valuable activity which produce both direct economic measurable advantages for cost-saving expenses related to the disposal of organic waste classified as “special waste”, and no easily quantifiable long-term benefits related to the recovery of soil fertility. Despite the inherent problems of a direct inference of composting process and SOM management on the economic balance, the overall positive evaluation from local stakeholders has fostered an acknowledgement of the reliability of the proposed methodologies by local farmers and producer’s organizations, thereby promoting the realization of additional composting facilities in Campania region. Six composting facilities have been realized in collaboration and with the assistance of the personnel of the Beneficiaries of LIFE CarbOnFarm (AZIENDA CERRO, LA COLOMBAIA, ORTOMAD, PUNZI, ROMANFRUIT, COOPERATIVA NUOVO CILENTO), with an estimated investment in facilities and associated equipment which round up to about 700.000 €

c. Long-term / qualitative social benefits

As outlined in the previous paragraph the replication of proposed methodologies has promoted a local investment on additional on-farm composting facilities. This effect has as additional outcome the creation of dedicated personnel which may be estimated at minimum as 1 FTE unit for each composting facility. Besides the already discussed issues on the treatment of local organic wastes, for the social point of view the correct application of composting process and the related demonstration activities performed during the project course, have as consequence the decline of the current mistrust of public and private stakeholder on the use of composts and the increase of the awareness on its positive environmental and productive benefits. The expertise created in the LIFE CarbOnFarm project for the realization and management of the LIFE on-farm composting prototype, have been conveyed to a planned development of Spin-off consortium of the University of Basilicata. The Spin-off formed by young Researchers involved in the activities of LIFE CarbOnFarm with the Beneficiary UNIBAS. is the AGES s.r.l.s.–Agro-ecosystems services (http://www.ecoages.it/) which currently include about 3 FTEs units

d. Continuation of the project actions by the beneficiary or by other stakeholders.

The production of on farm compost will be continued by the Beneficiary Prima Luce as well as the distribution of on-farm compost in the demonstrative fields of associated farmers and producers

The field activities related to the demonstrative and innovative practices included in Action B.3 which involve the soil addition with compost and biomimetic catalyst will be continued after the end of the project in the public farms pertaining to the University of Napoli and University of Torino. On these project sites the monitoring activities related with Action C.2 (Monitoring of Soil organic carbon stabilization) Action C3 (Monitoring the agronomical, phytopathological and practical sustainability of proposed strategies) and C.4 (Monitoring greenhouse gases emissions in field) will be continued.

In line with the After LIFE communication plan also the dissemination activities will be pursued by the Beneficiaries. The Project Committee has conceived the possibility to present a proposal for the either next call within LIFE and/or Bio Based Industry frameworks. This proposal will act as a update and continuation of LIFE CarbOnFarm based on the improvement of value chain related to the on-farm compost with an additional integration of circular economy, represented by the application of compost and compost derivatives as bio-stimulation, bio-pesticides and to produce a recyclable packaging (e.g. bio-plastic) of agricultural products with modified natural organic components

3. Replicability, demonstration, transferability, cooperation: Potential for technical and commercial application (transferability reproducibility, economic feasibility, limiting factors) including cost-effectiveness compared to other solutions, benefits for stakeholders, drivers and obstacles for transfer, if relevant: market conditions, pressure from the public, potential degree of geographical dispersion, specific target group information, high project visibility (eye-catchers), possibility in same and other sectors on local and EU level, etc.

As stated in the corresponding paragraph of Mid tern report, the *integration of a sustainable productive cycle based on local resources is expected to be easily transferred and adapted to the need and requirements of different agro-ecosystems characterized by the local availability of organic biomasses and with a decline of soil quality. In particular the feasibility of on farm compost facility strongly benefit from the possibility to be placed in a farm association exploiting the economical and processing advantages to use large amounts of different agricultural residues and to produce a large amount of composts easily conveyable to associated farms, thereby reducing the economic and environmental limiting factors related to the long range transport of organic materials*

This evaluation has been confirmed with the replication of the proposed methodologies already activated in Campania e in Basilicata region. As previously outlined additional on farm composting facilities have been realized and are currently operative. Moreover, the Regione Basilicata has started an official process for the development of a network of local small-sized composting plan for the effective recycle of agricultural residues and urban organic wastes

Besides the attainment of high-quality compost, an additional sector is represented by compost by products and derivatives (e.g humic molecule, compost tea, lignin fraction). These materials may be effectively applied as natural biostimulant, biofertilizers, bio-pesticides and for an innovative application as film packaging. The real interest for a commercial application of these materials is already included in the Annual working plan 2018 of Bio-Based Industry programme.

4. Best Practice lessons: briefly describe the best practice measures used and if any changes in the followed strategy could lead to possible adjustment of the best practices

Following the indications obtained at the intermediate stage of project activities, a refining of the on-farm composting processes was performed with a more careful planning of the sources of organic biomasses with a steady supplying of ligneous/woody fractions in order to increase the yield of final compost thereby improving the cost efficiency of the process. The result of LCA evaluation clearly indicated that for a composting plant based on farm association, it has been calculated in 10 km the extension for the transport of either fresh biomasses and final compost, for the environmental and economical sustainability. Moreover, the expertise acquired at the LIFE composting prototype in the production of green compost have been transferred to the on-farm composting plan of the University of Napoli at the project site of Castel Volturno; currently an agreement between Cermanu with a National coffee company (Kimbo) was achieved for the allocation of the coffee residues for the recycling in green compost

The agronomic results indicate the progressive approaching to the new steady state of soil equilibrium based on the annual amendment with organic materials. On the other hand, the initial contrasting response of the various cropping systems to the SOM managements (e.g. maintenance of overall productivity on horticultural crops, increase productivity on peach and kiwi orchard, decrease in maize yields) focus the necessity to conceive the modification of soil management in agro-ecosystem with a transition-state approach. Although the positive effect on SOM content may have a quick response, the conversion of conventional systems based on intensive inputs (e.g. chemical fertilizer) to organic farming result in a unavoidable time-lag response of the overall soil fertility and of cropping systems for the adaptation to the new soil management, before to reach the new steady state. This means to carefully plan the intervention (e.g. with an initial possible mixing of conventional and innovative practices) and to strongly support the public awareness of involved stakeholders (farmers, farmer associations, technician) by combining and actualize the possible short-term effect on crop productivity with the long term beneficial effect of agronomical and environmental benefit. In this respect the direct involvement of commercial farms has greatly supported the reliability and the effectiveness of the proposed project strategies and the consequent demonstrative characteristic and make the possible local replication and transfer as real options

5. Innovation and demonstration value: Describe the level of innovation, demonstration value added by EU funding at national and international level (including technology, processes, methods & tools, organisational & co-operational aspects);

The basic demonstration value is represented by the application of feasible and reliable SOM managements based on the valorisation of local resources.

In this respect a significant goal is represented by the LIFE on-farm composting facility at PRIMA LUCE. This objective face at the same time innovative aspects, for the large scale of on-farm composting process, and the demonstration value for the attainment of of high-quality compost for SOM managements rom residual biomasses

The EU economic support allow to display a large range of monitoring activities that produce a significant array of data that allow a comprehensive evaluation of the effects of SOM managements practices, with a particular innovation represented by the evaluation of compost and SOM quality and on the analyses of GHG emissions from agricultural soils. In this respect an important innovation is represented by the development of the new systems for the analysis if field GHG emission based on a remote control, used by UNIBAS at the project site of Mellone farm

Although the technological innovative activity based on the soil application of biomimetic catalyst underwent to an operative reorganization in respect to the objective of an in-house synthesis, thus still maintaining an economical limiting factor, the possibility to upscale such highly technological practices from the level of scientific research to a real field application represents an evident and important support of public sector to the introduction of innovative scientific approaches in to agro-ecosystems.

The attainment of the Handbook and Kit for self-soil evaluation, have already received a large attention of the technician, farmers, teachers, students for the combination of easy application and interpretation of proposed methodologies and the correspondence to the main technical daily problems faced in the soil management

Long term indicators of the project success: describe the quantifiable indicators to be used in future assessments of the project success, e.g. the conservation status of the habitats / species.

✓ Amount of recycled organic biomasses in the area of Salerno (agricultural residues and by-products) 15.000 tons/year; Amount of composts produced: 7.500 tons/year

- ✓ Amounts of recycled organic biomasses in Basilicata region (agricultural residues, by products and urban organic fraction (15.000-20.000 tons/year Amounts of compost produces 10.000 tons/year
- ✓ Amount of new on farm composting process activated in local territories: in the next 3-5 years from the current indications is possible to conceive to attain a number of about 15 new small-medium on farm composting facilities in the province of Salerno, Matera and Potenza
- ✓ Steady evaluation of public and institutional awareness on compost production and utilization
- ✓ Effective and stable improvement of SOM quantity (SOC sequestration) and quality: 0.5 to1.5 g Kg OC kg-1 year -1; increase of microbial biomasses, enzymatic activities,
- ✓ Maintenance of crop productivity: stable yields with reducing traditional inputs fertilizer -30%, pesticides -30%, irrigation -15-20%)
- ✓ Diffusion of compost application as current SOM management practices
- ✓ Refining and updating of regional rules and administrative obligation to speed the realization of medium-large scale on-farm composting process

6. Comments on the financial report

In respect to the Financial Issues raised in the Commission letters (Communication related to: Mid-term Report Ares(2016)4741396 23/08/2016), Fourth Monitoring visit Ares(2016)6152590 - 27/10/2016; Fifth Monitoring visit Ares(2017)5584494 - 15/11/2017; Last Note Ares(2018)4574194 - 06/09/2018) please find a dedicated Financial Annex including the requested explanations and the list of requested supporting documents (**Annex 8.3 Answer to EC's issues**)

Additional reporting Table is included in the present section to highlight the main transfers of allocated budgets between Category and Actions (Budget transfers); a supplementary information was attached in the Financial Annexes to outline the list of incurred and expected costs, divided by Actions and Cost category for each Beneficiary: Annex 8.4 Itemized project budget).

6.1. Summary of Costs Incurred

PROJECT COSTS INCURRED				
Cost category		Budget according to the grant agreement*	Costs incurred within the project duration	%**
1.	Personnel	1,513,725	1,696,409	112.1
2.	Travel	43,040	35,873	83.3
3.	External assistance	643,110	683,127	106.2
4.	Durables: total non-depreciated cost	296,200	317,548	107.2
	- *Infrastructure sub-tot.*			
	- *Equipment sub-tot.*	30,000	26,922	89.7
	- *Prototypes sub-tot.*	266,200	290,626	109.2
5.	Consumables	349,540	263,371	75.3
6.	Other costs	26,700	29,120	109.1
7.	Overheads	178,950	174,003	97.2
	TOTAL	3,051,265	€ 3,199,449	104.9

*) If the Commission has officially approved a budget modification indicate the breakdown of the revised budget Otherwise this should be the budget in the original grant agreement.

**) Calculate the percentages by budget lines: e.g. the % of the budgeted personnel costs that were actually incurred

With respect to the indications included in the Common Provisions point 24.2, "*the sum of the public organisations' contributions (as coordinating beneficiary and/or associated beneficiary) to the project must exceed (by at least 2 %) the sum of the salary costs of the civil servants charged to the project*", the final averaged rate of Public Beneficiaries is 2.30%.

Please be aware that two Public beneficiaries CERMANU and ALSIA are under the rate of 2%

Personnel:

The main discrepancy in respect to the expected budget is represented by the category Personnel.

This difference is mainly related to the larger amount of Personnel allocated by the Beneficiary PRIMA LUCE for the implementation of Action B.1, for the management of the prototype and the production of on-farm compost, and the implementation of Action B3 for the field distribution of compost in the demonstrative fields (Annex 8.4 Itemized project budget).

This increase is composed by both the larger amount of requested working hours and the higher daily rates, as compared to the corresponding quantities assigned in the original budget (Annex 8.2e Financial Report Prima Luce; Annex 8.4 Itemized project budget). With regard to the daily rates, as already pointed out in the Financial section of the Mid Term Report, different salaries were introduced following the replacement of the original Beneficiary occurred with the Amendment to Grant Agreement; the original daily rates were based on the general basic salary (union scale averaged salary) of not specialized farm workers employed by the original Beneficiary Terra Orti; the current daily rates refer to the actual salaries of qualified operators for specific farm activity (e.g. tractor driver) which may differ from the basic rate employed by the Beneficiary PRIMA LUCE.

In respect to the working time, as already outlined in the Technical part the prototype realized in LIFE CarbOnFarm project represented the first example of such large facility for the attainment of on-farm compost. Therefore, no reliable data were available on the technical requirements and needs for the estimation of the working units required for compost production and management, in the preparation of the proposal.

Additional variations in the Personnel were introduced for Actions B.3 and D.3 (+719 and +321 working hours, respectively) by the Beneficiary UNIBAS (Annex 8.4 Itemized project budget). As pointed out in the technical part these modifications are related to the need to replace the peach cultivation for the project site of MELLONE/IDEA-NATURA (Action B.3), and to allocate a large amount of personnel to fulfil the increased amount of dissemination activities, performed by the involved beneficiary, associated to the transfer of on-farm composting procedures and to the demonstrative days focused on both the composting activities and on the procedures included in the *Kit and Manual for the Self-evaluation of soil properties*

F2/Travel

1) Economies produced by the Beneficiary REGCAMP (€ 6640) have been transferred to other Beneficiaries (ALSIA, AGROSELVIT-DISAFA. CREA-OF, UNIBAS) to improve the activities included in actions D.3, D.4, D.5, E.1, according to the following scheme: ALSIA €1500, AGROSELVIT-DISAFA € 640, CREA-OF € 2500, UNIBAS € 1500 (Table: Budget transfers)

F3/External assistance

As pointed out in the first Progress Report, and further discussed in the Fourth monitoring visit, the public Beneficiaries CERMANU, AGROSELVIT, CREA-OF, have allocated in this category the budget for the high qualified personnel, specifically hired for the required expertise in the monitoring activities. In compliance with the reporting rules, the corresponding amounts were currently monthly recorded in the financial statements.

An exception is represented by the costs associated with Vincenza Cozzolino in the Financial Report of CERMANU. The total cost (€104,397), related to a triannual contract to carry out specific monitoring activities, was transferred to the University centrals administration in a unique solution and the global amount has been accordingly included in the External Assistance

The main modifications, already outlined in the Mid Term Report, introduced in the External Assistance are related to the following budget transfers (Table Budget transfers)

1) part of the costs related to the attainment of biomimetic catalyst (Action B.2), initially included in Consumables, have been allocated in F3, for a global amount of € 32,932 (+4.5% of F3 and -8.5% for F6). These changes are associated to the outsourcing of the synthesis of biomimetic catalyst, as explained in the corresponding action in the technical part of the present document:

- agreement with Siena Biotech total costs € 13,932
- agreement with Dept. di Scienze e Tecnologie Chimiche Università di Roma Tor Vergata € 18,300 (Annex 8.2a Financial report CERMANU; Annex 8.4 Itemized project budget)

2) additional costs not foreseen in the Grant Agreement, were sustained by Prima Luce for the external services related to the collection, transport and distribution of organic residues (woody residues and orchard trimming) from other farms, to supply the requirements of ligneous fraction for on farm composting process (global amount = € 30000; + 3.1 % Budget Category). A compensation has been envisaged, always as beneficiary contribution, with the removal of corresponding amounts associated with these activities in Category F4 Consumables (Annex 8.2e Financial report PRIMA LUCE; Annex 8.4 Itemized project budget)

F4b Equipment

A minor budget variation was introduced by the Beneficiary ALSIA for the acquisition of supporting equipment to carry out the dissemination activities in Action D3, D4, D5, not foreseen in the original budget (€ 2,829)

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F4c/Prototype

As outlined in the Mid term report, the actual total costs for the attainment of the prototype for the on-farm compost production have exceeded the provisional budget allocated in the Grant Agreement: + € 24,426 ~+9.2 % of original budget (Annex 8.2e Financial report PRIMA LUCE; Annex 8.4 Itemized project budget). The difference is mainly related with the attainment of the carrying facility structure and with the acquisition of monitoring systems for temperature, oxygen, moisture (probes, connection, electric panel). In order to not modify the planned overall project budget a reduction has been envisaged in the expenses charged on Consumables which were already included as Beneficiary own contribution, without change in the technical commitments

F6/Consumables

An overall decrease of total expected budget occurred in this category. The difference is mainly related to the budget modifications described in the foregoing points, for CERMANU (Consumables to External Assistance = - € 32,232) and PRIMA LUCE (Consumables to External Assistance =- € 30,000 and to Prototype = - € 24,426)

F7/Other costs

The economies produced by the Beneficiary REGCAMP in this category (€ 7,016) for the activities related to Action D.3, have been moved in F6/Consumables to improve the services related to the production of Layman's report (Table Budget transfer Annex 8.2f Financial report REGCAMP; Annex 8.4 Itemized project budget)

An additional cost, not foreseen in the original budget, was sustained by Beneficiary CREA-OF for the acquisition of toolkit for the DNA sequencing used for the Metagenomic analyses carried out in Action C.1 (€ 3,757)

Budget transfer **involving category changes**

Increased Category	Amount	DESCRIPTION	Lowered Category	Difference
Travel and subsistence	€ 5,000	The economies obtained for the Mid-term conference (Action D5) were moved in Travel for Action E1 and Actions D3, D5 (workshops and demonstrative events and to Mid Term and Final Conference) **beneficiary ALSIA**	External assistance	0
Equipment	€2,829	Part of the economies in Other costs were used to acquire equipment used in the Dissemination activities (workshops and demonstrative events and to Mid Term and Final Conference) **beneficiary ALSIA**	Other costs	0
External assistance	€ 32,232	Outsourcing for Synthesis of biomimetic catalyst (Action B.2) 1. Contract with Siena Biotech company 2. Contract with II University of Rome Tor Vergata **(beneficiary CERMANU)**	Consumables	0
External assistance Consumables	€ 5,000	The costs related to the Proceedings and to Consumables for Action D5 were used for the organization of Summer school and moved to Other costs (**beneficiary CERMANU**)	Other costs	0
External assistance	€ 30,000	External services related to the collection, transport and distribution of organic residues (woody residues and horchard trimming) from other Farms, to supply the requirements of ligneous fraction for on farm composting process (**beneficiary PRIMA LUCE**)	Consumables	0
Consumables	€ 6,800	Economies produced in Other costs were used to improve the final Layman's report (**beneficiary REGCAMP**)	Other costs	0

- Budget modifications involving transfer between Actions, **without category changes**

FROM	TO	DESCRIPTION	AMOUNT
Action A.1 External assistance	Action C.4 External assistance	Development of collecting system for field GHG data acquisition (**beneficiary UNIBAS**)	€ 10,000
Action A.1 External assistance	Action C.5 External assistance	The organization of LCA evaluation (Action C.5) is started with the set-up of project sites, after the completion of the technical planning (A.1); this change has not produced neither any delay nor	€ 8,442

		technical modification in the project activities (**beneficiary AGROSELVIT**)	
Action C.2/C.4 Travel and subsistence	Action E1/D5 Travel and subsistence	Part of the travel costs included in the Action C.2 and C.4 for sample and data collection were transferred to Actions D5/ E1 for the travel costs related to the Mid tem and final conference, Project meetings and Monitoring visits (**beneficiary CERMANU**)	€ 2,000
Action C.4 Travel and subsistence	Action D3 Travel and subsistence	In order to face with the large number of initiatives dedicated to the Communication activities an aliquot of travel costs was moved from Action C.4 to Action D.3 (**beneficiary UNIBAS**)	€ 4,000
Action D.3 Other costs	Action D.5 Other costs	The expenses previously included as “Conference fees”, were used for the Organization of Final Conference (**beneficiary REGCAMP**)	€ 2,500
Action. D.5 External assistance	Action. D.3 External Assistance	The expenses for the Mid-term conference organization were reduced without any prejudice for the event; these financial resources were used to improve other dissemination events (Actions D.3, D.5) (**beneficiary ALSIA**)	€ 5,000
Travel and subsistence	Travel and subsistence	Economies produced by the Beneficiary REGCAMP were transferred to other Beneficiaries to improve the activities included in actions D.3, D.4, D.5, E.1 (ALSIA, CRA-ORT, UNIBAS)	€ 6,900

6.2. Accounting system

- Brief presentation of the accounting system(s) employed and the code(s) identifying the project costs in the analytical accounting system,

The accounting systems of different beneficiaries are organised in accordance with the respective obligations included in National legislation. The involved University departments (CERMANU, AGROSELVIT, UNIBAS) have an electronic accounting system that comply with the rules issued by the corresponding Minister (MIUR) and by the respective University administration.
In particular, the accounting system of University departments is currently based on the Unique University Balance systems, (Bilancio Unico di Ateneo - Decreto Legislativo 27/01/2012 n° 18). All the funds are received by the central administration and subsequently allocated and assigned to the specific Departments and Research Centres which act as Expenditure Center (Centri di Spesa)
The REGCAMP use the accounting system established with the Decreto Legislativo (Regional Legislative Decree) n. 118 del 23/06/2011. The CREA-ORT is a component of Minister of Agriculture and must comply with the rules of national legislation for the public sector (D.L. 14 marzo 2013, n. 33). The ALSIA follow the Regional provisions which regulate the accounting systems in compliance with D.L. 14 marzo 2013, n. 33.
The accounting system of private beneficiary PRIMA LUCE s.a.s. are related to the Articles of National Civil Code artt. 2251-2290, which regulate the legislative obligations for the “Società Semplice” organization. For Prima Luce two different bank accounts are used for the project, referred to Banco di Napoli (BDN) and Banca di Credito Cooperativo (BCC)

The project costs in each accounting systems are identified by the following codes:

✓ Project code (LIFE12 ENV/IT 000719); for the accounting systems of University departments the project code is automatically associated with the full project title.
✓ Codice Unico di Progetto (CUP) E78C12000150006 assigned by the National Authority from the start of project activity (08/07/2013).
✓ For the Public beneficiaries all the costs and transactions related with acquisition of external goods and services are further associated with the CIG code Codice Unico di Gara (Tender Unique Code), unique for each specific tender procedure. (Legge n. 136/2010 for the traceability of financial fluxes)

- Brief presentation of the procedure of approving costs,

For the acquisition of goods and services the National and Regional legislation oblige all public beneficiaries to fulfil the compliance with the rule of the best value for money. In this respect the Public beneficiaries refer to the authorized public service of CONSIP spa (MEPA system Electronic Market for Public Administration) as first option. When the MEPA may not fulfil the requirements (e.g. lack of products or not correspondence with the required characteristics), the administration may implement the procedure related with the acquisition of different offers, depending on the own rules of respective central administration

CERMANU, CREA-ORT, REGCAMP and UNIBAS comply with a minimum of three and five requested offers with no threshold; AGROSELVIT must present three requested offers with a threshold of € 40,000; ALSIA may proceed with the acquisition of three offers for a threshold of € 20,000 and with a minimum of 5 offers for an amount ranging from € 20,000 to € 80,000.

In case of unique supplier for the specific good or service a declaration of uniqueness is provided.

- the type of time recording system used, i.e. electronic or manually completed timesheets,

The time recording system of all Beneficiaries is based on electronic completed timesheets, filled on mothly bases, based on an Excel file organized in agreement with the acknowledged by the LIFE toolkit system. The following beneficiaries, ALSIA, CREA-OF and REGCAMP, produce an internal daily independent electronic registration system. For the University departments (AGROSELVIT, CERMANU, UNIBAS) the Technical permanent staff (Technicians) involved in project activities, in addition to the project time sheets, have also a daily electronic registration system

- Brief presentation of the registration, submission and approval procedure/routines of the time registration system,

Each project beneficiary has an appointed administrative responsible (see point 4.1 "Management scheme" in the present report) which, in combination with the technical manager, is entrusted to verify the timing and correctness of periodic registration of the own personnel involved in project activities. In order to avoid, as much as possible, mistakes and time consuming remaking of inappropriate documents, the timesheets are recorded in electronic version; the copy is hence initially checked by the Beneficiary management and subsequently, at monthly interval, submitted to the Coordinating management (Administrative responsible and Coordinator) for the final approval after the evaluation of the technical compliance with requested mandatory information and with scheduled activities. The approved timesheet is finally signed by the Beneficiary responsible. The hard-original copies are kept by the administration of each corresponding Beneficiary, while the electronic true consistent copies, are stored in the recording system of Coordinating beneficiary

- Brief explanation how it is ensured that invoices contain a clear reference to the LIFE+ project showing how invoices are marked in order to show the link to the LIFE+ project

For each accounting system of Public beneficiaries, the funds related to specific projects have a dedicated section of the Budget and of the registration system, that warrant the linear traceability of the assigned incomes and expenditures. Since the acquisition of good and services are mainly based on the above mentioned MEPA system, the documents related to project costs and expenses (i.e. offers, invoices, contracts etc) are produced in electronic form by the centralized registration system; it is therefore not possible include automatically the project code; however as already explained all the costs are included in the dedicated section for the specific project. In this eventuality to comply with the LIFE Common provision is mandatory for all beneficiaries to include a stamp with a clear reference to the project based on both identification code and acronym (LIFE12 ENV/IT000719 – LIFE CarbOnFarm).

6.3. Partnership arrangements (if relevant)

Please briefly explain how financial transactions between the coordinating beneficiary and the associated beneficiaries have taken place. How is financial reporting implemented (do associated beneficiaries themselves enter the information in the financial tables or is this done by the coordinating beneficiary?)

The transfer of the first and second payments of EC contribution to each Associated beneficiary was performed accordingly with the indications provided in the specific Partnership agreements. An equal percentage quote, corresponding to the 40% of specific requested EC contributions, have been assigned to each Beneficiary. For the second payment, following an intermediate recalculation of requested budget, the Beneficiary REGCAMP have formally reduced its 2nd EU contribution to € 9,760. The Project Management Committee have hence decided to utilize the economies produced by the Beneficiary REGCAMP to improve the project activities with a redistribution of the budget, as hereby summarised:
Expected Economies of REGCAMP= € 14,684
Transfer to other beneficiaries: AGROSELVIT-DISAFA € 640 (Travel); ALSIA € 1,500 (Travel); CERMANU € 8,444 (External Assistance and Consumables); CREA-OF € 2,500 (Travel); UNIBAS € 1,500 (Travel)
The respective amounts were added in the Funding page of Financial Statement of each involved beneficiary

In respect to the Final payment, according with the decision to by-pass the 3rd Leaflet (Deliverable Action D.3), the Project Committee Meeting decided to reduce a quote of EU contribution related with dissemination activity which has been withdrawn from the final request of payment assigned to the beneficiary REGCAMP responsible of the Action: -4722 € (Annex 8.2f Financial Report REGCAMP)

The financial reporting scheme were currently updated by each Beneficiary following the specific project activities. A periodic check is performed on the electronic versions of individual financial reporting systems by the Administration of Coordinating Beneficiary. Each beneficiary retains the hard copies of administrative documents, while the corresponding certified electronic copies are periodically delivered to the Coordinating beneficiary with the contemporary updating of Project’s financial reporting system.

6.4. Auditor's report/declaration

Marco Mensitieri N° iscrizione Registro Revisori: 37710; Cross Hub srl via dei Mille 47, 80121 Napoli (IT), www.crosshub.it

6.5 Summary of costs per action

This table should present an allocation of the costs incurred per action. It should be presented in both paper and Excel format.

(Projects submitting final reports after 1 January 2014 must use this format.)

Action no.	Short name of action	1. Personnel	2. Travel and subsistence	3. External assistance	4.a Infra-structure	4.b Equip-ment	4.c Prototype	6. Consumables	7. Other costs	TOTAL
A1	Technical Planning	39,762	0	0		0		0	0	39,762
B1	Composting plant	217,881	1,770	30,000		0	290,626	30,855	0	571,132
B2	Biomimetic Catalyst	46,617	0	32,232		0		13,047	0	91,896
B3	Set up project sites	215,617	1,427	37,437		0		81,840	8,638	344,960
C1	Monitor. compost quality	91,503	3,332	68,520		0		13,030	3,757	180,142
C2	Monitor SOC	59,610	1,511	131,188		4,880		44,996	0	242,184
C3	Monitoring Agronomic impact	149,570	39	121,857		8,803		8,186	0	288,454
C4	Monitoring GHG	113,579	5,535	101,952		1,027		51,364	0	273,458
C5	Monitoring Env, Econ,sust.	53,859	81	76,753		7,604		0	0	138,296
C6	Mon. awareness	15,202	0	0		0		0	0	15,202
D1	Website	22,148	0	3,660		1,779		0	0	27,587
D2	Notice boards	4,371	0	0		0		1,010	0	5,381
D3	Workshops	158,571	8,039	55,773		2,829		1,963	4,423	231,599
D4	Info days	28,376	98	4,201		0		0	6,058	38,732
D5	Conferences	77,013	1,976	11,026		0		2,780	488	93,283
D6	Layman's	27,431	1,054	0		0		14,298	0	42,784
D8	Networking	15,950	415	0		0		0	3,416	19,781
E1	Management	359,349	10,596	8,528		0		0	2,341	380814
Over-heads										174,003

	TOTAL	1,696,409	35,873	683,127		26,922	290,626	263,371	29,120	**3,199,449**

(Projects submitting final reports after 1 January 2014 must use this format.)

Please comment on any major discrepancies between this table and the summary of costs per action set out in the grant agreement (form FB or R2).

As pointed out in the previous section, the main discrepancies between planned and actual costs are related to the implementation of Action B1 associated with the realization and management of on-farm composting prototype. The principal increased categories are represented by Personnel followed by External Assistance and Prototype, while lower expenses were charged in Consumables (Annex 8.x Itemized project budget).

As already outlined in the previous progress report, the lower amount recorded in A1 action are related to the transfers of the budget originally included in this action, to Actions C4 and C5 by UNIBAS and AGROSELVIT-DISAFA, (see Table Budget transfers)

7. Annexes

7.1 Administrative annexes

- At the stage of the final report most administrative annexes, including all **Partnership agreements** (if relevant), should have already been submitted to the Commission. For such previously submitted documents, a list indicating with which report they were already forwarded to the Commission is sufficient.

Partnership Agreements delivered with Inception report: AGROSELVIT-DISAFA; CREAOF; REGCAMP; UNIBAS; Partnership Agreement delivered with 1st Progress report: ALSIA; PRIMA LUCE

7.2 Technical annexes

.

Annex 1 Closing report on Action C.1
Annex 2 Closing report on Action C.2
Annex 3 Closing report on Action C.3
Annex 4 Closing report on Action C.4
Annex 4b Manual for the "*Automatic remote sensing software and system for field GHG sampling and analyses*"
Annex 5 Closing report on Action C.5
Annex 6 Closing report on Action C.6

7.3 Dissemination annexes

7.3.1 Layman's report

7.3.2 After-LIFE Communication plan – for LIFE+ Biodiversity and LIFE Environment Policy and Governance projects

7.3.3 Other dissemination annexes

- In hard format: *Project Brochure, Project Leaflet; Project bag; Manual on On farm composting; Handbook on self soil evaluation*
- In Electronic format: - *Project Brochure, Project Leaflet; Manual on On farm composting; Handbook on self soil evaluation; Resume of Dissemination events; - Videos of on-farm composting; Video of Final Conference; Video of Final Workshop Torino; Videos of training workshops on On-Farm composting; Application letters for Farm and Association and collaboration with LIFE CarbOnFarm; Delibera Regione Basilicata*

7.4 Final table of indicators

Annex 7.4 LIFE CarbOnFarm Final Table of indicators

8. Financial report and annexes

- 8.1 "Payment request -

- 8.2 " Financial Statements (Excel and signed PDF formats)
  8.2a CERMANU with Consolidated cost statement
  8.2b AGROSELVIT-DISAFA
  8.2c ALSIA
  8.2d CREA-OF
  8.2e PRIMA LUCE
  8.2f REGCAMP
  8.2g UNIBAS

  Annex 8.3 Answers to EC's issues
  Annex 8.4 Itemized project budget
  Annex 8.5 Gannt scheme of project activities
  Annex 8.6 Project management chart
  Annex 8.7 Auditor's report

Annex 1– Closing Report on C1 Action

-

Monitoring of composting processes and characterization of compost quality

List of Abbreviation

$^{13}C/^{31}P$-CPMAS NMR (solid state Cross Polarization Magic Angle Spinning Nuclear Magnetic Resonance on ^{13}C); **CFU**: colony-forming unit; **EC:** electrical conductivity; **FDA**: hydrolase activity with fluorescein diacetate method; **N:** nitrogen; **OC** organic carbon; **OM** organic matter; **thermochemolysis-GC-MS**: off-line thermally assisted hydrolysis and methylation pyrolysis GasChromatography Mass Spectrometry **CMP:** compost from solid digestate-Marco Polo-AGROSELVIT; **SSMP**:fresh solid digestate- Marco Polo-AGROSELVIT; **CV**: on farm compost from project site of CastelVolturno-CERMANU; **L:** on farm composts from LIFE on-farm composting plant of PRIMA LUCE; **WEOM**: Water Extract Organic Matter

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1.1 Sampling strategies and sample preparation

A sampling strategy was defined in order to perform an adequate analysis and monitoring of both composting processes and chemical quality of the starting organic substrates. Organic matrixes and final compost, representative of lots, were sampled according to procedures extrapolated from the available literature (CEN EN 12579 Rule, 1999; ANPA, 2002).
The following sampling steps were defined taking into account the composting technique used at the "Prima Luce" composting plant, logistics of organic material discharge and their types and volumes:
• picking up at least 5 subsamples (subsample is the amount of material from each taking of the lot in question) from equidistant points of the truck/compost pile (from external and middle areas) and at different depths avoiding the taking from exposed surfaces (sampling below the 10 cm layer). The subsamples for batch were then mixed to form an homogeneous composite sample to be analyzed;
• sampling points have to be more numerous in case of heterogeneous materials;
• mix again the samples manually, if necessary coarsely shredding and drying them on the tray in an oven at about 65°C;
• finely grind the sample with a suitable reel, mix thoroughly and keep in polyethylene containers.

1.2 Composts

The following composts were analysed for the chemical, biological and molecular properties.

Piemonte: project sites Tetto Frati and Grandi, supplier Marco polo company: fresh solid residues (SSMP)and mixed compost (CMP) from bio-digestate process of cow slurries from livestock farms

Campania:

- project site Castel Volturno: on farm mixed compost (CV) from cow and buffalo manure, maize straw, woody fractions from recycled woody boxes
- project sites Prima Luce/Mellone: on farm green compost (L) from
- nutritional/green materials: vegetable residues, from seasonally available crops with high nutritional capacity as easily degradable
- heavy structuring material: ligneous residues from horchard trimming, woody residues from silviculture a and from the final sieving refinement of mature compost

The composition of green and woody fractions (expressed as m3) of the twelve composts achieved from the LIFE on farm composting plant of Prima Luce were as follows:
(L stands for **lot** followed by a number (between 1 and 10) that indicated the composting productive lines and by a letter that identified the various production cycles on the same line)
L1: start 25/07/2014, end 28/10/2014
heavy (ligneous) fraction: (Quercus cerris) Turkey oak 25
nutritional: basil 20; watermelon 15; salads 15; tomato 30
L2 start 30/07/2014, end 29/09/2014
heavy (ligneous) fraction: artichoke 25; Turkey oak 5

nutritional: basil 16; watermelon 25; salads 5
L3 start 30/07/2014, end 21/10/2014
heavy (ligneous) fraction: artichoke 25; Turkey oak 5
nutritional: basil 16; watermelon 25; salads 5
L4 start 24/08/2014. end 14/11/2014
heavy (ligneous) fraction: Turkey oak 40
nutritional: 50; endive, endive scarole, lettuce 30; watermelon, melon, pepper 20
L1A: start 02/09/2014, end 06/11/2014
heavy (ligneous) fraction: 10 (Quercus cerris) Turkey oak
nutritional: lettuce 12,5; endive scarole, salads 22.5; rocket 12.5; pumpkin/watermelon 3.5; basil 11; sorghum 50; solanaceous 9
L2A start 02/10/2014, end 22/12/2014
heavy (ligneous) fraction: Turkey oak 15
nutritional: lettuce 55; endive scarole, salads 104.5; rocket 16.5; pumpkin 2.5; basil 24
L3A start 23/10/2014 end 23/01/2015
heavy (ligneous) fraction: trimming residues 36
nutritional: basil 64; lettuce, endive scarole 103; rocket 16.5; pumpkin 6; broccoli 2
L4A start 13/11/2014, end 19/02/2015
heavy (ligneous) fraction: Turkey oak 16; artichoke 4
nutritional: rocket 152.5; parsley 4; salads 4; tangerine 14
L5/6A start 21/11/2014 end 16/02/2015
heavy (ligneous) fraction: Turkey oak 19; fennel 25
nutritional: rocket 126; salads, endive scarole 28; lettuce 6.5; tangerine 28; broccoli 1,5
L7A start 11/12/2014, end 14/03/2015
heavy (ligneous) fraction: Turkey oak 10; walnut shell 30
nutritional: rocket 104.5; endive scarole, salads 25.5; cabbage, spinach, parsley 6.5; tangerine 14
L7/8 start 30/06/2014, end 05/09/2014
heavy (ligneous) fraction: olive tree trimming 40
nutritional: 50 m3 ; basil, endives, lettuce 50.
L2C start 21/11/2014 end 16/02/2015
heavy (ligneous) fraction: Turkey oak 27; fennel 50
nutritional: rocket 189.5; red chard, cabbage, spinach 7.5; lettuce 2.5; tangerine 7

2 Material and methods

Compost samples were analysed for pH, electrical conductivity, total N, OC, heavy metals (Cadmium-Cd, Chromium-Cr, lead-Pb, Nickel-Ni, Zinc-Zn, Copper-Cu), FDA, total bacteria, fungi, and phytotoxicity/biostimulation and suppressivity on Lepidium sativum, ^{13}C-CPMAS-NMR, Themochemolysis GC-MS

2.1 Chemical analyses

Elemental analyses for C, N and H, were performed with Fisons Elemental Analyzer EA 1108, using Acetanilide as standard reference for instrument calibration.

The determinations of the concentration of total heavy metals were performed on initial organic samples matrices. Analysis were performed on ICP-OES spectrometer (iCAP 6000 Series - Thermo Scientific) following sample mineralization with step-wise acidification process.

2.2 Biological analyses

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 CO2 was measured using a CO2 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 CHCl3/ CH3OH (2:1 v/v). The reaction mixture was centrifuged (700×g) and the absorbance of the aqueous supernatant measured at 490 nm.

Populations of fungi, yeast, total bacteria, and pseudomonads were evaluated in by plating serial ten-fold dilutions. Fungi were counted on PDA (Oxoid) pH 6, to which 150 mg L−1of nalidixic acid and150 mg L−1of streptomycin were added. Yeast was counted onrosebengal medium (Oxoid) 32 g L−1, to which 0.1 g L−1of chloram-phenicol (Oxoid) was added.Total bacteria were counted on selective medium (glucose1 g L^{-1}, proteose peptone 3 g L^{-1}, yeast extract 1g L^{-1}, K_2PO_4 1g L^{-1}, agar 15g L^{-1}) to which actidione (cycloheximide)100 mg $L^{-1}$was added. Pseudomonads were counted on selectiveagar medium without iron, to which actidione was added. Finally, spore-forming bacteria were counted onNutrient Agar using compost preparations previously heated at 90◦C for 10 min. Population densities are reported as logc.f.u. $ml^{-1}$of organic matrices.

The method used for the phytotoxicity assessment will be based on evaluating growth of a test plant (Lepidium sativum) on a mixture containing compost plus a base substrate consisting of sand and peat. The procedure involves the preparation of the base substrate by mixing silica sand and peat in a 1:1 volume ratio. Compost is then be added to the base substrate, at 4 increasing amounts. Each treatment is replicated four times. The different mixtures thus obtained are placed in 2 liter pots containing a bottom layer (1 cm) of expanded clay, to ensure drainage. An unfertilized control treatment (base substrate without compost addition) is also be included. The mixture is covered with a layer of 1 cm of sand where seeds will be posed to germinate, to detect the toxicity only on plant development. The number of seeds per pot is adjusted to ensure the germination of at least 100 seeds per pot. Seeds are covered with layer (1 cm) of perlite. At the end of the vegetative development (about 21 days) plants are cut at the collar to determine biomass yield.

The suppressive capability of different composts towards soil borne pathogens *Rhizoctonia solani* and *Sclerotinia minor* damping-off, was evaluated by pot assays on peat as common horticultural supporting matrices, using Lepidium sativum L. as host plant. Peat was amended with composts at rate of 20% (v/v), with un-amended peat as control.

All bioassays were carried out with sterile (twice autoclaved) and not sterile medium.

Several pathogen isolates were obtained from diseased lettuce (Lactuca sativa L.) (S. minor), and cabbage (Brassica oleracea L.) (R. solani) and maintained on PDA medium. Isolates of each species were preliminarily tested for pathogenicity on lettuce, L. sativum, and showed very similar behavior. Pathogen inocula were prepared as follows: common millet seeds were placed in 0.5 L flasks, saturated with a PDB (potato dextrose broth) solution (1/10 w/w) and twice autoclaved.

Flasks were inoculated with fungi cultured on PDA (potato dextrose agar) for 7 days, and incubated for 21 days at 20 C. The resulting fungal millet inoculum was air-dried for 3 days, powdered in a mortar, and added at four levels to the potting mixtures. Pathogen inoculums was used at different concentrations (0%, 0.3%, 1% and 3% w/w, dry weight) to test the effect of different inoculum density and to avoid the ‘‘flattening effect” of the results often observed when only one concentration is used. In the controls, non-inoculated common millet prepared as described above was added. Pots (7 cm diameter and 100 ml volume capacity) were filled with the different organic mixtures and sown with 20 *L. sativum seeds cv. Comune (Blumen*), moistened to field capacity and arranged in greenhouse (25 °C) following a complete randomized design. Pot distribution was rearranged randomly every 2 days to avoid the effects of environmental heterogeneity in the greenhouse. After 7 days disease incidence was recorded as percentage of diseased plants. Damping-off percentage was calculated as

$$\%DO = \frac{HPo\text{-}HPi}{HPi} \times 100$$

where HPo is the number of healthy plants in the non-inoculated control mixture and HPi is the number of healthy plants in the inoculated potting mixes

Bioassay test

A specific analysis on biostimulant activity of compost materials was carried out by CERMANU with bioassay test on maize and tomato seedlings in a growth chamber testing the water soluble components (Water Extract Organic Matter) of compost materials. The development of shoot and root tissues was performed with the *WinRhizo* software system. The following compost have been tested: on-farm manure compost used at CastelVolturno (CMP CV, Compost from digestate used at Tetto Frati and Grandi CMP TF, on-farm green composts (summer A and winter B) used at Mellone (CMP a and CMP B). WEOM was obtained from each 1 kg compost samples as it follows:100 g of each air-dried compost was suspended in 1000 ml of distilled water and mechanically shaken for 24 h. The suspension was then centrifuged at 1000g for 15 min and finally filtered through a 0.45 μm Whatman filter. For each compost, the WEOM extraction was carried out in triplicate. An aliquot of each WEOM extract was freeze-dried prior to further analytical analyses.

A bioactivity assay on seedling growth was conducted in a growth chamber with the following parameters: 75% of humidity, 20–2 7 °C temperature range and a photoperiod comprising 8 h of darkness and 16 h of light. Maize (Zea mays L. var. 30.21, Limagrain) and tomato (cultivar Pullrex) seeds were soaked in distilled water for one night and germinated in the dark at 25 °C on filter paper moistened with distilled water. After germination, maize seedlings (four days old)with uniform size, shape and healthy aspect were selected and transferred into 15 tubes filled with a modified Hoagland solution composed of: 40 μM KH_2PO_4, 200 μM $Ca(NO_3)_2$, 200 μM KNO_3, 200 μM $MgSO_4$, 10 μM FeNaEDTA, 4.6 μM H_3BO_3, 0.036 μM $CuCl_2 \cdot 2H_2O$, 0.9 μM $MnCl_2 \cdot 4H_2O$, 0.09 μM $ZnCl_2$, 0.01 μM $NaMoO_3 \cdot 2H_2O$. After 8 d from transplanting, plantlets were treated as it follows: 1. control with only nutrient solution, 2. addition of the WEOM extract diluted 1:10 into the nutrient solution.

Plants were harvested 72 h after the application of WEOM treatments. Fresh and dry weight of shoots and roots and root length were measured. Plantlets of growth test were first scanned with an

Epson Perfection V700 modified flatbed scanner and, then, their length was measured by the WinRhizo software, version 2012b (Regent Instruments, Inc.).

2.3 Molecular characterization

The molecular characterization have been performed by solid state Nuclear Magnetic Resonance (CPMAS-NMR) and off-line thermochemolysis-Gas Chromatografy Mass Spectrometry (THM-GC-MS). The combined application of 13C cross-polarization magic angle spinning nuclear magnetic resonance spectroscopy (13C-CPMAS-NMR), and off-line pyrolysis followed by gas chromatography-mass spectrometry (THM-GC-MS), are updated and powerful tools for the molecular investigation of complex organic materials.

2.3.1 13C CPMAS NMR

The nondestructive NMR techniques provide the distribution of organic carbons in a wide range of different matrices and are properly applied to characterize the composition and transformation, of natural organic materials. Besides the basic distribution of C functionalities, the solid state NMR application allows the implementation of specific experiments dedicated to the evaluation of steric arrangement and conformational behavior of complex materials. In particular the analytical appraisal of specific parameters related to cross polarization dynamics, such as cross-polarization time (*tCH*) and the spin-lattice proton relaxation in the rotating frame ($t1\rho H$), are suitable to evaluate structural properties and modification in complex substrates.

Pyrolysis in the presence of tetramethyl ammonium hydroxide (TMAH) is commonly used to study the detailed molecular composition of either natural and synthetic biopolymers. It involves the cleavage of covalent bonds combined with the solvolysis and methylation of ester and ether groups, in complex mixture of organic macromolecules and biopolymers, thereby enhancing the thermal stability of acidic, alcoholic, and phenolic groups and allowing a suitable chromatographic detection of pyrolytic products.

Fine-powdered composite samples of bulk compost samples, were analyzed by solid-state NMR spectroscopy (13C CPMAS NMR) on a Bruker AV300 Spectrometer equipped with a 4 mm wide-bore MAS probe. The NMR spectra of initial and composted substrates were obtained by applying the following parameters: 13,000 Hz of rotor spin rate; 2 s of recycle time; 1H-power for CP 92.16 W: 1H 90° pulse 2.85 µs; 13C power for CP 150,4 W; 0.5 to 2 ms of contact time; 30 ms of acquisition time; 4000 scans. Samples were packed in 4 mm zirconium rotors with Kel-F caps. The cross-polarization pulse sequence was applied with a composite shaped “ramp” pulse on 1H channel in order to account for the inhomogeneity of Hartmann-Hann condition at high rotor spin frequency. The Fourier transform was performed with 4k data point and an exponential apodization of 100Hz of line broadening. Solid-state 13C Nuclear Magnetic Resonance (NMR) is a powerful tool for non destructive study of solid samples. The analysis of solid materials, however, implies the occurrence of unavoidable technical drawbacks, such as chemical shield anisotropy and dipolar coupling effects, with a loss of signal resolution. Although the application of Magic Angle spinning and high power decoupling, produce a significant improvement of spectral quality, the solid state spectra of organic materials are characterized by a large signal broadening and an overlapping of carbon functionalities. As a consequence, the main natural organic components, are conventionally grouped into the following chemical shift regions: Alkyl-C: 0-45 ppm; Methoxyl-C/C-N: 45-60 ppm; O-Alkyl-C: 60-110 ppm; Aromatic-C: 110-145 ppm; O-Aromatic-C: 145-160 ppm, and

Carboxyl-C: 190-160 ppm. The relative contribution of each region was determined by integration (MestreNova 6.2.0 software, Mestre-lab Research, 2010), and expressed as percentage of the total area. In order to summarize the modification occurring with incubation time, two indices of the extent of decomposition, named Hydrophobic index (HB) and Aromatic ratio (Ar) were determined from the combination of relative area of different NMR spectral regions (Table 1) as follows:

HB=Σ [(0-45)+1/2(45-60)+(110-160)] / Σ [1/2(45-60)+(60-110)+(160-190)]

Ar=Σ [1/2(45-60)+(110-160)] / Σ (0-160)

In order to determine the conformational behavior and the best NMR acquisition parameters of complex organic materials, Variable Contact Time (VCT) experiments have been performed on different composts. The analytical conditions were the following:

VCT 13,000 Hz of rotor spin rate; 2 s of recycle time; 92.16W 1H-CP pulse power; Variable contact time: from 0.01 to 10 ms for a total of 20 steps; 30 ms of acquisition time; 2000 scans

The equation used to fit the experimental data and to evaluate the molecular CP parameters was the following:

1) VCT $I=[I0/\alpha]\ x\ [exp\ (-tCP/\ t1\rho H)]\ x\ [1-(exp\ (-\alpha\ tCP/\ tCH)]$

I=experimental signal intensity; I0=theoretical max signal intensity; α=(1-tCH/t1ρH)] tCP=instrumental variable contact time(s); tCH = molecular cross polarization time; t1ρH= molecular proton-lattice relaxation time (in the rotating frame)

2.3.2 Off-line TAHM-GC-MS

For off line-TAHM-GC-MS about 0.2 g of bulk compost samples were placed in a quartz boat with 0.5 mL of TMAH (25% in methanol w/v) solution. After drying under a stream of nitrogen, the mixture was introduced into a Pyrex tubular reactor (50 cm × 3.5 cm i.d.) and heated at 400 °C for 30 min in a circular oven (Barnstead Thermolyne 21100 Furnace, Barnstead International, Dubuque, IA, USA). The gaseous products from thermochemolysis were flowed into two chloroform (50 mL) traps in series, kept in ice/salt baths. The chloroform solutions were combined and rotoevaporated to dryness. The residue was dissolved in 1 mL of chloroform and transferred in a glass vial for GC-MS analysis. The GC-MS analyses were conducted with a Perkin Elmer Autosystem XL by using a RTX-5MS WCOT capillary column (Restek, 30 m × 0.25 mm; film thickness, 0.25 mm) that was coupled, through a heated transfer line (250 °C), to a PE Turbomass-Gold quadrupole mass spectrometer. The chromatographic separation was achieved with the following temperature program: 60 °C (1 min. isothermal), rate 7 °C min−1 to 320 °C (10 min. isothermal). Helium was used as carrier gas at 1.90 mL min−1, the injector temperature was at 250 °C, and the split-injection mode had a 30 mL min−1 of split flow. Mass spectra were obtained in EI mode (70 eV), scanning in the range 45–650 m/z, with a cycle time of 0.2 s. Compound identification was based on comparison of mass spectra with the NIST-library database, published spectra, and real standards

3 Results

3.1 Chemical analyses

The data related to pH, EC and elemental composition of analyzed composts are shown in Table 1. Almost all the composted materials were characterized by a sub-alkaline or alkaline pH, which ranged from 6.95 to 9.10, characteristic of the increasing concentration of minerals components, such as alkaline metals (e.g. Ca, Mg, K, Na), with the progressive decrease of total dry weigh during the composting processes; the EC ranged from 1.3 to 3 µS cm^{-1} with the exception of the on farm compost from Castel Volturno which showed a largest salt concentration (6.591 µScm^{-1}) may be related to the inclusion of buffalo manure in the starting matrix components.

The solid digestate and the mixed composts made from cattle manure, used in Piemonte (SSMP, CMP) and in the project site of Castel Volturno (CV) showed an larger content of total N in respect to the green composts of Prima Luce, except for the composts L7A (Table 1). This finding is related to the different source of organic materials involved in the composting processes. In fact the large amount of fresh nutritional easily degradable matrices used for the attainment of green compost, may have promoted a larger decomposition of bio-labile components, such as peptide moieties. However no large differences were found in the final C/N ratio which ranged from 18 to 23, a part for the fresh solid digestate which larger OC content raised the C/N ratio to about 27

Table 1 Chemical characteristics of composts

Compost	pH	EC (µS cm^{-1})	OC (%)	N (%)	C/N[a]
SSMP	7.92	1.904	43.8	1.91	26.8
CMP	8.38	2.982	35.1	1.96	20.9
CV	8.57	6.591	34.3	2.0	20.0
L1	7.77	1.722	26.7±0.8	1.30	24.0
L2	8.43	1.316	29.7±1.2	1.28	27.1
L3	9.10	2.639	32.1±0.9	1.55	24.2
L4	8.73	2.258	34.2±2.2	1.76	22.7
L1A	7.73	1.621	23.9±1.3	1.20	23.2
L2A	9.02	1.514	28.9±1.0	1.68	20.1
L3A	8.59	1.564	31.0±3.2	1.52	23.8
L4A	7.00	1.339	30.0±2.1	1.89	18.5
L5/6A	6.95	1.950	25.2±0.6	1.32	22.3
L7A	8.20	2.324	33.0±0.8	2.31	16.7
L7/8	8.82	2.684	26.0±0.5	1.68	18.1
L2C	8.21	1.85	31.8	1.85	20.1

a atomic ratio

All the analyzed compost showed a concentration of heavy metal, below the legal limits fixed for both, green and mixed composted materials (Table 2)

Table 2. Average heavy metals content (mg kg^{-1} dry matter basis) found in fresh materials

Element	Units	Range	law limits *
Cd	mg/kg d.m.	0 - 0.41	1.5
Cr	mg/kg d.m.	0.43 - 5.85	0.5 **
Cu	mg/kg d.m.	2.29 - 21.22	150
Ni	mg/kg d.m.	0.4 - 4.86	50
Pb	mg/kg d.m.	0.66 - 11.52	140
Zn	mg/kg d.m.	2.98 - 122.8	500

*Limits according to – D.lgs. 75/2010 – Composted soil improvers. **Cr VI

3.2 Biological analyses

Notwithstanding the differences among composts all organic matrices showed an effective biological activity as revealed by the total hydrolase properties (FDA) (Table 3). Also different responses were found in the phytotoxicity/biostimulation assays on Lepidium, with all composts revealing a positive effect on the plant development at larger doses concentrations (Table 3)

Table 3. Biological analyses: hydrolase activity (FDA), bioactivity, quantification of total bacteria (Tot B), Pseudomonas-like (Ps Lb) and Bacillus-like bacteria (BLb) and total fungi (TotF).

Compost	FDA ($\mu g\ g^{-1}$d.m)	Biostimulation (%)			Tot B	Ps Lb	BLb	Tot F
		50 g/L[a]	15 g/L[a]	5 g/L[a]	(Log_{10} CFU[b] g^{-1})			
SSMP	16.2	28.7	61.8	44.0	6.0	5.3	4.6	3.8
CMP	6.8	25.3	19.3	5.2	8.8	12.1	5.7	4.2
CV	7.7	25.9	22.6	32.1	6.2	7.2	5.5	4.8
L1	3.2	8.8	17.9	-23.5	8.6	7.8	8.6	4.7
L2	1.8	7.9	-3.7	-13.3	7.9	12.4	6.5	4.2
L3	2.9	23.3	7.8	-0.8	7.5	7.8	6.9	5.9
L4	8.3	34.6	64.4	19.1	12.6	12.6	6.4	5.2
L1A	7.0	140.5	129.1	92.6	8.0	7.8	5.6	6.0
L2A	12.0	44.2	39.4	40.0	6.8	6.7	6.1	3.3
L3A	11.4	9.8	1.5	-7.4	6.6	7.6	5.8	4.0
L4A	2.6	30.9	32.3	32.9	8.1	8.2	5.8	6.0
L5/6A	2.7	12.3	24.9	20.7	8.2	8.2	6.8	5.2
L7A	2.7	12.3	24.9	20.7	5.9	5.7	5.9	5.23
L7/8	3.8	5.6	20.9	-1.2	7.6	7.7	6.8	6.4
L2C	2.7	17.3	0.5	2.2	7.0	7.0	6.3	4.6

a compost doses

b Colony-forming unit

All composts were analysed for concentration of total bacteria and fungi, Pseudomonas spp. and thermal resistant bacteria (such as Bacillus spp.) (Table 3) and for presence or counting of potentially harmfull bacteria, such as Enterobacteria, Clostridium spp., Escherichia coli, Salmonella, total and faecal Coliforms and Streptococcus spp. (Table 4). Total bacteria and fungi ranged from 5.88 to 12.56 and from 3.30 to 6.36 log10 C.F.U. g^{-1} of compost, respectively. Pseudomonas spp. and thermal resistant bacteria ranged from 5.30 to 12.62 and from 4.60 to 8.56 log10 c.f.u. g^{-1} of compost, respectively. No Enterobacteria and no Salmonella were detected in all the compost, whereas total and faecal Coliforms were detected in almost all the composts. Streptococcus were detected only in one compost and Escherichia coli was detected only in three composts. Eight composts containing Clostridium spp.

Table 4. Microbiological analyses: detection of harmful bacteria (*Enterobacteria*- Ent; *Clostridium*-Clos; *Escherichia Coli* E-Coli; *Salmonella*-Sal; *Total Coliform*-TC, *Fecal Coliform*-FC; *Sptreptococcus*-Sptrc)

Compost	**Ent**	***Clos***	***E. coli***	***Sal***	**TC**	**FC**	**Sptrc**

	(Log_{10} CFU[a] g^{-1})				MPN[b]		
SSMP	0	0	0	0	4.5	0.9	0
CMP	0	0	0	0	4.5	0	0
CV	0	0	0	0	9.5	0	0
L1	0	0	0	0	15.0	0.9	0
L2	0	4.5	0	0	9.5	4.5	0
L3	0	4.0	0	0	25.0	4.5	0
L4	0	0	0	0	140.0	45.0	0
L1A	0	2.6	0	0	9.5	0.4	0
L2A	0	3.7	0	0	0.9	0.4	0
L3A	0	3.4	0	0	0	0	0
L4A	0	3.8	0	0	0.4	0	0
L5/6A	0	0	0	0	7.5	2.5	0
L7A	0	3.2	0	0	9.5	0.9	0
L7/8	0	3.7	0	0	9.5	4.5	0
L2C	0	2.5	0	0	9.5	0	0

a Colony-forming unit b Most probable number

On farm composts were analyzed for their potential suppressive activity against two "soil-borne" phytopathogenic fungi, *Rhizoctonia solani* and *Sclerotinia minor*, using Lepidium sativum as host plant. Bioassays were carried out under laboratory conditions, in artificially infected soil-peat amended with raw or heat-sterilized compost samples at 20% vol. concentration. The reduction of damping-off disease incidence found in each treatment for all the analysed composted materials, revealed a significative level of disease suppressiveness, , compared with the not-amended controls. The in vivo assays evidenced a differential responses of the fungal infections on seedling among the various examined composts (Fig. 1). The duplication of the bioassays with *R. solani* and *S. minor* on L. sativum using additionally autoclaved composts, allowed to estimate the relative contribution of the biotic component of the amendment, that was completely eliminated by heating, on the whole suppressive effect displayed by raw samples.

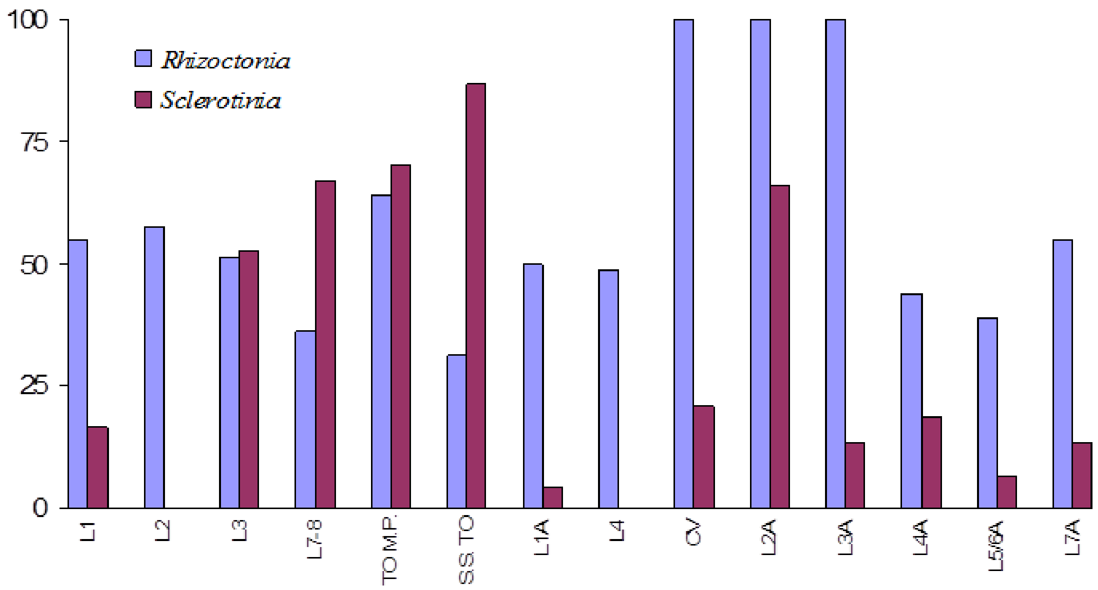

Figure 1. *Compost suppressivity against Rhizoctonia solani and Sclerotinia minor.*

Moreover, the two pathogens helped to differentiate the magnitude and the variability of this peculiar property of on farm composts among both the samples and the pathogens with different aggressiveness. In order to evaluate the plausible mechanisms underlying on farm compost suppressiveness, data of bioassays will be computed in a broad view with all other measured chemical, biochemical and microbiological characteristics of each sample. However, starting a deep investigation of the biological component of composts able to cause pathogen suppression, antagonistic bacteria populations has been isolated, collected and assayed for their in vitro activity against the two model pathogens (Table 5).

Table 5. *Selection of Bacillus-like bacteria for their antibiosis activity against Rhizocotonia solani and Sclerotinia minor.*

Compost	N° Heat-resistant isolates	N° Antagonistic isolates	
		Rhizoctonia	*Sclerotinia*
SSMP	14	13	10
CMP	16	0	0
CV	11	8	8
L1	6	0	0
L2	11	6	7
L3	24	24	5
L4	8	7	6
L1A	14	4	4
L2A	4	1	0
L3A	21	6	9
L4A	17	1	1
L5/6A	6	0	0
L7A	13	7	5
L7/8	19	5	12
L2C	nd	nd	nd

Bioassay test

All the water extracts (WEOM) of compost materials showed positive stimulation effects on both shoot and root development and on the total biomass of maize ad tomato plantlets (Figs. 2, 3, 4). This biostimulation has been related tot the hormone-like components included in the humified fractions of compost materials during the stabilization phase of composting processes. Although a defined correspondence between the molecular features of complex organic matter and plant cellular pathways has not yet been fully clarified, recent studies indicated the positive role of aromatic and phenolic components of soluble humic and humic-like fractions as potential molecular bio-effectors. range of different metabolic intermediates and pathways were claimed to be involved in the humic/plant interactions, such as modulation of phosphorus availability, P-transporters, aquaporins system, IAA, ABA, PM-H + -ATPase, NO, ROR. The results of bioassay test suggested that a positive the role may be played by lignin derivatives in stimulating plant growth. However, not only the content of aromatic moieties in HS from composts, but also the cooperative occurrence of different variables, such as WEOM concentration and its partitioning equilibrium in soil solution, should be responsible for the bioactivity of the humic derived organic substances. In fact, it is the release of bioactive molecules from the humic supramolecular associations in the rhizosphere system that confers the plant bio-stimulation capacity to the humic substances from composts. In fact, the dynamic equilibrium of contiguous hydrophilic and hydrophobic domains as controlled by the soil/plant conditions (pH, moisture, ionic strength, root exudates, active mineral surfaces, etc.), regulates the release and mobility of small bioactive molecules

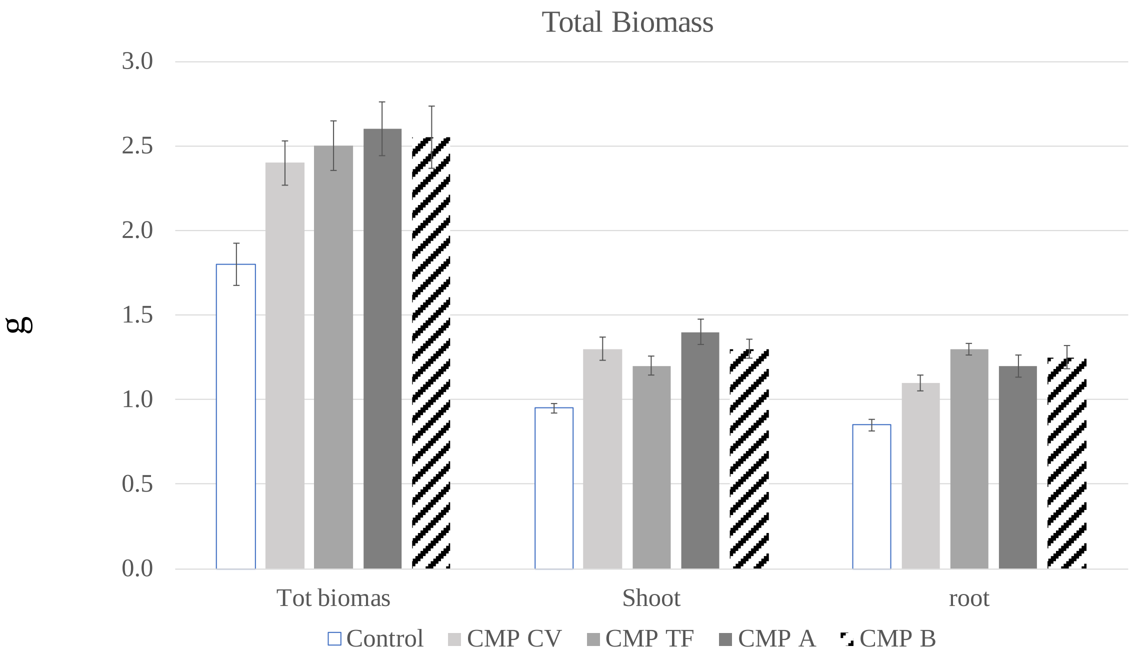

Figure 2 Yield of fresh biomass (g) of maize seedlings in bioassay test with WEOM from compost materials

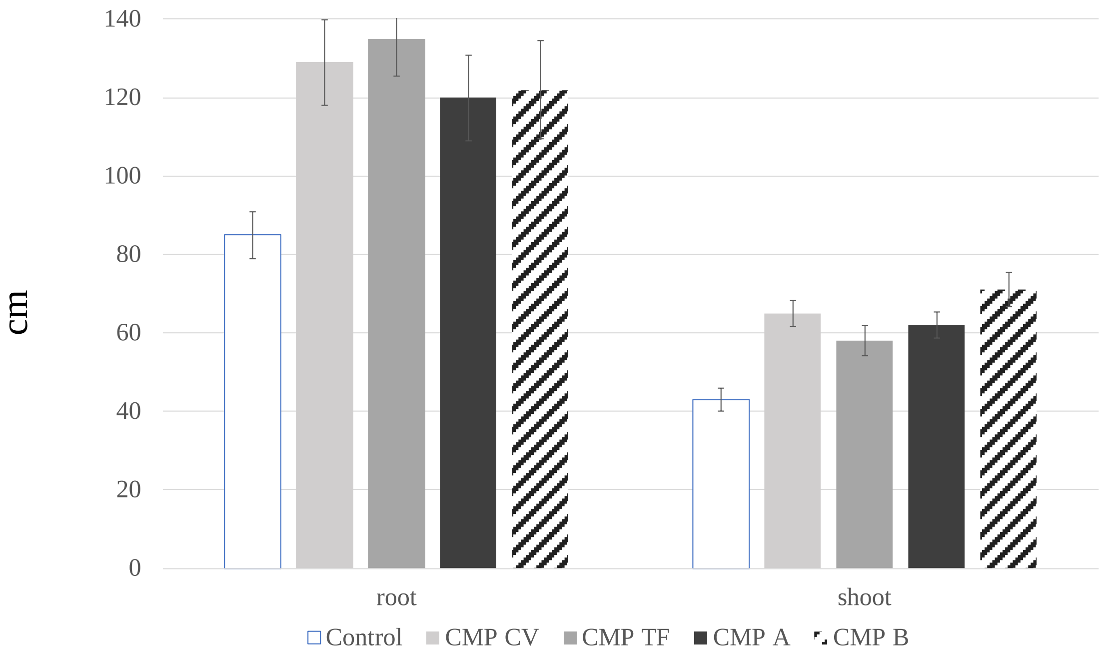

Figure 3 Shoot and rood development (cm) of maize seedlings analysed by winrhyzo in bioassay test with WEOM from compost materials

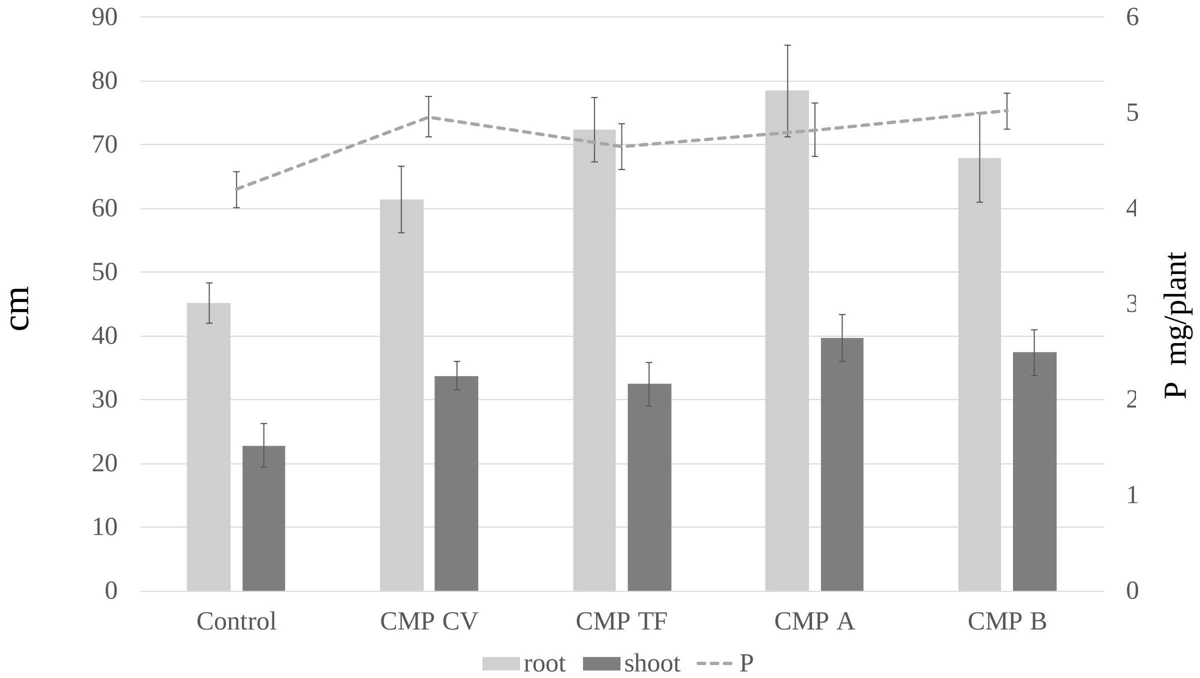

Figure 4 Root and shoot development (cm) and P content (mg) in tomato plantlets in bioassay test with WEOM from compost materials

3.3 Molecular characterization

3.3.1 CPMAS 13C NMR analysis

The spectra in Figure 4 show representative VCT experiments carried out to determine the best NMR acquisition parameters for compost samples. A comparable optimum contact time around 1 ms was found for the various organic materials from either manure derived (SSMP, CMP and CV) or green based composts. This fitting value correspond to the average contact time usually applied in NMR analyses of natural organic matter. In fact the proton density associated complex organic matrices allow an averaged spin diffusion among the different molecules thus promoting a common similar cross polarization dynamics.

The NMR spectra of bulk compost are reported in Figures 5a, 5b, 5c

The different resonances in the O-alkyl-C region (60-110 ppm) are currently assigned to monomeric units in oligo and polysaccharide chains of plant tissue . The intense signal around 72 ppm corresponds to the overlapping resonances of carbon 2, 3, and 5 in the pyranoside structures in cellulose and hemicelluloses, whereas the signal at 105 ppm is the specific mark of anomeric carbon 1 of linked glucoses in cellulose. The shoulders at 62/64 and 82/88 ppm represent, respectively, carbon 6 and 4 of carbohydrate rings, the low field resonances (higher chemical shift) of each couple being related to the presence of crystalline forms of cellulose, while the high field ones (lower chemical shift) are typical of amorphous forms and/or hemicellulose structures.

In the alkyl-C interval (0-45 ppm) the shoulder marked by peaks at 19 and 31 ppm, indicated, respectively, the presence of terminal methyl groups of aliphatic chains and that of bulk methylenes (CH2) segments of different lipid molecules, like wax, sterol and cutin components, while the shoulder around 26 ppm is associated with the CH2 group in α and β position of alcoholic portion in aliphatic esters. The less intense and broader signals at 39-40 ppm, are mainly attributable to the inclusion of tertiary (CH) and quaternary (C-R) carbons in assembled rings of sterol derivatives.

The signal at 56 ppm is related to the methoxyl substituent on the aromatic rings of guaiacyl and syringyl units in lignin, with a possible partial contribution of the C-N groups in peptidic moieties.

The broad band included in the 110-130 ppm shift interval, derive from protonated and C-substituted phenyl carbon of lignin monomers, as well as from the typical aromatic moieties in lignans and flavonoids. The slight shoulder shown in the phenolic region (140-160 ppm) indicated the initial lower relative contribution of O-substituted aromatic carbons, pertaining to lignin and lignan components. Finally, the signals in the carbonyl region (190-160 ppm) result from the overlapping of different carboxyl groups related to aliphatic acids, amide groups in amino acid moieties and acetyl substituent in carbohydrates components of hemicelluloses.

The NMR spectra of bulk compost samples, revealed an effective stabilization of organic biomasses, characterized by the relative preservation of recalcitrant molecules. Although the direct correlation of quantitative NMR outcomes with decomposition processes and their extensive application to different organic materials may be questionable, the evaluation of NMR characteristics have revealed to be reliable probing tools to estimate, either, reactivity or recalcitrance of decomposing biomasses such as composts, litters and soil OM inputs. The marked decrease of O-alkyl- carbon atoms (60-110 ppm), combined with the increase of NMR signals associated to alkyl (0-45), aromatic (110-160) and methoxyl (45-60) regions, (Table 6), is in line with the selective preservation of stable hydrophobic organic compounds in final mature composts, currently observed in aerobic composting processes. The degradation of polysaccharide constituents, in final compost samples, was further highlighted by the flattening shown for the shoulders related to the C4 atoms, involved in the glycosidic bond (82-84 ppm), associated with a corresponding broadening of the C1 resonances (104-105 ppm) (Fig. 5), that suggested the progressive breakage of the 1-4 linkage and the consequent shift of the residual unbound carbons to less deshielded position. Moreover, the molecular modification of bulk compost samples, summarized by the structural HB and Ar indexes, confirmed the significant improvement of the overall hydrophobic character associated with the OM stabilization of composting matrices (Table 6).

Table 6 Relative distribution (%) of signal area over chemical shift regions (ppm) in 13C-CPMAS-NMR spectra of initial biomasses (t0) and final bulk compost samples

Samples	190-160	160-145	145-110	110-60	60-45	45-0	HB[a]	Ar[a]
SSMP t0	4.4	4.6	14.3	62.9	7.8	6.1	0.41	18.8
CMP	5.2	4.5	13.7	54.6	10.1	11.8	0.54	18.3
CV t0	8.6	1.6	9.7	59.1	11.1	10.0	0.40	11.3
CV	6.6	3.6	13.7	49.1	11.1	16.0	0.63	17.3
L1 t0	8.4	2.0	6.7	49.6	9.5	23.8	0.64	8.7
L1	6.4	3.0	10.7	37.6	12.5	29.8	0.99	13.7
L2 t0	8.8	2.6	9.1	52.2	10.6	16.6	0.55	11.8
L2	5.8	3.6	12.1	42.2	13.6	22.6	0.82	15.8
L3 t0	9.2	2.8	13.2	56.2	9.8	8.9	0.45	15.9
L3	7.2	4.8	17.2	46.2	9.8	14.9	0.71	21.9
L4 t0	7.1	2.7	12.8	54.9	11.2	11.2	0.52	15.6

L4	5.1	4.7	15.8	46.9	11.2	16.2	0.74	20.6
L1A t0	4.3	1.6	12.3	60.7	8.8	12.2	0.47	13.9
L1A	5.3	4.6	15.3	45.7	11.8	17.2	0.76	19.9
L2A t0	5.6	3.1	12.8	58.5	7.8	13.2	0.51	15.9
L2A	3.6	5.1	16.8	48.5	9.8	16.2	0.75	21.9
L3A t0	5.7	1.6	12.0	52.8	14.0	13.9	0.59	13.6
L3A	4.7	3.6	14.0	43.8	14.0	19.9	0.80	17.6
L4a t0	8.3	1.5	14.0	49.8	11.9	14.6	0.62	15.5
L4A	6.3	3.5	18.0	39.8	11.9	20.6	0.92	21.5
L5/6A t0	6.0	3.6	9.5	60.4	9.1	11.4	0.44	13.1
L5/6A	4.0	3.6	11.5	55.4	11.1	14.4	0.54	15.1
L7A t0	4.6	5.5	18.4	50.9	12.0	8.6	0.69	23.9
L7A	6.6	7.5	21.4	35.9	14.0	14.6	1.02	28.9
L7/8 t0	5.7	2.2	7.9	58.2	10.9	15.1	0.48	10.1
L7/8	6.7	4.2	12.9	41.2	12.9	22.1	0.84	17.1
L2C t0	6.2	2.3	7.4	51.0	8.4	24.6	0.67	9.7
L2C	5.2	3.3	12.4	39.0	11.4	28.6	1.00	15.7

a HB=Σ [(0-45)+1/2(45-60)+(110-160)] / Σ [1/2(45-60)+(60-110)+(160-190)]

Ar=Σ [1/2(45-60)+(110-160)] / Σ (0-160)

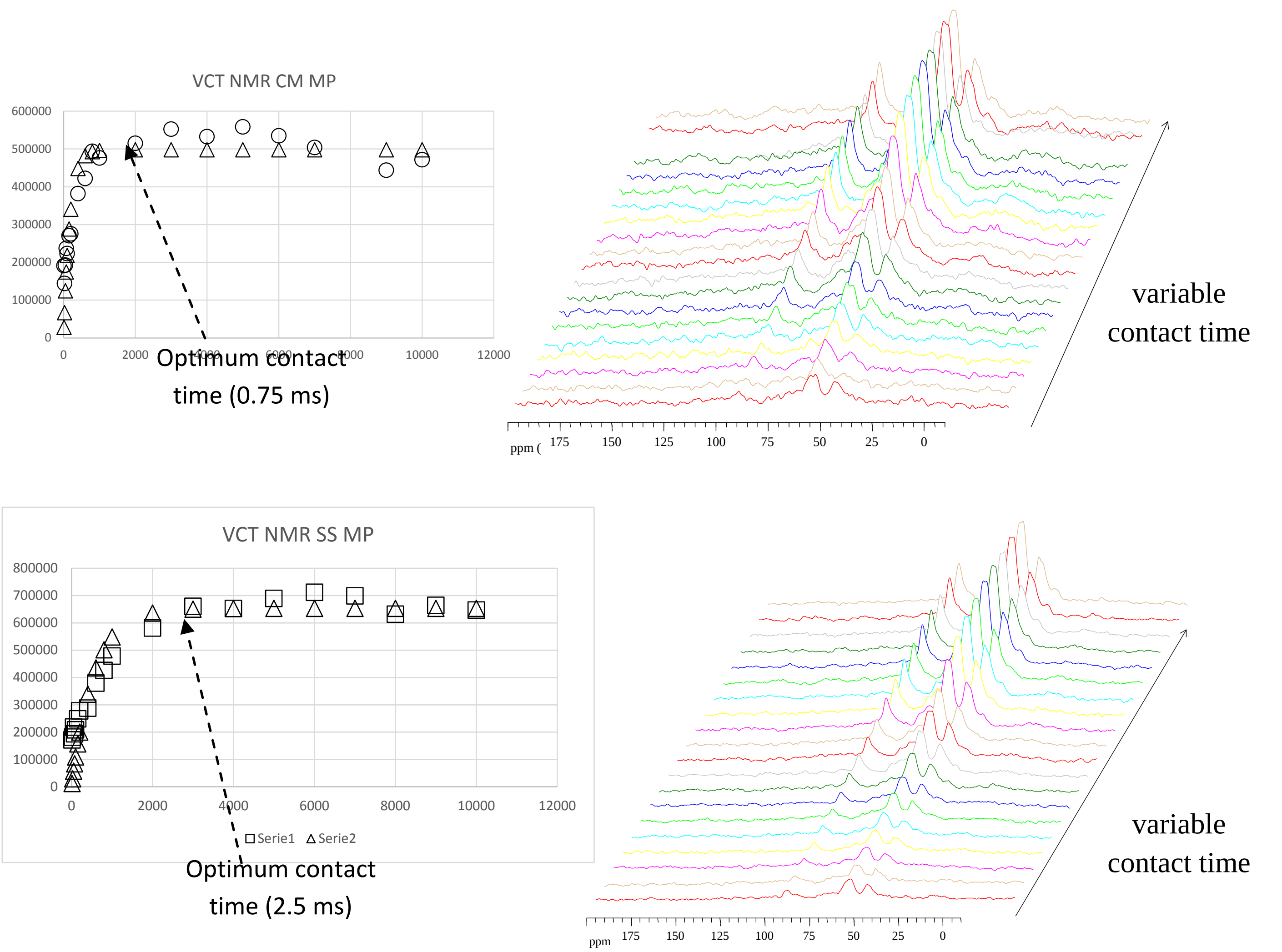

Figure 4 Pseudo bidimensional VCT NMR experiments and VCT curves of SSMP and CMP samples

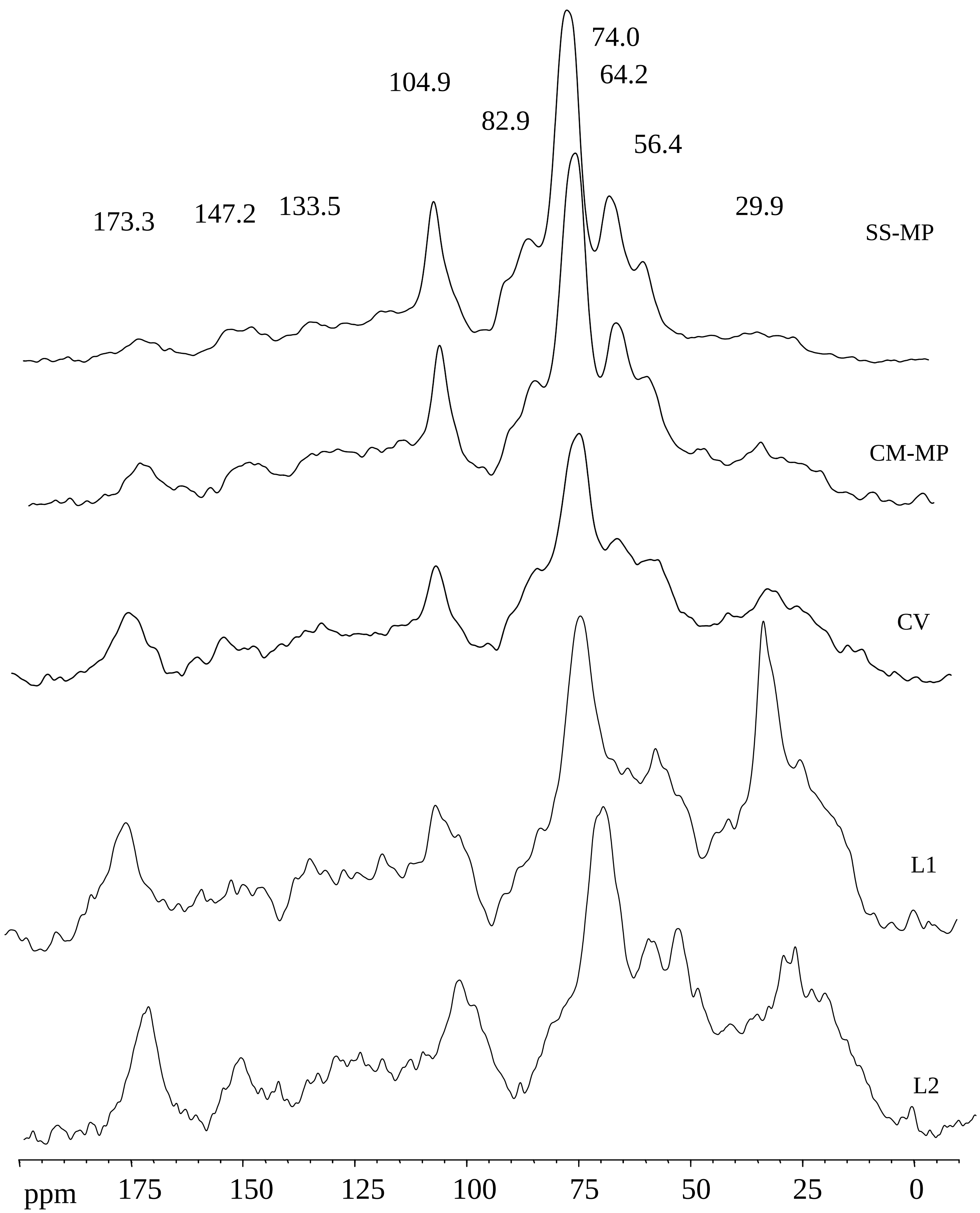

Figure 5a 13C CPMAS spectra of mature compost

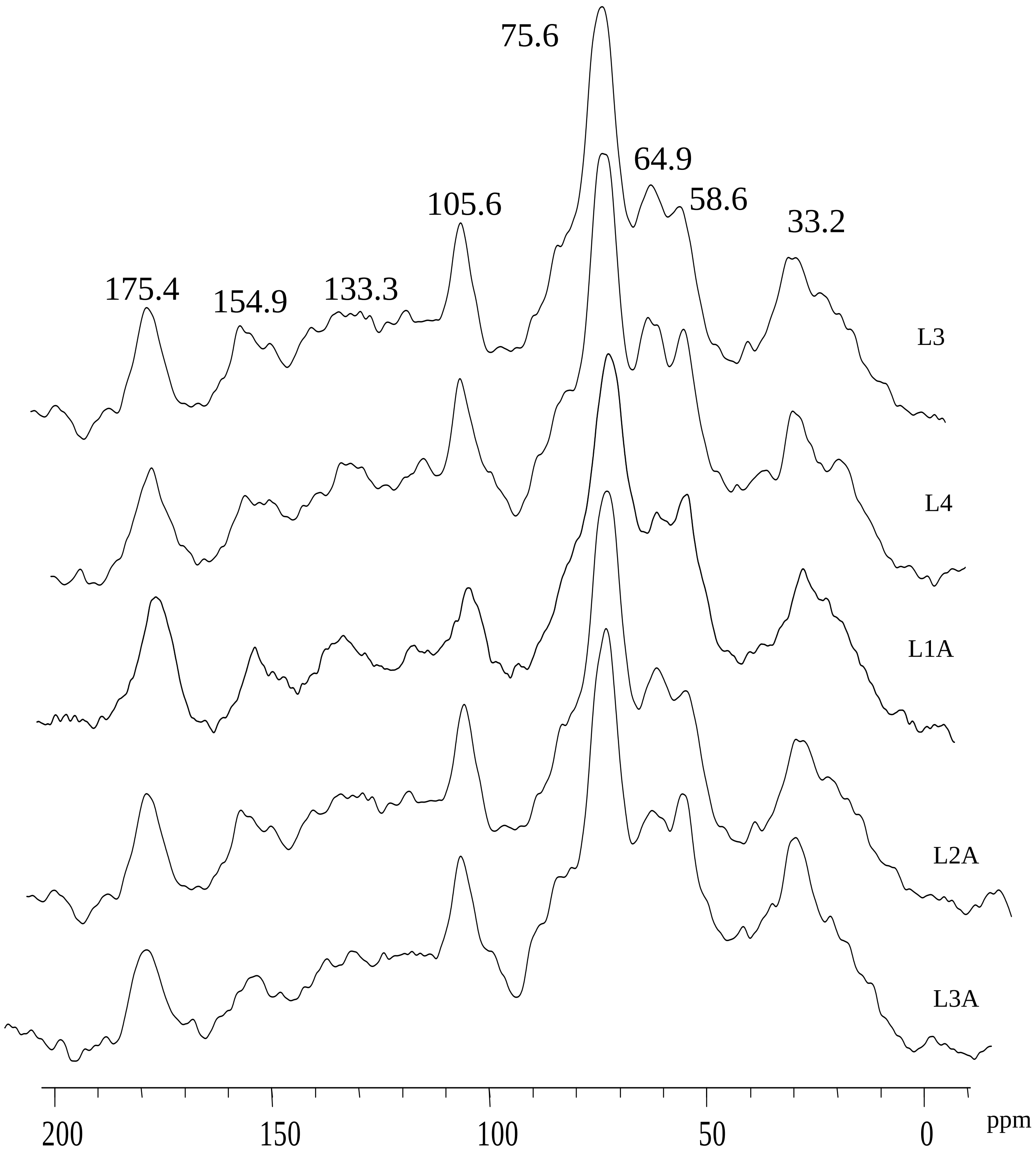

Figure5b 13C CPMAS spectra of mature compost

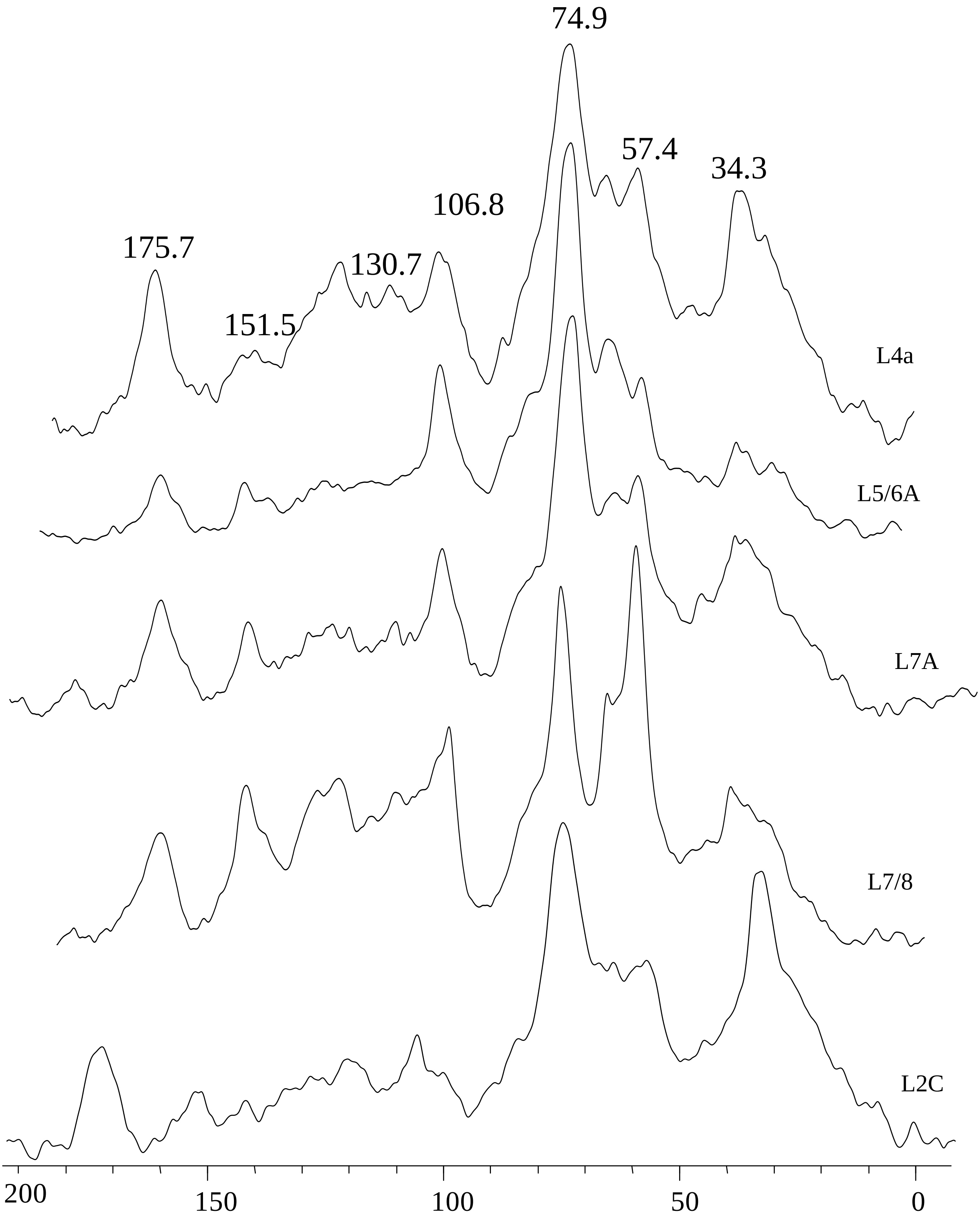

Figure 5c 13C CPMAS spectra of mature compost

3.3.2 off-line TAHM-GC-MS

The list in Table 7 show the representative monomers release by thermochemolysis analyses, while the data in Tables 8 show the yield of main molecular components found in organic materials.

The thermochemolysis applied to bulk compost samples, released more than hundred recognizable different molecules, which were identified as methyl ethers and esters of natural compounds (Table 7), mainly represented by lignin derivatives, fatty acids, aliphatic biopolymers, hydrocarbons and alcohols. Amount and distribution of the most representative monomers were comparable with previous results obtained from the thermochemosys of different organic biomasses. In respect to NMR data, a significant lower yield of carbohydrates was found among the pyrolysis products of compost samples. This finding has been related to the lower efficiency of pyrolysis techniques to detect carbohydrate units of polysaccharides in complex matrices. The thermal behavior and pyrolitic rearrangement of poly-hydroxy components, combined with the alkaline reaction condition of TMAH reagent solution, are believed to negatively interfere in the release and subsequent chromatographic detection of carbohydrates and polysaccharides.

The identified lignin constituents (Table 7) are associated with current symbolism applied in thermochemolysis analysis to identify lignin basic structures: P, p-hydroxyphenyl; G, guaiacyl (3-methoxy, 4-hydroxyphenyl); and S, syringyl (3,5-dimethoxy, 4-hydroxyphenyl). As expected from the initial composition of starting biomasses, the even contribution of the three different forms of lignin components to pyrolitic products indicated both herbaceous and woody tissues of angiosperm species as main sources of organic lignin materials (Table 7). This finding was confirmed by the prevalence, as most representative monomer, of the propenoic acid derivative [2-propenoic acid, 3-(4-methoxyphenyl)-methyl ester] (P18), which is a basic component of lignified tissues of annual crops and grasses.

An increase was found, in each mature compost samples, for the global yield of lignin compounds, with respect to initial biomasses (Table 8), thereby confirming the occurrence of selective preservation of aromatic and phenolic components in the OM stabilization process highlighted by NMR analyses. The extent of lignin preservation or decomposition may be estimated by structural indexes that are based on the relative amount of specific thermochemolysis products associated with the presence of, either, microbially processed organic materials or to undecomposed plant debris. In particular, the aldehydic (G4 and S4) and acidic (G6 and S6) forms of guaiacyl and siryngyl components derive from the progressive degradation of lignin polymer, involving the ongoing oxidation of propyl chain. Conversely the corresponding homologues with integral hydroxylated side chains (G14/15, S14/15) are indicative of unaltered lignin components, which retain the typical β-O-4 ether intermolecular linkages (Table 7). Therefore the indexes obtained (Table 4) by dividing the amount found for the acidic structures over that of, respectively, G4 and S4 aldehydes (Ad/AlG = G6/G4, Ad/AlS = S6/S4) and for the global yield of threo/erythro isomers ($\Gamma G = G6/[G14 + G15]$; $\Gamma S = S6/[S14 + S15]$) are regarded as suitable indicators of the bio-oxidative transformation of lignin polymers. The larger the values of dimensionless indexes, the wider the decomposition process of lignin substrates. The lower intensity and the substantial evenness shown by the decomposing indexes, determined at initial and final composting time (Table 8), further indicated an overall maintenance and preservation of hydrophobic aromatic and phenolic constituents.

Aliphatic and alicyclic lipid compounds were the principal alkyl components found in the pyrograms of compost samples (Table 7). The most abundant products were the methyl ester of linear fatty acids, dominated by the hexadecanoic and octadecanoic saturated and unsaturated homologues. These compounds may derive from the breakdown of long chain ester as well as from the terminal oxidation of linear hydrocarbons and aliphatic alcohols. Notwithstanding the multiple possible origins of the C16 and C18 acids, the amount of unsaturated monomers suggested the plant lipids as prevalent source of the straight chain aliphatic acids. The offline pyrolysis of initial biomass, produced also a notable yield of the methylated form of ω–hydroxy alkanoic acids and alkan-dioic acids (Tables 7 and 8). These molecules are the main building blocks of the external hydrophobic protective barriers of fresh and lignified plant tissues, namely cutin and suberin. No clear predominance of particular monomer was revealed by both of these compound classes, which instead showed an almost uniform distribution of even carbon-numbered long chain components (Table 7). Conversely, the di- and tri-hydroxy substituent of the C16 and C18 homologues were the unique representatives of mid-chain-hydroxy alkanoic acids (Tables 7 and 8). The 9,16-/10,16-dihydroxyhexadecanoic isomers, and the 9,10 epoxide 18hydroxy-octadecanoic acid were the most abundant released monomers of these important structural units of plant cuticles, frequently used also as plant biomarkers. The bio-polyesters may also contribute to the large content of the substituted aromatic acids (P18 and G18) found in initial and final compost samples.

The relatively least abundant lipid compounds were the high molecular weight tetra- and pentacyclic triterpenes (Tables 7 and 8), that have been tentatively identified as methyl ethers and esters of both methyl/ethyl cholest(di)en-3-ol structures, and of ursane, lupeane and oleanane derivatives.

The contribution of microbial input was shown by the pyrolytic release of phospho-lipid fatty acids (PLFA) and 2-hydroxy aliphatic acids, which are basic structural components of microbial cells (Table 3). The most representative PLFA monomers were, in order of elution, the 12- and 13-methyl tetradecanoic (iso/anteiso pentadecanoic), the 14- and 15-methyl hexadecanoic (iso/anteiso heptadecanoic) acids and the cyclopropane-(2-hexyl)-octanoic acid (C17 cy FAME), commonly found as characteristic microbial markers of natural organic matter.

As noted for the aromatic derivatives also the relative amount of lipid alkyl molecules of final compost sample, showed a relative increase of about the 50% on total dry weight basis, revealing a differential behaviour for different compound classes (Table 8). The decrease found for linear fatty acids may be attributed to the decomposition of bio-available free components, which undergo to a most favourable decomposition during the active phase of composting processes. Conversely a large preservation was found for the hydrophobic and structural recalcitrant biopolyester constituents, made up by hydroxyl and alkyl dioic acids (Table 8), which form the stable alkyl fraction of the inert SOM pools and may play an important role in determine the bioactivity of compost materials.

Table 7. Main hermochemolysis products released by bulk composts

R.t.[a]	Assignment[b]		R.t.	Assignment	
6.8	2-CH_3O phenol	Carb.	19.9	Benzaldehyde, 3,4,5-triCH_3O	Lig S4
7.5	CH_3O benzene	Lg P1	20.6	*cis*-2-(3,4-DiCH_3Ophenyl)-1-CH_3O ethylene	Lg G7
7.8	Benzene, 1,3-diCH_3O	Lg G1	20.9	*trans*-2-(3,4-DiCH_3Ophenyl)-1-CH_3O ethylene	Lg G8
8.0	Benzene, 1-Ethenyl-4- CH_3O	Lg P3	22.1	*trans* 2-Propenoic acid, 3-(4-CH_3O Phenyl) M.e.	LgP18
9.9	3,4-diCH_3O Toluene	Lg G2	23.1	Benzoic acid, 3,4,5-triCH_3O M.e.	Lg S6
10.1	Phenol, 3,5-diCH_3O	Carb	23.6	*trans*-1-(3,4-DiCH_3O phenyl)-3-CH_3O-1-propene	LgG13
10.8	1H-Indole, 1-Methyl-	N der	24.5	cis-1-(3,4,5-triCH_3O phenyl)-2-CH_3O ethylene	Lg S7
11.8	1,3,5-triCH_3O Benzene	Carb	24.8	C15 *iso* FAME	Mic.
12.1	2-CH_3O-4-Vinylphenol	Lg G3	24.9	threo/erythro-1-(3,4-diCH_3O phenyl) -1,2,3-triCH_3O propane	LgG14
12.5	Carbohydrates derivative	Carb.	24.9	C15 *anteiso* FAME	mic
13.2	Carb.	Carb	25.0	cis-1-(3,4,5-Tri CH_3O phenyl)-2- CH_3O ethylene	Lig S8
13.4	Benzene, 4-Ethenyl-1,2-diCH_3O	Lg G3			
13.5	1,2,3- CH_3O Benzene	Lg S1	25.2	threo/erythro-1-(3,4-Di CH3O phenyl) -1,2,3-tri CH3O propane	LgG15
13.7	Benzoic acid, 4-CH_3O M.e.	Lg P6	25.5	cis-1-(3,4,5-tri CH_3O phenyl)-1- CH_3O -1-propene	LgS11
13.9	2-Propenoic acid, 3-Phenyl M.e.	Biop.	25.7	C15 *n*-FAME	lip
14.2	Benzene, 1,2,3-triCH_3O-5-Methyl	Lg S2	26.1	isomer of G14	Lg G
15.5	4-CH_3O-1-Methylindole	N der.	27.4	2-Propenoic acid, 3-(3,4-diCH_3O Phenyl) M.e.	LgG18
15.7	*trans* Phenol, 2-CH_3O-4-(1-Propenyl)	Lg G	27.5	trans-1-(3,4,5-tri CH_3O phenyl)-3- CH_3O -1-propene	LgS13
16.7	Benzaldheyde 3, 4 diCH_3O	Lg G4	27.6	threo/eryth-1-(3,4,5-triCH_3O phenyl) -1,2,3-triCH_3O propane	LgS14
16.9	Benzene, 1,2-diCH_3O-4-(1-Propenyl)	Lg G21	27.9	threo/erythro-1-(3,4,5-triCH_3O phenyl) -1,2,3-triCH_3O propane	LgS15
17.4	3,4,5-triCH_3O Styrene	Lg S3	28.3	C16 FAME	Lip
17.6	C12 FAME		29.8	C17 iso FAME	Mic.
17.8	Benzenepropanoic acid, 4-CH_3O M.e.	LgP12	30.0	C17 anteiso FAME	Mic
19.0	Ethanone, 1-(3,4-diCH_3O Phenyl)	Lg G5	30.5	cis-1-(3,4,5- triCH_3O phenyl) -1,3-diCH_3O prop-1-ene	LgS16

19.5	*cis* 2-Propenoic acid, 3-(4-CH_3O phenyl)-, M.e.	LgP18	30.6	C17 n FAME	Lip.
			31.4	Carbohydrates derivative	Carb.
19.7	Benzoic acid, 3,4-diCH_3O M.e.	Lg G6	32.3	C18:1 FAME	Lip.

Table 3. Continue

R.t.[a]	Assignment[b]		R.t.	Assignment	
32.4	C18:1 FAME	Lip	44.4	C27 alkane	Lip.
33.0	C18 FAME	Lip.	45.0	C24 FAME	Lip.
33.5	C16 □CH	Biop	45.5	C22 ωCH3O FAME	Biop
33.8	C19 br. FAME	Mic.	46.2	C28 alkane	Lip.
34.5	C22 alkane	Lip.	46.3	squalene	Lip.
34.8	C19 cyclopropane FAME	Mic.	46.7	C26 alcohol	Lip.
35.7	C16 dioc acid DIME	Biop	47.0	C24 2 CH_3O FAME	Mic
36.1	Labd-7-en-15-oic acid, 6-oxo-, M.e.	Lip	47.2	C 22 dioic acid DIME	Biop
			47.3	sterol	Lip.
36.4	C16 (9)10-16 diCH_3O FAME	Biop.	47.9	C29 alkane	Lip.
36.7	C23 alkane	Lip.	48.4	C26 FAME	Lip.
36.8	Carbohydrates derivative	Carb	48.9	C24 ωCH3O FAME	Biop
37.2	C18:1 ωCH3O Fame	Biop.	49.0	sterol	Lip.
37.2	C20 FAME	Lip.	49.4	sterol	Lip.
37.8	C16 diCH3O FAME	Biop.	49.6	C30 alkane	Lip.
38.7	C24 alkane	lip	49.8	sterol	Lip.
39.1	Carbohydrates derivative	Carb.	50.1	C28 alcohol	Lip.
39.2	C18:1 dioic acid DIME	Biop	50.6	sterol	Lip.
39.4	C18 12,13-Epoxy-18-CH_3O, FAME	Biop	51.1	C24 dioic acid DIME	Biop
40.7	C25 alkane	Lip.	51.7	C28 FAME	Lip.
41.2	C22 FAME	Lip.	52.0	sterol	Lip.
41.8	C20 ωCH_3O FAME	Biop	52.2	C26 ωCH3O FAME	Biop.
42.1	C18 diCH_3O FAME	Biop	52.7	sterol	Lip.
42.6	C26 alkane	Lip.	53.1	sterol	Lip.
43.1	C23 FAME	Lip.	53.4	sterol	Lip.
43.3	C18 tri CH_3O FAME	Biop			
44.4	C27 alkane	Lip.			

a Rt Retention time (min)

b Biop.= biopolymers; Carb. = Carbohydrates; CH_3O = methoxy; DIME= dimethyl ester; FAME = fatty acid methyl ester; Lg= Lignin; Lip.= lipid; M.e. = methyl ester; Mic. Microbial; N der. = nitrogen compounds;

Table 8. Composition[a] and yields (μg g^{-1}) of TAHM products released from initial biomasses and final composts

Compound	SSMP	CMP	CV t0	CV	L1 t0	L1
Lignin[b]	2190	3470	1710	3230	2123	4946
Ad/Al_G	1.2	0.9	2.4	1.8	2248	4168
Γ_G	1.4	1.5	2.0	1.9	1.2	0.8
Ad/Al_S	2.4	2.7	4.2	3.7	4.5	4.4
Γ_S	1.4	1.3	1.9	1.8	0.9	1.2
Linear FAME C_{12}-C_{30} ($C_{18:1}$)	6820	3740	9575	5740	9540	5420
Microbial FAME C15-C24 (C_{17})	785	889	780	595	1170	895
Hydroxy+ Dioic acids ME C_{16}-C_{26} (C_{16})	1160	3209	2990	3520	2575	4670
Alkanes C_{17}-C_{31}	300	418	252	350	570	995
Alcohols C_{22}-C_{26}	135	92	757	420	1950	900
Sterols	100	195	440	360	230	470

a Total range varying from Ci to Cj; compounds in parentheses are the most dominant homologues; numbers after colon refer to double bond.

b Structural indices: Ad/Al = G6/G4, S6/S4; ΓG = G6/(G14 + G15); ΓS = S6/(S14 + S15).

Table 8. Composition[a] and yields (μg g^{-1}) of TAHM products released from initial biomasses and final composts

Compound	L2 t0	L2	L3 t0	L3	L4 t0	L4
Lignin[b]	2248	4168	1912	4157	2123	4946
Ad/Al_G	1.2	0.8	1.9	2.0	1.0	0.9
Γ_G	4.5	4.6	2.6	3.6	2.7	3.1
Ad/Al_S	3.8	2.8	2.1	2.2	3.0	2.5
Γ_S	0.5	0.3	1.2	1.4	1.1	0.9
Linear FAME C_{12}-C_{30} ($C_{18:1}$)	9575	4820	10672	7820	8540	4210
Microbial FAME C15-C24 (C_{17})	995	573	875	780	960	540
Hydroxy+ Dioic acids ME C_{16}-C_{26} (C_{16})	1949	4909	2309	4715	2520	3510
Alkanes C_{17}-C_{31}	975	850	450	395	740	580
Alcohols C_{22}-C_{26}	1120	850	950	640	1620	960
Sterols	210	145	250	185	220	150

a Total range varying from Ci to Cj; compounds in parentheses are the most dominant homologues; numbers after colon refer to double bond.

b Structural indices: Ad/Al = G6/G4, S6/S4; ΓG = G6/(G14 + G15); ΓS = S6/(S14 + S15).

Table 8. Composition[a] and yields (μg g^{-1}) of TAHM products released from initial biomasses and final composts

Compound	L1A t0	L1A	L2A t0	L2A	L3A t0	L3A
Lignin[b]	3415	4300	1980	2390	2150	3550
Ad/Al_G	2.2	3.0	2.9	2.7	3.2	2.4
Γ_G	4.0	2.8	2.3	1.7	0.8	1.1
Ad/Al_S	2.4	1.4	1.9	2.1	3.2	2.7
Γ_S	1.5	1.5	1.0	1.2	0.7	1.1
Linear FAME C_{12}-C_{30} ($C_{18:1}$)	14751	8535	8690	4560	8750	6220
Microbial FAME C15-C24 (C_{17})	436	399	985	680	1120	915
Hydroxy+ Dioic acids ME C_{16}-C_{26} (C_{16})	2470	5800	1620	4110	2115	3980
Alkanes C_{17}-C_{31}	540	255	250	280	360	295
Alcohols C_{22}-C_{26}	1120	980	1475	1040	1212	850
Sterols	250	185	420	90	550	350

a Total range varying from Ci to Cj; compounds in parentheses are the most dominant homologues; numbers after colon refer to double bond.

b Structural indices: Ad/Al = G6/G4, S6/S4; ΓG = G6/(G14 + G15); ΓS = S6/(S14 + S15).

Table 8. Composition[a] and yields (μg g^{-1}) of TAHM products released from initial biomasses and final composts

Compound	L4A t0	L4A	L5/6 t0	L5/6	L7/A t0	L7A
Lignin[b]	1960	3473	2670	3620	2980	4270
Ad/Al_G	2.6	2.1	4.2	3.7	2.8	1.9
Γ_G	1.6	1.9	0.7	1.1	2.1	1.6
Ad/Al_S	2.9	2.3	3.5	2.6	2.7	2.2
Γ_S	0.7	0.8	1.2	1.4	0.9	0.7
Linear FAME C_{12}-C_{30} ($C_{18:1}$)	8540	5420	6540	4850	11085	8750
Microbial FAME C15-C24 (C_{17})	650	480	865	540	470	465
Hydroxy+ Dioic acids ME C_{16}-C_{26} (C_{16})	3620	8200	2985	4500	2750	5345
Alkanes C_{17}-C_{31}	890	1100	850	645	650	250
Alcohols C_{22}-C_{26}	2200	1850	685	780	755	590
Sterols	230	195	125	190	360	285

a Total range varying from Ci to Cj; compounds in parentheses are the most dominant homologues; numbers after colon refer to double bond.

b Structural indices: Ad/Al = G6/G4, S6/S4; ΓG = G6/(G14 + G15); ΓS = S6/(S14 + S15).

Table 8. Composition[a] and yields (μg g^{-1}) of TAHM products released from initial biomasses and final composts

Compound	L7/8 t0	L7/8	L2C t0	L2C
Lignin[b]	2190	4470	1980	3390
Ad/Al_G	1.2	0.9	1.4	1.2
Γ_G	4.0	2.8	2.3	1.7
Ad/Al_S	2.4	2.7	2.9	2.5
Γ_S	1.3	1.3	0.8	1.1
Linear FAME C_{12}-C_{30} ($C_{18:1}$)	4760	3820	6590	4870
Microbial FAME C15-C24 (C_{17})	960	790	690	480
Hydroxy+ Dioic acids ME C_{16}-C_{26} (C_{16})	2150	3290	1680	4110
Alkanes C_{17}-C_{31}	540	255	250	280
Alcohols C_{22}-C_{26}	530	270	675	440
Sterols	210	115	195	220

a Total range varying from Ci to Cj; compounds in parentheses are the most dominant homologues; numbers after colon refer to double bond.

b Structural indices: Ad/Al = G6/G4, S6/S4; ΓG = G6/(G14 + G15); ΓS = S6/(S14 + S15).

Annex 2 –Closing Report on C.2 Action

Monitoring of SOC stabilization and the improvement of physical and biological soil fertility

Project responsible. CERMANU

List of abbreviations

AMF: arbuscular mychorrhyza fungi; ^{13}**C/**^{31}**P-CPMAS NMR:** solid state Cross Polarization Magic Angle Spinning Nuclear Magnetic Resonance on ^{13}C; **FAME**: Fatty acids methyl ester; **HA**: Humic acids; **N/PLFA:** Neutral/Phospho-lipid fatty acids; **MWDw**: mean weight diameter in water;
N: nitrogen; **S/TOC** soil/total organic carbon; **13C-OC**: 13C isotopic content; **SOM:** Soil organic matter; **TAHMs-GC-MS**: off-line thermally assisted hydrolysis and methylation pyrolysis GasChromatography Mass Spectrometry

The report on C.2 Action include the following analyses
Project sites Tetto Frati, Grugliasco, Castel Volturno, Prima Luce
Soil Organic matter

- soil aggregate stability:
- SOC distribution and 13C-OC content
- SOM characterization by off line pyrolysis GC-MS:
- Humic acids (13C CPMAS NMR)

Project site Mellone: SOC distribution and 13C-OC content

Biological analyses

- Project sites Tetto Frati, Grugliasco, Castel Volturno: PLFA,
- Project site Prima Luce: Enzimatic activities (β-glucosidase, β-galactosidase, glucosaminidase invertase phosphomonoestarase, arylsulphatase, urease, nitrate reductase and protease)

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1. Codes of soil treatments according with Project sites

Piemonte: Tetto Frati,

Trad: traditional agronomic technique **0 N**: no Nitrogen fertilization; **SSB** and **SSA** low (1000 kg of C ha^{-1} and high (2000 kg of C ha^{-1}) dose of fresh solid phases of digestate; **CMPB** and **CMPA** low (1000 kg of C ha^{-1} and high (2000 kg of C ha^{-1}) dose of compost; **Fe-P** : traditional agronomic technique with addition of biomimetic catalyst (0.5g m^{-2}).

Grandi

Trad: traditional agronomic technique **0 N**: no Nitrogen fertilization; **SSB** and **SSA** low (1000 kg of C ha^{-1} and high (2000 kg of C ha^{-1}) dose of fresh solid phases of digestate; **CMPB** and **CMPA** low (1000 kg of C ha^{-1} and high (2000 kg of C ha^{-1}) dose of compost;

Campania

Castel Volturno

Trad: traditional agronomic technique **CMPB** and **CMPA** low (10 tons ha^{-1} and high (20 tons ha^{-1}) dose of on farm compost; **Fe-P** : traditional agronomic technique with addition of biomimetic catalyst (0.5g m^{-2}).

Prima Luce

A control no fertilization; **B** traditional organo-mineral fertilizer (Oligomax 8/5/10) 250 kg ha^{-1}; **C** LIFE on-farm compost 10 tons ha^{-1}; **D** LIFE on-farm compost 20 tons ha^{-1}

Mellone
two orchard systems: *kiwi and peach*; two composts: *A "summer" and B "winter"* depending on the crop residues used in composting process; three compost doses *0-control , 1- 10 tons ha-1, 2 - 20 tons ha*$^{-1}$ For the evaluation of SOC dynamics with isotopic 13C-OC analyses, field plots in A0 and B0 were added with 10 tons ha-1 of on farm green compos added with sorghum residues

2. Materials and Methods

2.1 Soil aggregate stability

A modified procedure of the classical method described by Kemper and Rosenau (1986) was used to separate the water-stable aggregates. Forty grams of the <2.00 mm, air-dried soil samples were put in the topmost of a nest of three sieves of 1.00, 0.50 and 0.25mm mesh size and pre-soaked in distilled water for 30 min. Thereafter the nest of sieves and its contents were oscillated vertically in water 20 times using a 4 cm amplitude at the rate of one oscillation per second. Care was taken to ensure that the soil particles on the topmost sieve were always below the water surface during each oscillation. After wet-sieving, the resistant soil materials on each sieve and the unstable (<0.25 mm) aggregates were quantitatively transferred into beakers, dried in the oven at 50◦C for 48 h, weighed and stored.. The percentage ratio of the aggregates in each sieve represents the water-stable aggregates of size classes: 2.00–1.00, 1.00–0.50, 0.50–0.25 and <0.25 mm. Mean-weight diameter in water (MWDw) of water-stable aggregates was calculated as follow

$$MWDw = \sum_{i=1}^{n} Xi\ Wi$$

where Xi is the mean diameter of the *ith* sieve size and Wi the proportion of the total aggregates in the *ith* fraction.

2.2. Soil organic matter analyses

2.2 1Elemental analyses

TOC and total N in bulk soils and soil aggregates were determined by Fisons EA 1108 Elemental Analyzer (Fisons Instruments S.p.A., Rodana, MI, Italy). The soil samples were firstly dried in a oven at 40 °C and ground to a fine powder using a quartz agate mortar and pestle.

2.2.2 13C isotopic analyses

The measurements will be performed on bulk samples provided by sampling procedure from each project site and on soil fractions deriving from the determination of soil aggregate stability. The analyses will be carried out in triplicates with continuous-flow isotope ratio mass spectrometry (IRMS) Results were expressed in the relative δ per mil scale, according to the equation:

$$\delta 13C\ ‰ = (Rsample/Rstandard - 1) \times 1000$$

where R= 13C/12C, and the standard is referred to the international reference Pee Dee Belemnite (PDB). At initial and final sampling date, the amount of TOC derived compost addition in bulk soil and aggregate-size fractions is calculated as follows:

$$13C\text{-}OC = Ct \times (\delta s - \delta c)/(\delta 0 - \delta c)$$

where Ct indicates total C of sample, δ s is the δ13C value in the sample, δ0 is the δ 13C of the added compost and δc is the δ13C value of control sample.

2.2.3 TAHM -GC-MS

Pyrolysis in the presence of tetramethyl ammonium hydroxide (TMAH) is commonly used to study the detailed molecular composition of either natural and synthetic biopolymers. It involves the cleavage of covalent bonds combined with the solvolysis and methylation of ester and ether groups, in complex mixture of organic macromolecules and biopolymers, thereby enhancing the thermal stability of acidic, alcoholic, and phenolic groups and allowing a suitable chromatographic detection of pyrolytic products.

For off line-THM-GC-MS about 2 g of soil samples were placed in a quartz boat with 2 mL of TMAH (25% in methanol w/v) solution. After drying under a stream of nitrogen, the mixture was introduced into a Pyrex tubular reactor (50 cm × 3.5 cm i.d.) and heated at 400 °C for 30 min in a circular oven (Barnstead Thermolyne 21100 Furnace, Barnstead International, Dubuque, IA, USA). The gaseous products from thermochemolysis were flowed into two chloroform (50 mL) traps in series, kept in ice/salt baths. The chloroform solutions were combined and rotoevaporated to dryness. The residue was dissolved in 1 mL of chloroform and transferred in a glass vial for GC-MS analysis. The GC-MS analyses were conducted with a Perkin Elmer Autosystem XL by using a RTX-5MS WCOT capillary column (Restek, 30 m × 0.25 mm; film thickness, 0.25 mm) that was coupled, through a heated transfer line (250 °C), to a PE Turbomass-Gold quadrupole mass spectrometer. The chromatographic separation was achieved with the following temperature program: 60 °C (1 min. isothermal), rate 7 °C min−1 to 320 °C (10 min. isothermal). Helium was used as carrier gas at 1.90 mL min−1, the injector temperature was at 250 °C, and the split-injection mode had a 30 mL min−1 of split flow. Mass spectra were obtained in EI mode (70 eV), scanning in the range 45–650 m/z, with a cycle time of 0.2 s. Compound identification was based on comparison of mass spectra with the NIST-library database, published spectra, and real standards.

For quantitative analysis, due to the large variety of detected compounds with different chromatographic responses, external calibration curves were built by mixing methyl esters and/or methyl ethers of the following molecular standards: tridecanoic acid, octadecanol, 16-hydroxyhexadecanoic acid, docosandioic acid, *â*-sitosterol, and cinnamic acid. Increasing amounts of standard mixtures were placed in a quartz boat and moistened with 0.5 mL of TMAH (25% in methanol) solution. The same thermochemolysis conditions as for compost samples were applied for the standards. The percentage recovery of standards ranged from 82 to 91% of initial amount.

2.2.4 Humic acids

The HA were extracted by the initial control soils following the classical procedure of the International Humic Science Society (IHSS). Briefly 50 g of soil were extracted with 400 ml of NaOH (0.1 M) and Na4P2O7*10H2O (0.01M) 1:8 extraction (soil:solution). Thereafter, the bottles were put in a horizontal shaker at 120 rpm for 24 hours. The solution was centrifuged at 7000 rpm for 20 minutes to separate better the solution from the soil fractions. Subsequently, the supernatant solution was filtered with glass fibres and it was acidified at pH 1 with HCL (12M) to promote the precipitation of HA, since they are insoluble at pH < 2. The acidified solution was allowed to stand on a bench overnight, then it was centrifuged at 7000 rpm for 20 minutes to recover the HA fraction. The HA were treated with HF and HCl to remove co-extracted inorganic impurities. The HA were dissolved in 100 ml of solution containing 2.5 ml of HCL (37%) and 2.5 ml of HF (40%) in 1L of deionised water and left for 24 hours in a horizontal shaker at 120 rpm. Thereafter, the solution was centrifuged at 7000 rpm for 20 min: the supernatant was discharged, while the HA precipitated was re-dissolved in deionised water and transferred into dialysis membranes

(Spectrapore 3, 3500 Mw cut-off) and dialysed against deionised water until the dialysis water gives an electrical conductivity of 15 µS/cm. Once the dialysis was finished the sample were put in a freezer at -20 °C and subsequently freeze-dried.

The HA were characterized by 13C CPMAS NMR. Fine-powdered composite samples were analyzed by solid-state NMR spectroscopy (13C CPMAS NMR) on a Bruker AV300 Spectrometer equipped with a 4 mm wide-bore MAS probe. The NMR spectra were obtained by applying the following parameters: 13,000 Hz of rotor spin rate; 2 s of recycle time; 1H-power for CP 92.16 W: 1H 90° pulse 2.85 µs; 13C power for CP 150,4 W; 1 ms of contact time; 30 ms of acquisition time; 4000 scans. Samples were packed in 4 mm zirconium rotors with Kel-F caps. The cross polarization pulse sequence was applied with a composite shaped "ramp" pulse on the 1H channel in order to account for the inhomogeneity of Hartmann-Hann condition at high rotor spin frequency. The Fourier transform was performed with 4 k data point and an exponential apodization of 50 Hz of line broadening. The different carbon functionalities are conventionally grouped into the following chemical shift regions: alkyl-C: 0–45 ppm; methoxyl-C: 45–60 ppm; O-alkyl- C: 60–110 ppm; aryl-C: 110–145 ppm; phenol-C: 145– 160 ppm, and carboxyl-C: 190–160 ppm. The relative contribution of each region was determined by integra- tion (MestreNova 6.2.0 software, Mestre-lab Research, 2010), and expressed as percentage of the total area.

2.3 Biological analyses

2.3.1 PLFA

The neutral and phospholipid fatty acid (NLFA and PLFA) analysis are used to study microbial biomass and community structure of soil samples. This analytical method is based on the identification of fatty acids as biomarkers to determine the presence and abundance of broad functional microbial groups such as fungi, arbuscular mycorrhizal, gram positive (+) and negative (-) bacteria, actinomyces, etc. This method is often used to determine gross changes in the microbial community associated with different environmental conditions.

For the analysis, 50 gr of soil was collected from the rhizosphere of field plots The soil sampling was performed during crop cycle about 70 days after sowing. For the extraction of PLFA and NLFA 1 gram of dried soil was extracted for 2 hours with a mixture of chloroform/methanol/citrate buffer at pH 4 (1:2:0.8 v/v). After centrifugation, the upper phase was collected and split into two phases by adding chloroform and citrate buffer. The lower phase was recovered and dried with N flux. Thereafter, the lipids are fractionated into neutral, glycol- and phospho- lipids on a silica gel column by elution with chloroform, acetone and methanol, respectively. The neutral and phospholipids were dried under N2 flux at 37 °C and stored at -20 °C. Neutral and phospholipids were hydrolysed to free fatty acids by alkalization and consequently derivatized into fatty acids methyl esters. In this step methyl nonadecanoate fatty acid (19:0) was added to the sample as internal standard. The FAME are then separated from the head groups by using n-hexane and analysed by GC/MS. A PerkinElmer Autosystem XL (GC) equipped with a PE Turbomass-Gold quadrupole mass spectrometer was used.

The relative area of the chromatographic peak obtained for each PLFA and NLFA was divided by that of the internal standard (19:0). Each PLFA and NLFA content was expressed as nmol of PLFA per gram of wet soil. The concentration of PLFA were summed together to obtain the estimation of total biomass. In the case of arbuscular mycorrhiza (AM) fungi, C16:1ω5 NLFA were used as indicators of arbuscular mycorrhiza.(AM) fungi. In particular, this NLFA was taken into account as representative of spores and propagules of AM fungi, reflecting therefore the AM fungal growth. As marker of the AM fungi biomass the C16:1ω5 PLFA can be also considered. However, it is not entirely specific only for AMF, but it can also be representative of for some Gram- bacteria. To assess whether this PLFA indicates the presence of AM fungi or Gram- bacteria, the ratio between the NLFA and PLFA is calculated. If the NFLA/PLFA ratio is between 1 and 200 the PLFA is representing AM fungi, while if the ratio is lower than 1 it must be considered biomarkers of Gram- bacteria.

2.3.2 Enzimatic activity and ergosterol content

The ergosterol is a membrane lipid almost exclusive of fungi, homologous to cholesterol of animal cells. It represents a quite constant fraction of fungal hyphae (0.5-1.5 %) and is decomposed rapidly upon death of hypae. For this reasons, the ergosterol content is considered a good indicator of fungal biomass in soil. The utility of its measurement is related to the importance of fungi functions in soil ecosystem. The ergosterol in soil samples was extracted as follows: 2 g of fresh sieved (2 mm) soil in 10 ml test tubes were added with 4 ml of 10% KOH dissolved in methanol. The tubes were sonicated for 15 min and heated for 90 min at 70 °C. After cooling, 1 ml of ultrapure water and 2 ml of cyclohexane were added and the tubes were vortexed for 30 sec. The hydrophobic (cyclohexane) phase containing the sterols was separated from the hydrophilic phase by centrifuging for 5 min at 3000 rpm. The cycloexane (the upper) phase was collected by Pasteur pipette and transferred to new test tubes. The cyclohexane was evaporated under a gentle N flow and the dried samples were dissolved in 1 ml of methanol. The ergosterol was measured by HPLC equipped with a C18 reverse-phase column and UV light detector at 282 nm. Methanol was used as mobile phase (1 ml min^{-1} flow rate).

3 Results

3.1 Soil aggregate stability

The differences in aggregate distribution and aggregate stability (MWDw found among project sites have to be mainly referred to the effects of textural composition. In fact, the larger clay content in soil samples from Castel Volturno act as stabilizing agent, while the sandy soils from Piemonte show a lower aggregate stability, with the larger amount of soil fraction accumulating in the finest soil aggregates sizes (< 0.50 mm). The silty soil from Prima Luce is characterized by an intermediate behaviour (Figs 1, 2, 3, 4).

After two year of SOM management, distinct behaviours were found in the aggregate yields and stability index from the field treatments of different project sites, whose overall distribution were still affected by the large influence of different textural composition (Figs1, 2, 3, 4). At Tetto Frati (Fig. 1) the soil amendments with fresh solid digestate (SS-B and SS-A) and higher dose of composted material (CMPA), produced a decrease of unstable micro-aggregates (< 0.25 mm) which were incorporated in the intermediate macro-aggregate size fractions, with consequent increase of MWDw values. Similar trend was observed in the field plots of Grandi, where only the two soil treatments based on the addition of fresh solid digestate promoting an incorporation of finest soil fraction in the most stable macro-aggregates classes (Fig, 2) This effect may be associated with the availability of bio-available components which may favour the development of microbial biomass and related by-products that promote a transient binding action on soil aggregate.

The main influence of textural composition was shown in the soil distribution of field plots of Castel Volturno (Fig. 3), which did not reveal any valuable effect of soil treatment on aggregates and stability index. Conversely a steady improvement of soil aggregation was found in soil samples amended with on farm compost at Prima Luce (Fig. 4). For both years the input of exogenous OM added with low and high dose of compost, produced a decrease of unstable microaggregate (< 0.25 mm), shifted into upper size fraction (1-0.50 mm), thereby revealing an average 30% increase in the stability index of soil sample with large compos addition (MWD 0.6 –D plots) in respect to conventional management (MWD 0.44 –A plots)

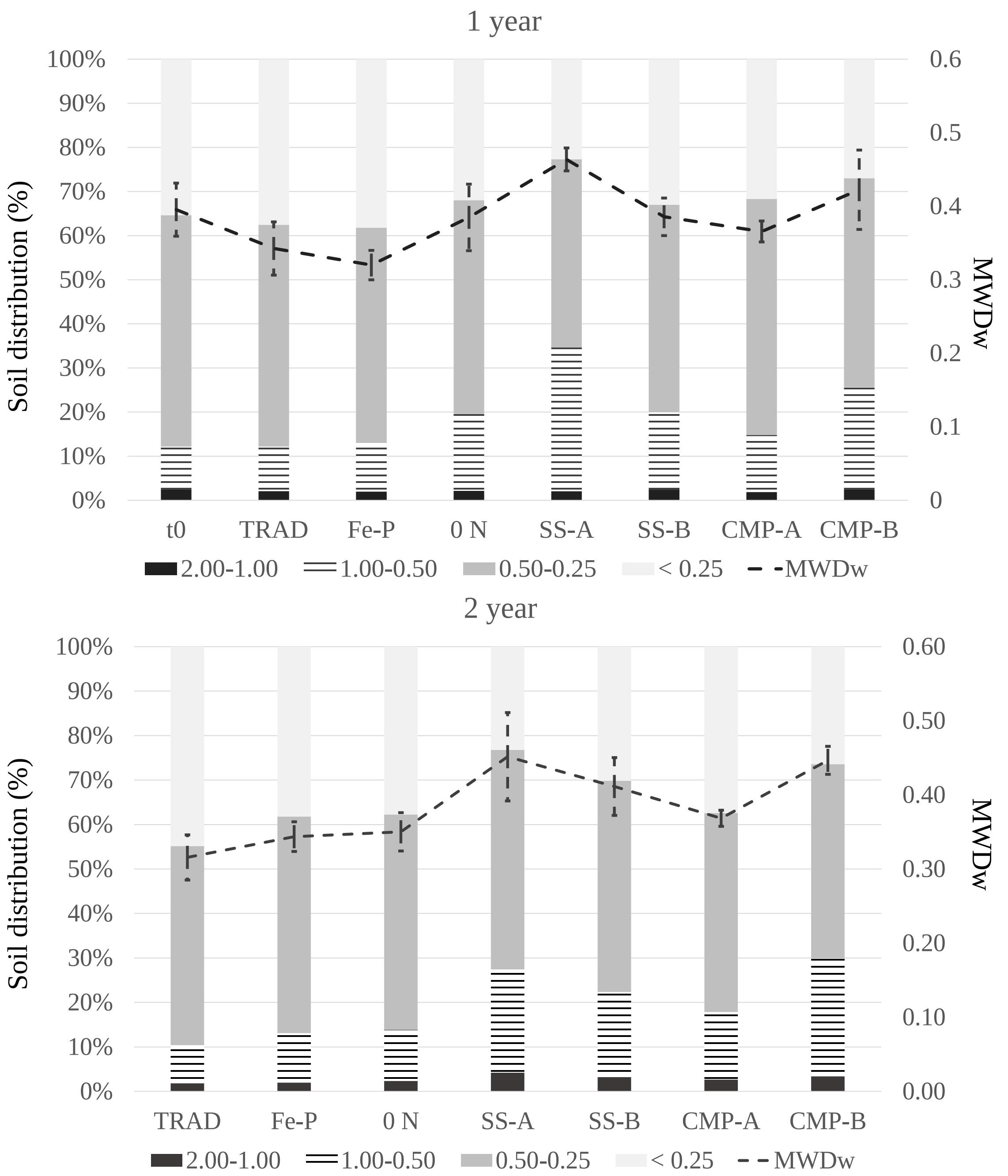
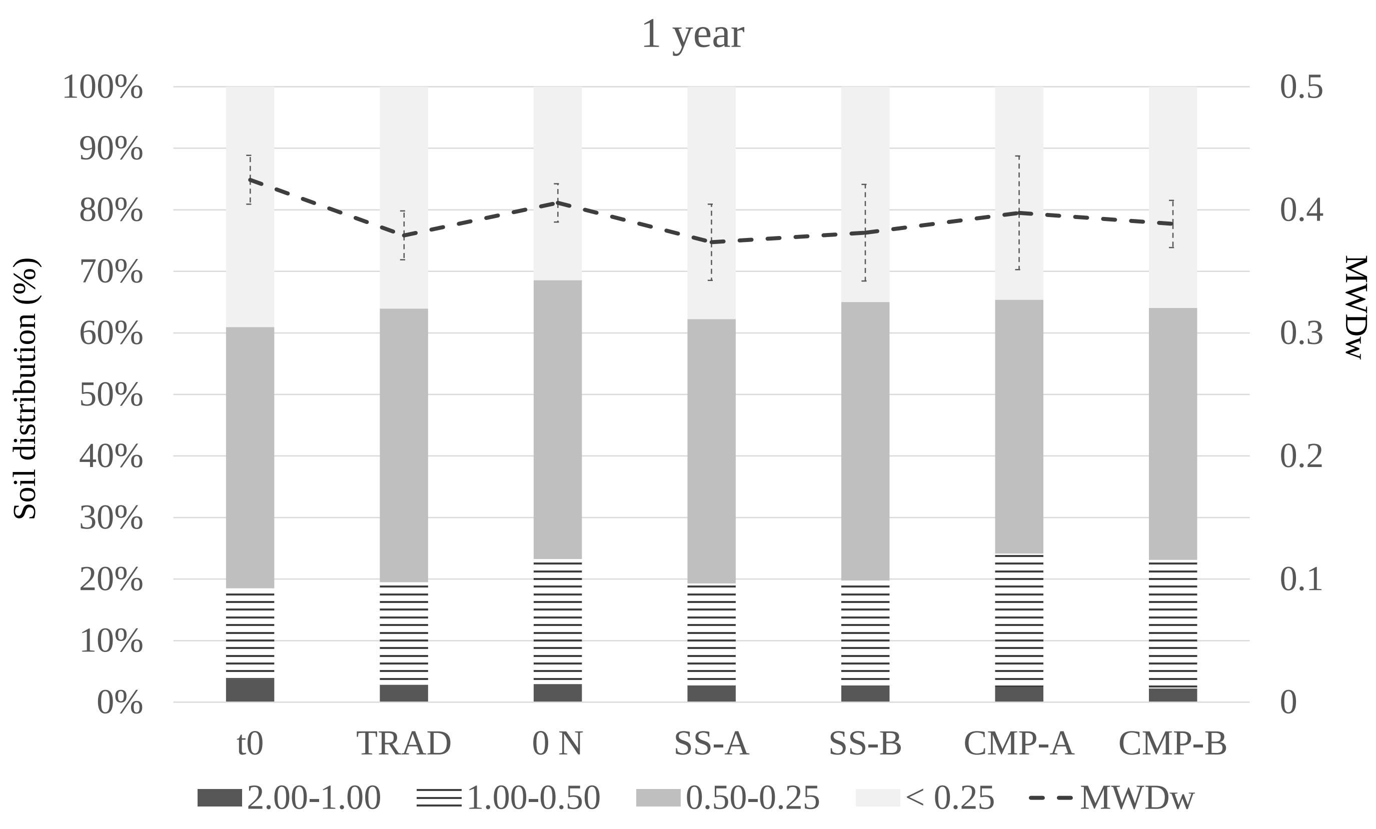


Figure 1. Tetto Frati: distribution (%) of water-stable aggregate sizes (mm) and stability index (MWD) in different field treatments



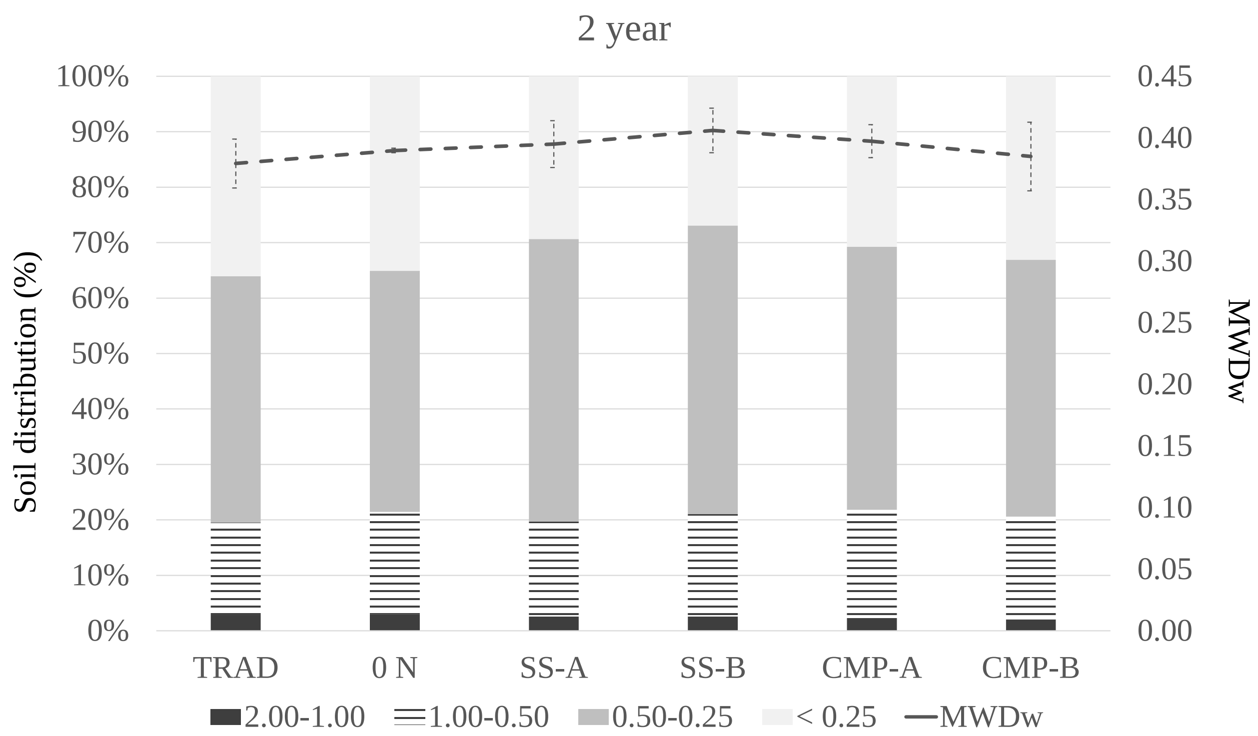
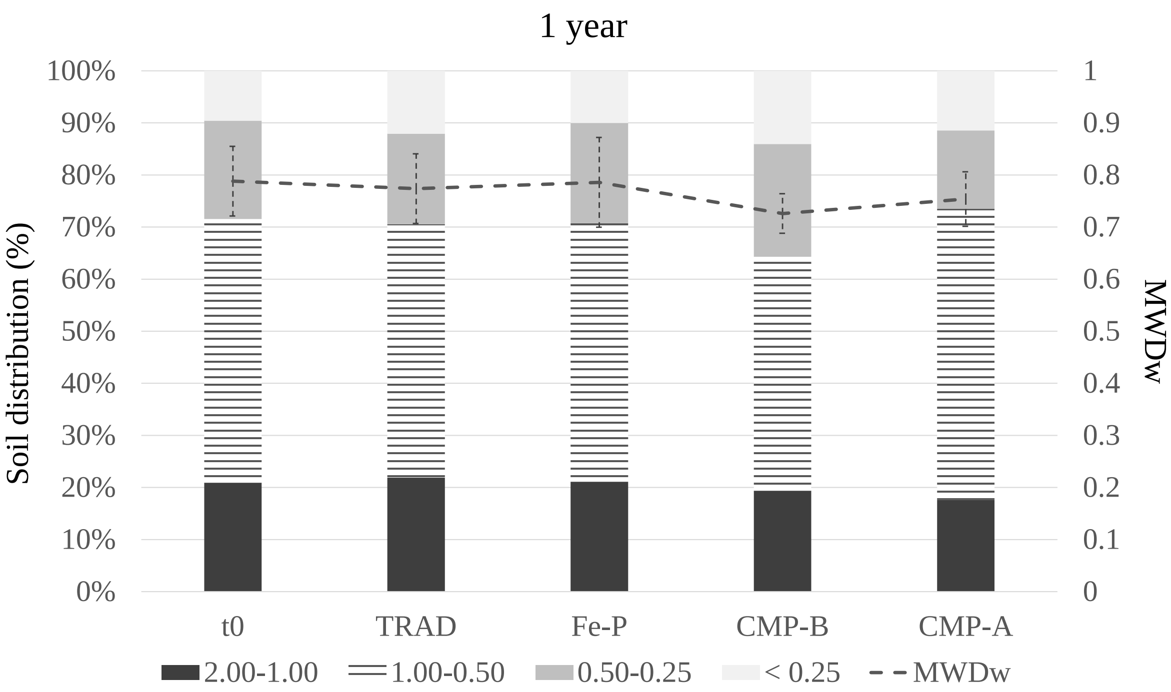


Figure 2. Grandi: distribution (%) of water-stable aggregate sizes (mm) and stability index (MWD) in different field treatments



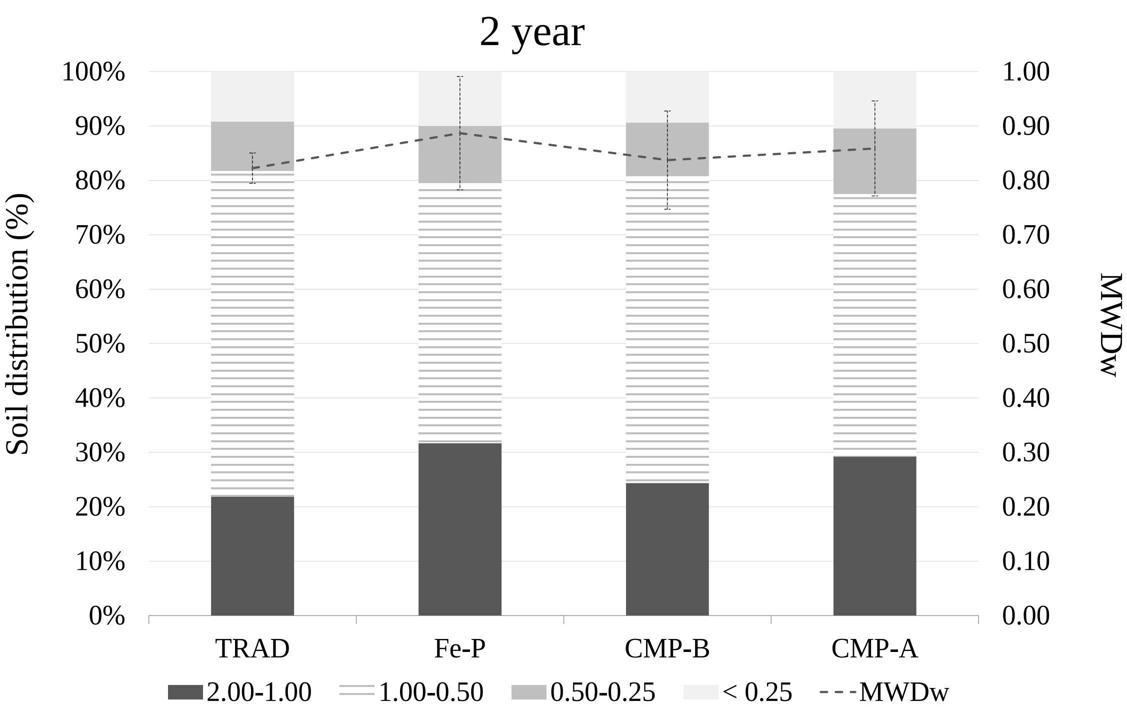


Figure 3 Castel Volturno: distribution (%) of water-stable aggregate sizes (mm) and stability index (MWD) in different field treatments

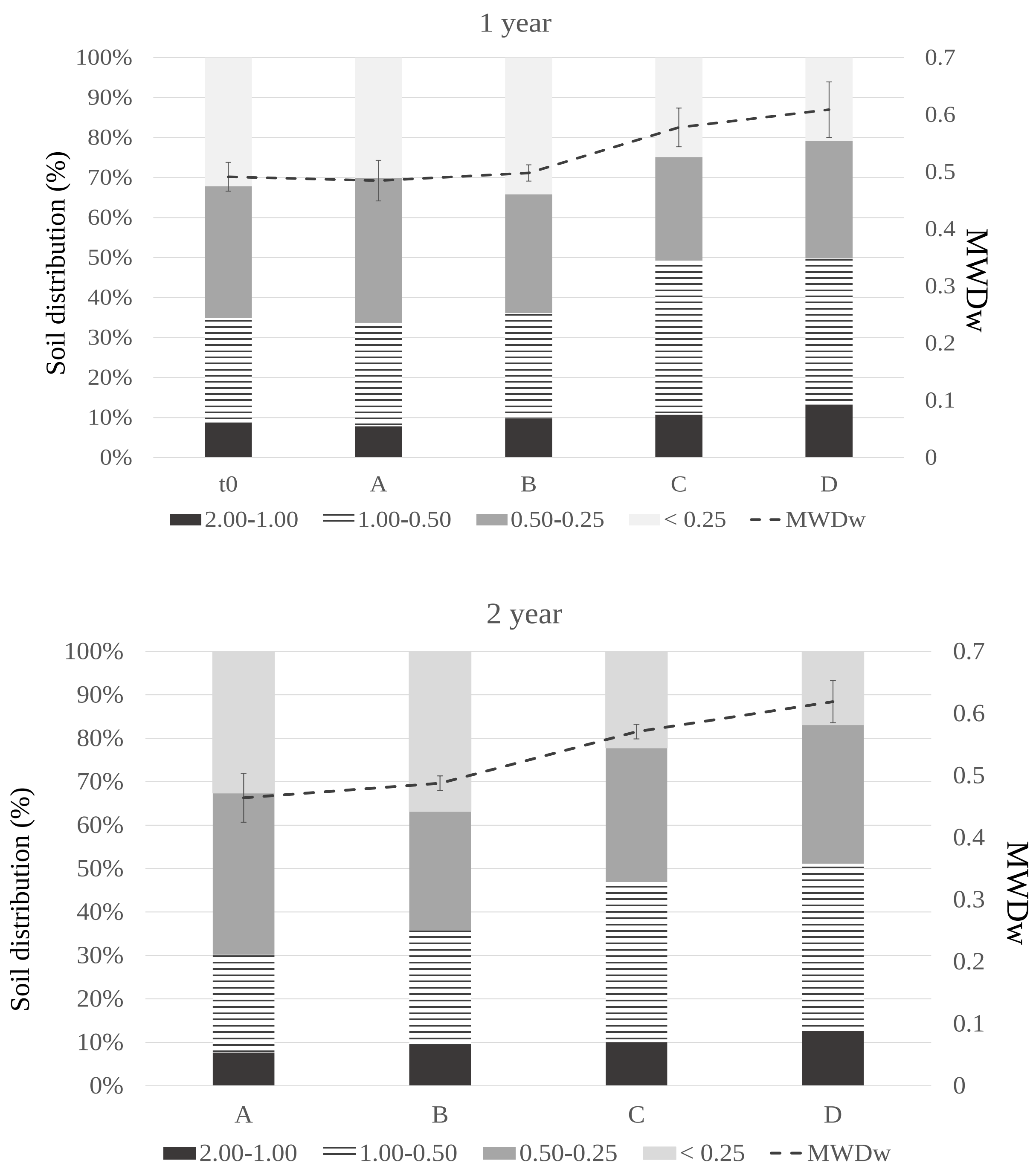
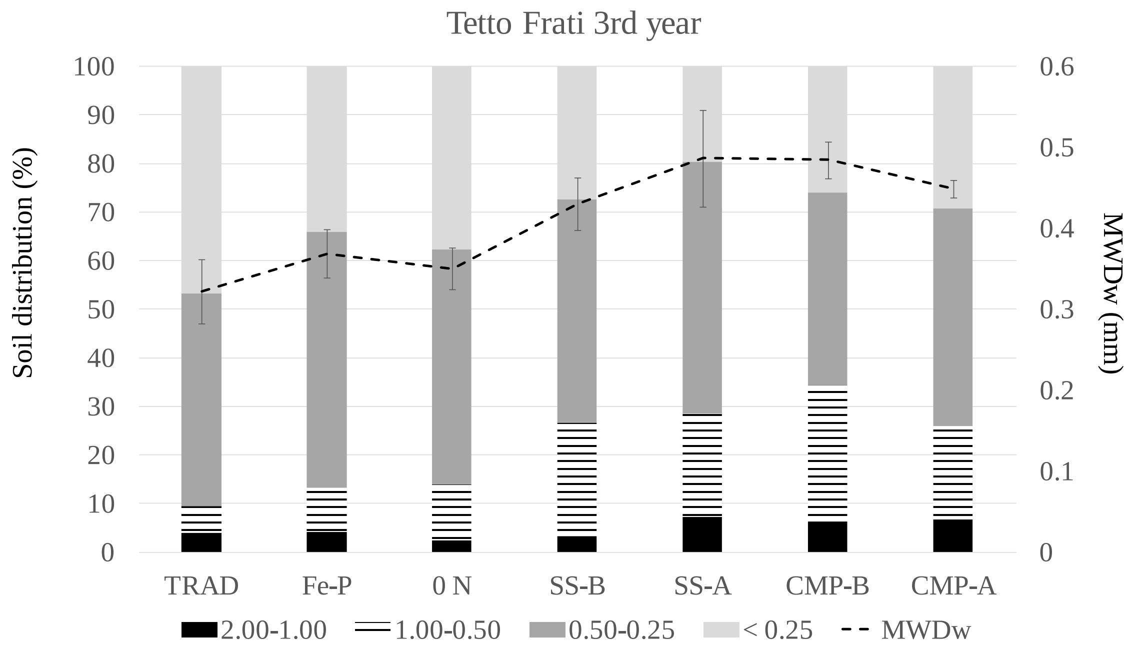


Figure 4 Prima Luce: distribution (%) of water-stable aggregate sizes (mm) and stability index (MWD) in different field treatments

In the second period of project activities, although the soil distribution among macro and micro aggregates found in the various project sites and the resulting values of aggregate stability index (MWDw), still show the main influence of different textural composition, a steady positive effects of OM addition was highlighted for the project sites of Tetto Frati and Grandi (Figs 5, 6). In fact for these soils, characterized by the lower content of finer soil fractions and lower aggregate stability, a slight increases were found in the yield of the larger macro-aggregates particles (1-0.5 mm and 1-2 mm) in soil treatments with either fresh solid digestate (SS-B and SS-A) or mature compost (CMP-B and CMP-A) thereby promoting also an improvement of the stability index. This finding may be related to stabilizing effect produced by the increase of TOC content found in soil plots added with either fresh digestate and compost; moreover, since the soil sampling is performed at the end of annual crop cycle, this aggregation may be hardly associated only with a temporary effect related to the microbial biomass.



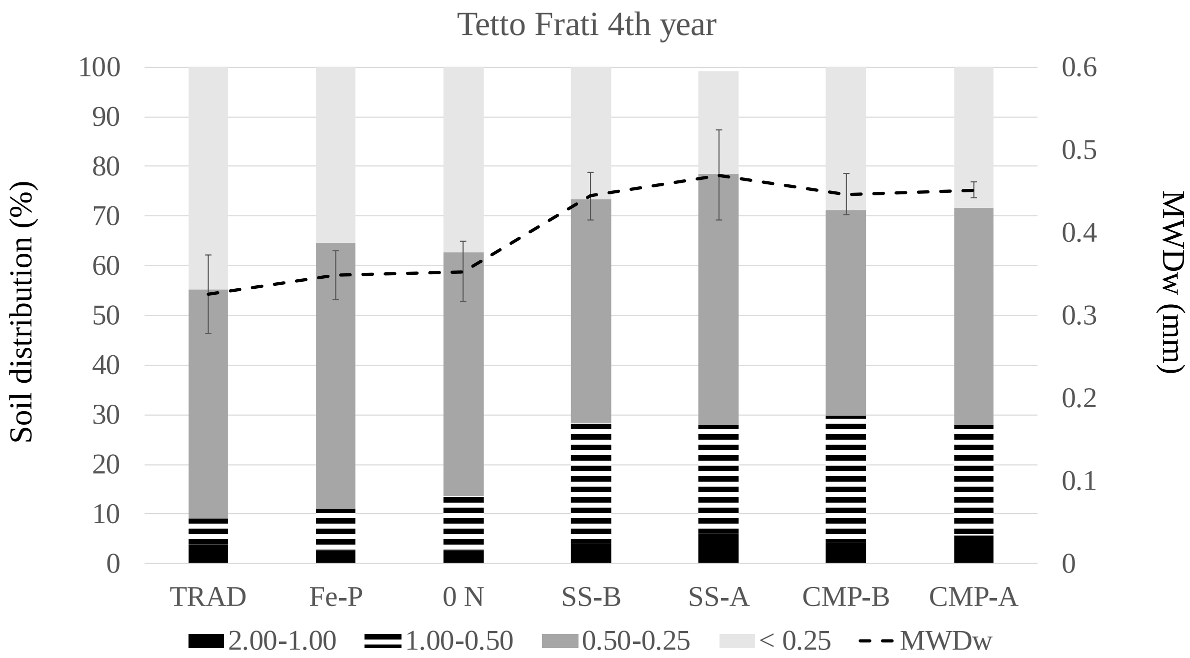
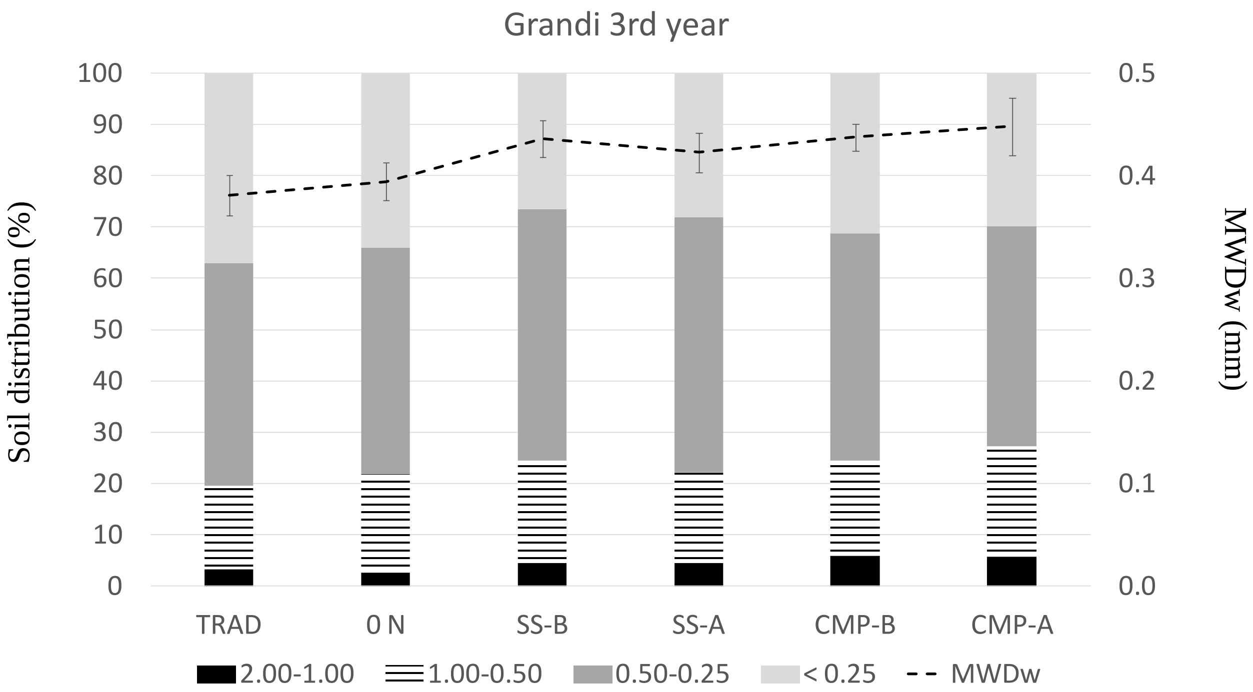


Figure 5. Tetto Frati: distribution (%) of water-stable aggregate sizes (mm) and stability index (MWD) in different field treatments for third and fourth years of SOM managements



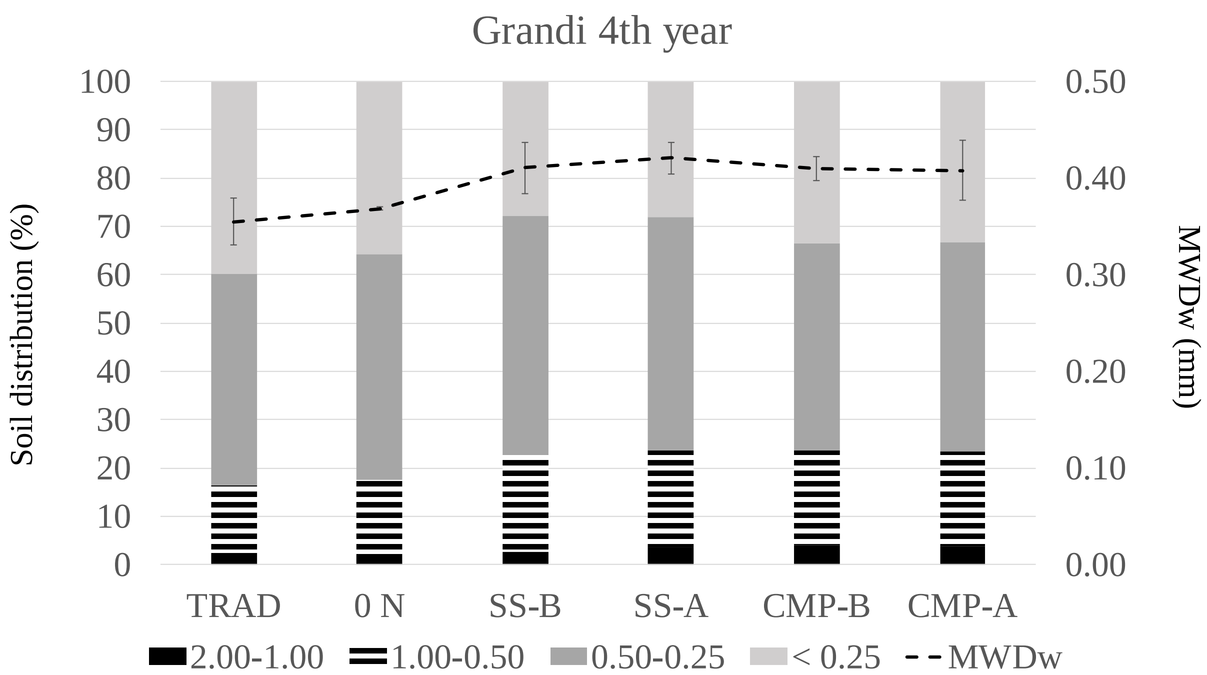
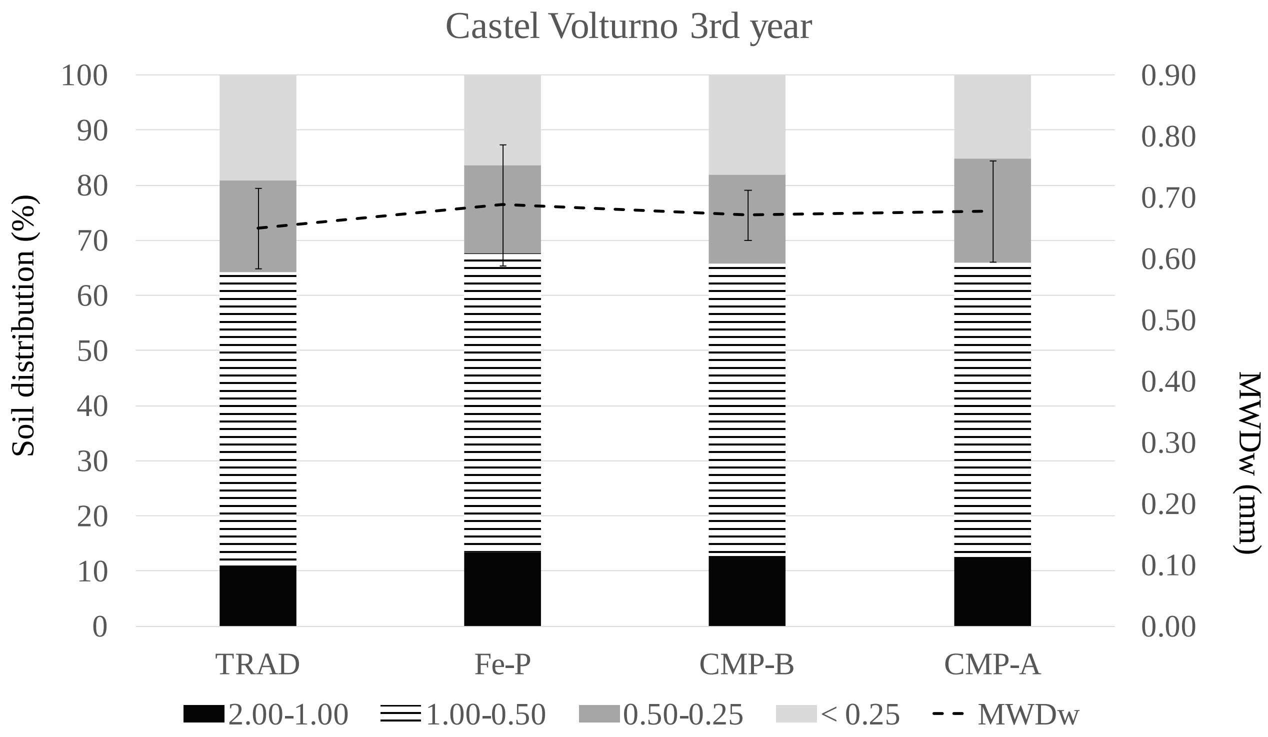


Figure 6. Grandi: distribution (%) of water-stable aggregate sizes (mm) and stability index (MWD) in different field treatments for third and fourth years of SOM managements

A similar trend with a steady positive effect on aggregate stability of both compost treatment was found for the project site of Prima Luce (Fig. 8), while conversely the data obtained for the project sites of Castel Volturno suggest that the larger clay content may act as a main stabilizing agent, thereby hindering the possible aggregation effect promoted by the increasing amount of TOC content in compost amended plots (Figs. 7, 8).



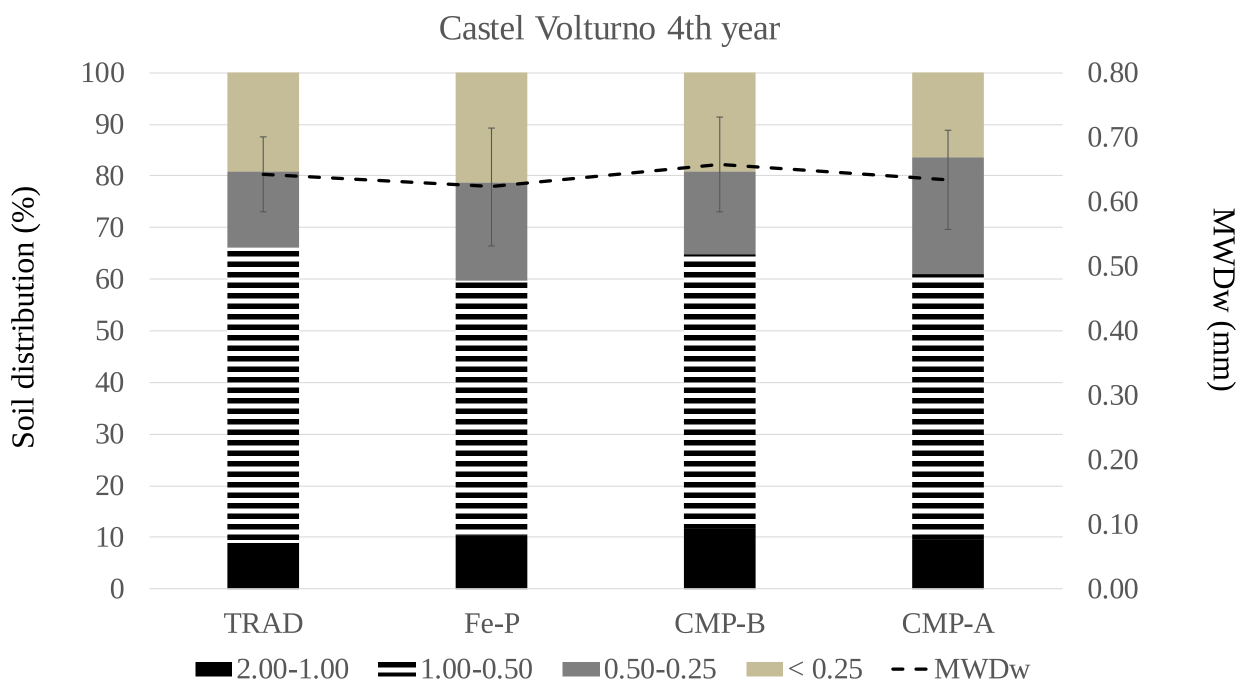
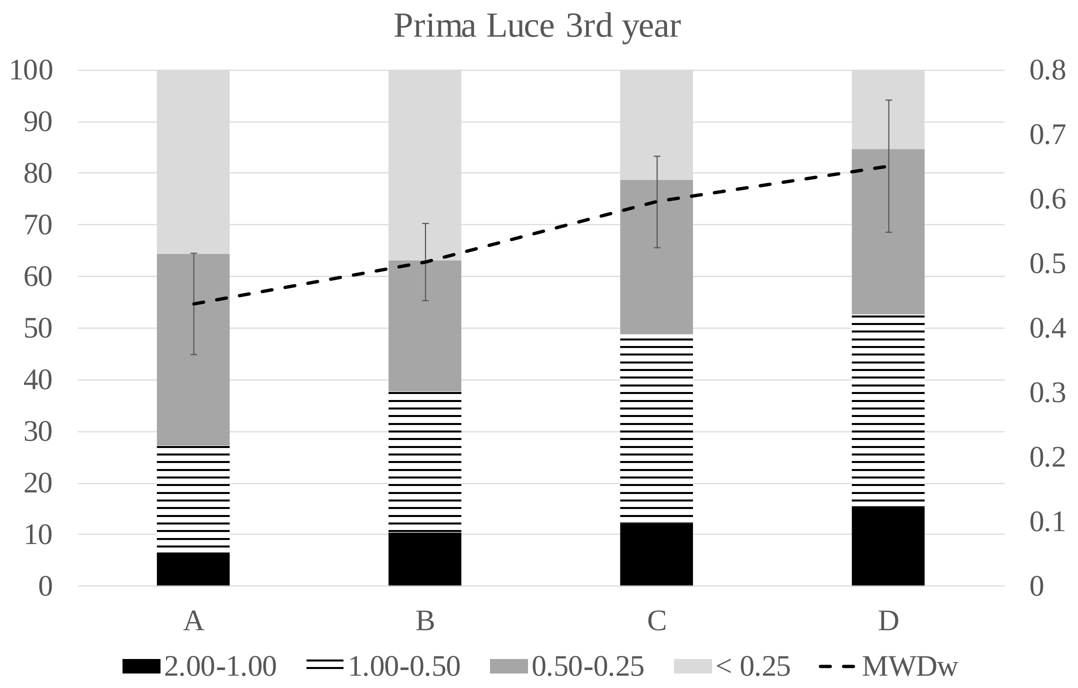


Figure 7. Castel Volturno: distribution (%) of water-stable aggregate sizes (mm) and stability index (MWD) in different field treatments for third and fourth years of SOM managements



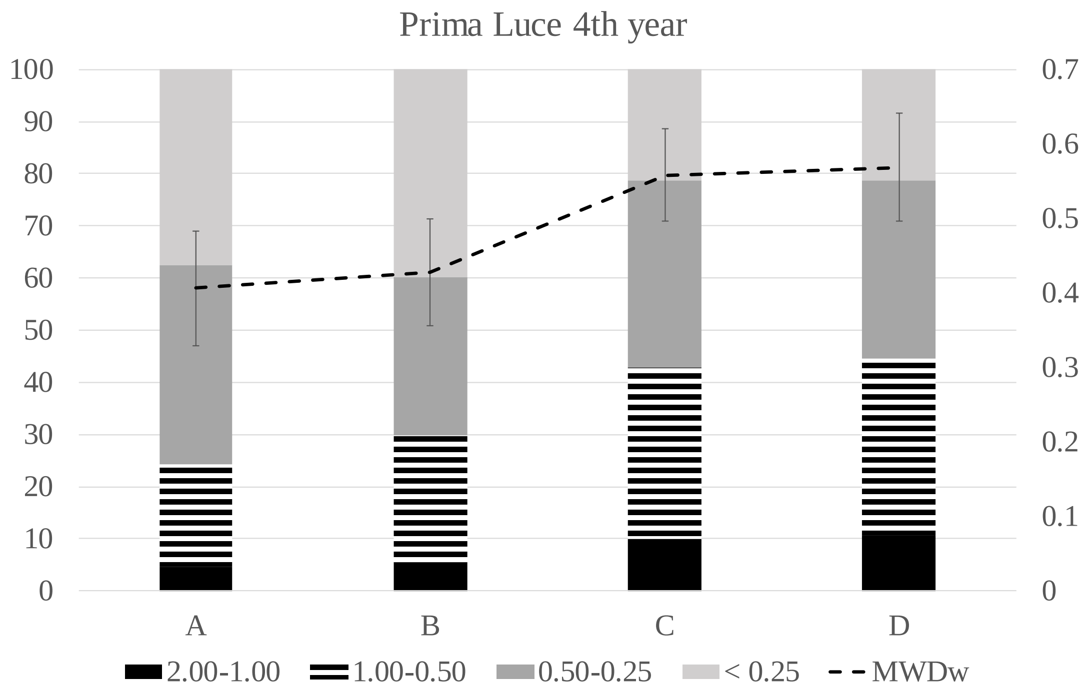
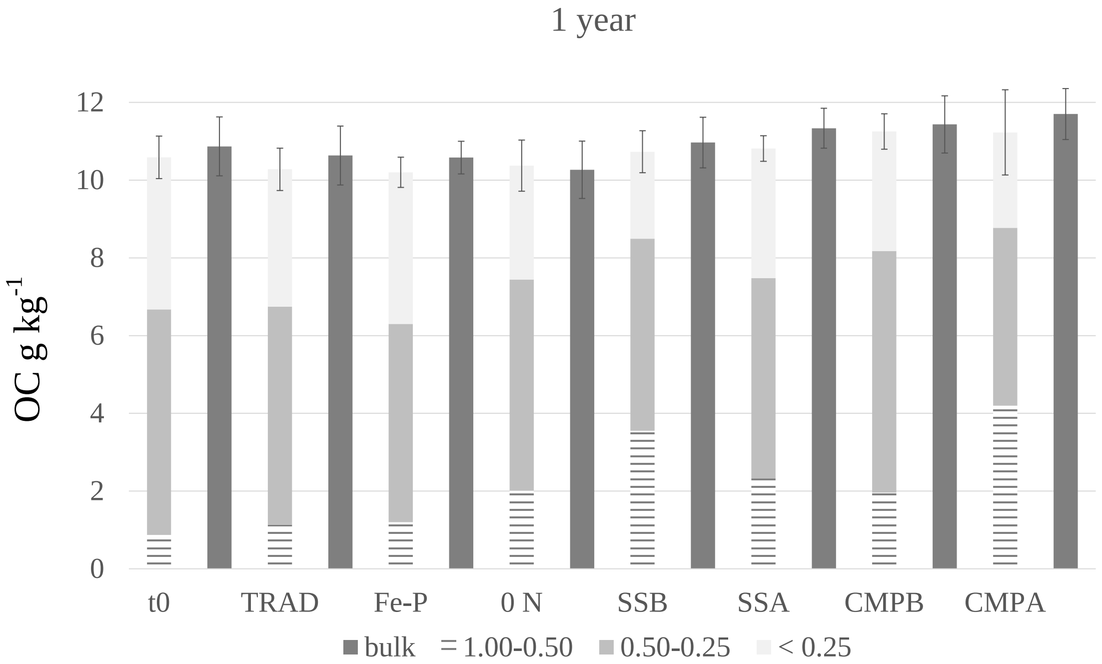


Figure 8. Prima Luce: distribution (%) of water-stable aggregate sizes (mm) and stability index (MWD) in different field treatments for third and fourth years of SOM managements

3.2 SOC and N distribution

After two year of SOM managements an average improvement of SOM content was found in field plots with organic amendments of each project sites in respect to conventional managed plots (Figs. 9, 10, 11, 12). The widespread increase of TOC found in different bulk soils ranged from 0.8 to 2.8 g kg-1, depending on soil type and dose of organic amendment, while no relevant differences may be associated with different compost composition. The stable incorporation of OM inputs was confirmed by the OM distribution found in aggregate size fractions which stacked amounts revealed the steady larger OC content in compost amended plots of each field site. With respect to SOC and N distribution, the lower aggregation capacity of the light textured soils of Tetto Frati and Grandi resulted in a null contribution of larger aggregate sizes (2.0-1-0 mm) to total SOM (Figs. 9, 10)



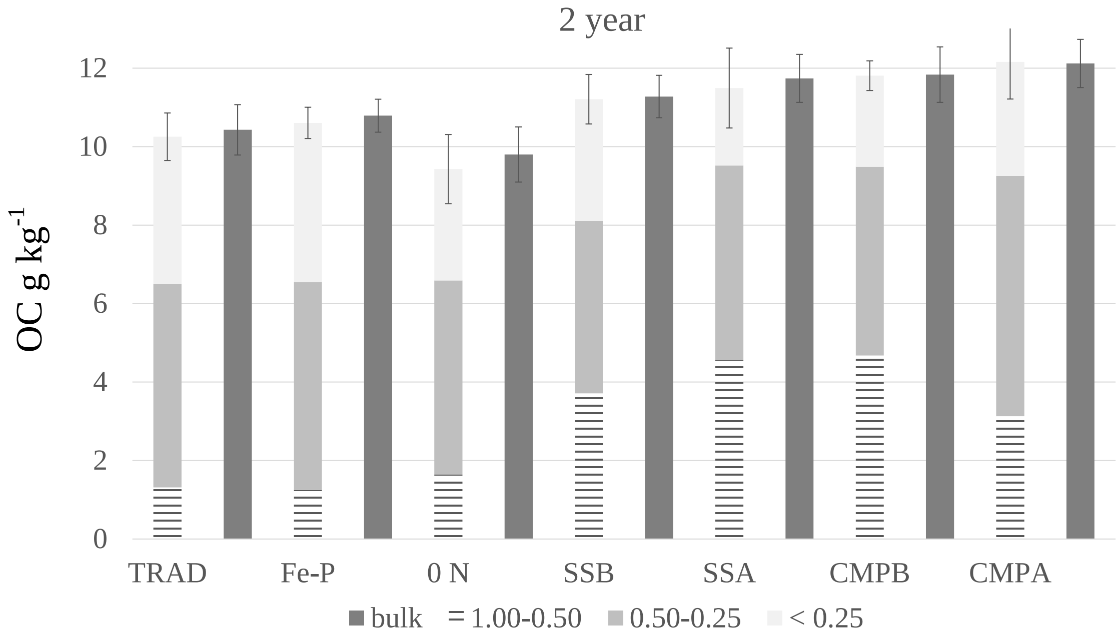
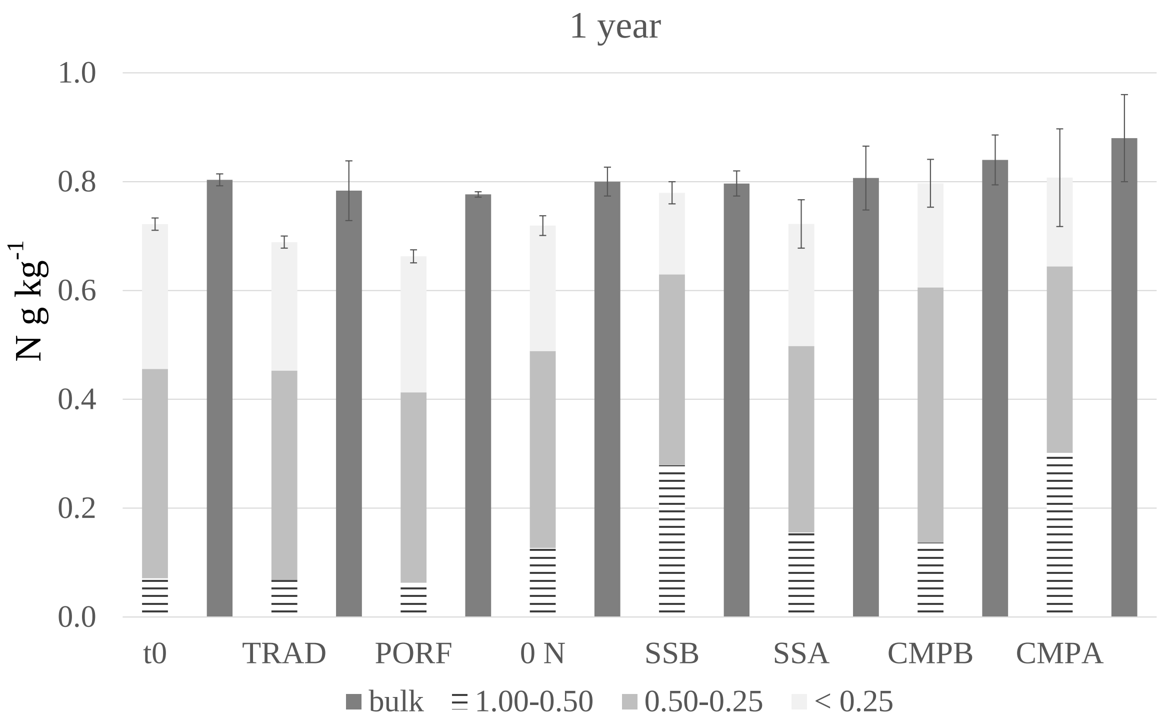


Figure 9a Tetto Frati: SOC content (g kg-1) in bulk soil and aggregates in different treatments for 1st and 2nd year of SOM management

At the end of second year in Tetto Frati farm site, the soil treatments with organic amendments showed an overall increase of about 1-1.2 g OC kg^{-1}, with minor differences among doses and OM types (Fig. 9a). The larger fraction of SOM in amended plots was incorporated in macro-aggregates, thereby supporting the aggregation process observed in aggregate distribution. For the N content in bulk soils only the larger dose of compost addition (CMPA) showed a significant increase in respect to control soil. Although not significant, the distribution in aggregated fractions revealed slight larger recovery of N in in soil treatments with organic matter addition, except for the SSA sample (Fig. 9b).



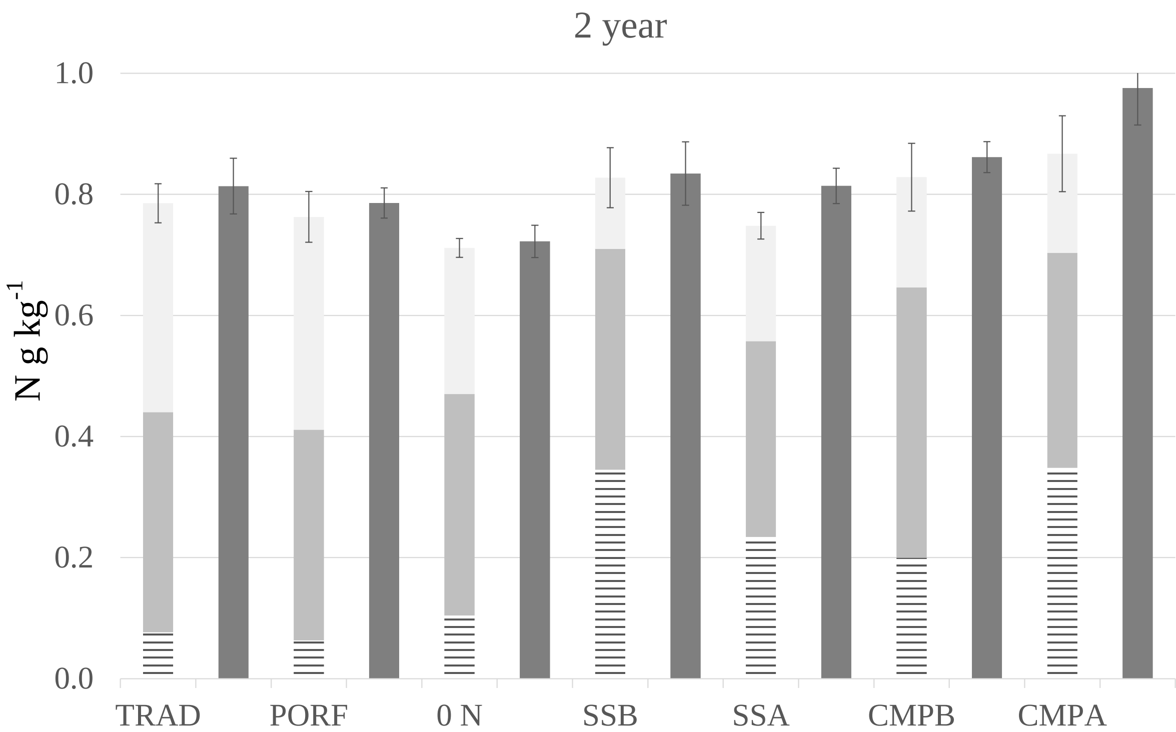


Figure 9b Tetto Frati: total N content (g kg-1) in bulk soil and aggregates in different treatments for 1st and 2nd year of SOM management

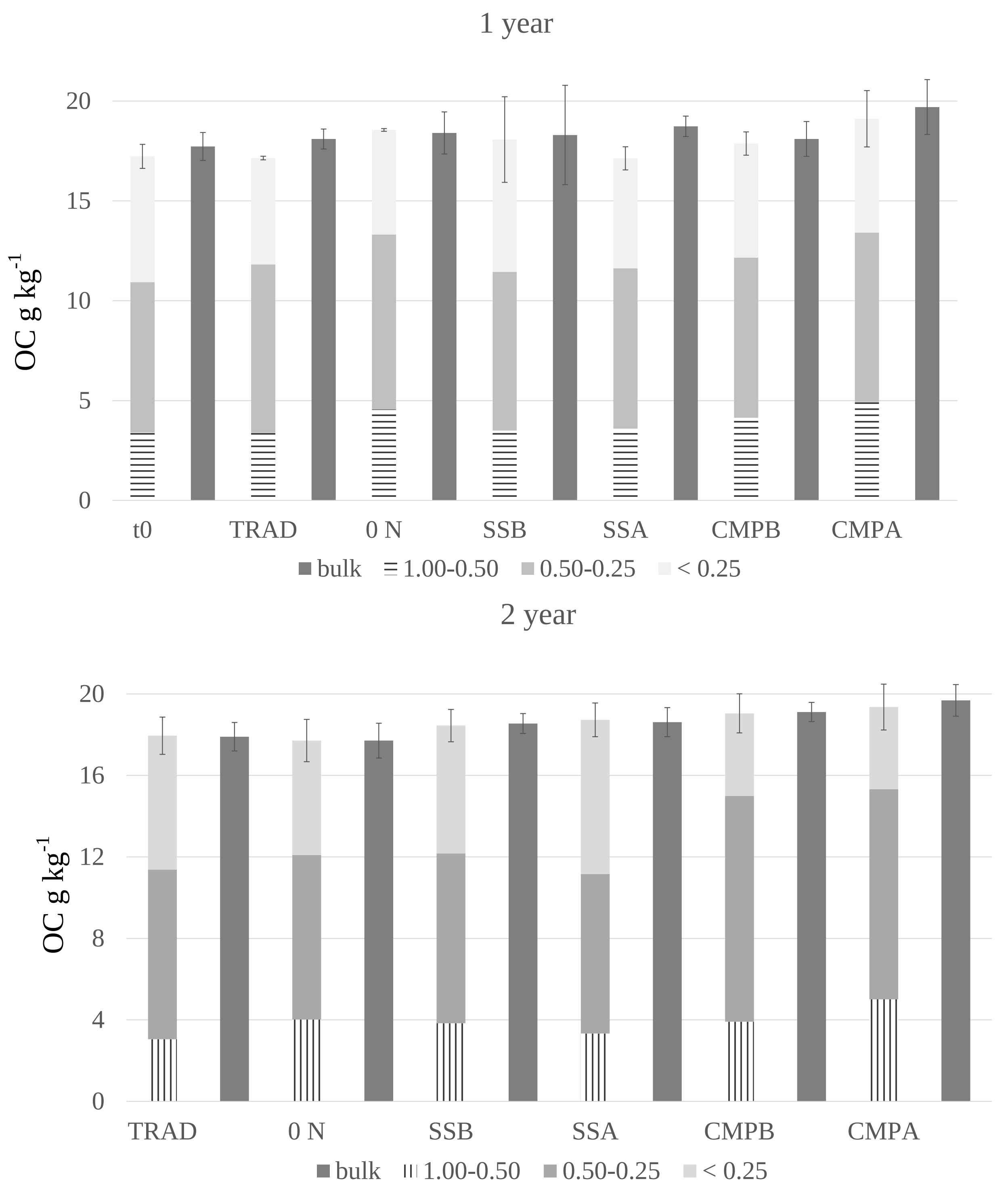


Figure 10a Grandi: total SOC content (g kg-1) in bulk soil and aggregates in different treatments for 1st and 2nd year of SOM management

In the Grandi farm at the end of second crop cycle, the soil treatments with organic matter additions showed an increase in SOC content which ranged from 0.6, to 1.6 g kg^{-1} from SSB to CMPA samples, in both bulk soils and combined size fractions, although not statistical differences were found in respect to traditional plots (Fig 10a). The OC distribution among soil aggregates revealed a large OC partition in finest particles for soil treatment with fresh solid digestate (SSB and SSA), while more than the 80% of TOC in CMPB and CMPA was incorporated in the water stable-macroaggregates (> 0.25 mm).

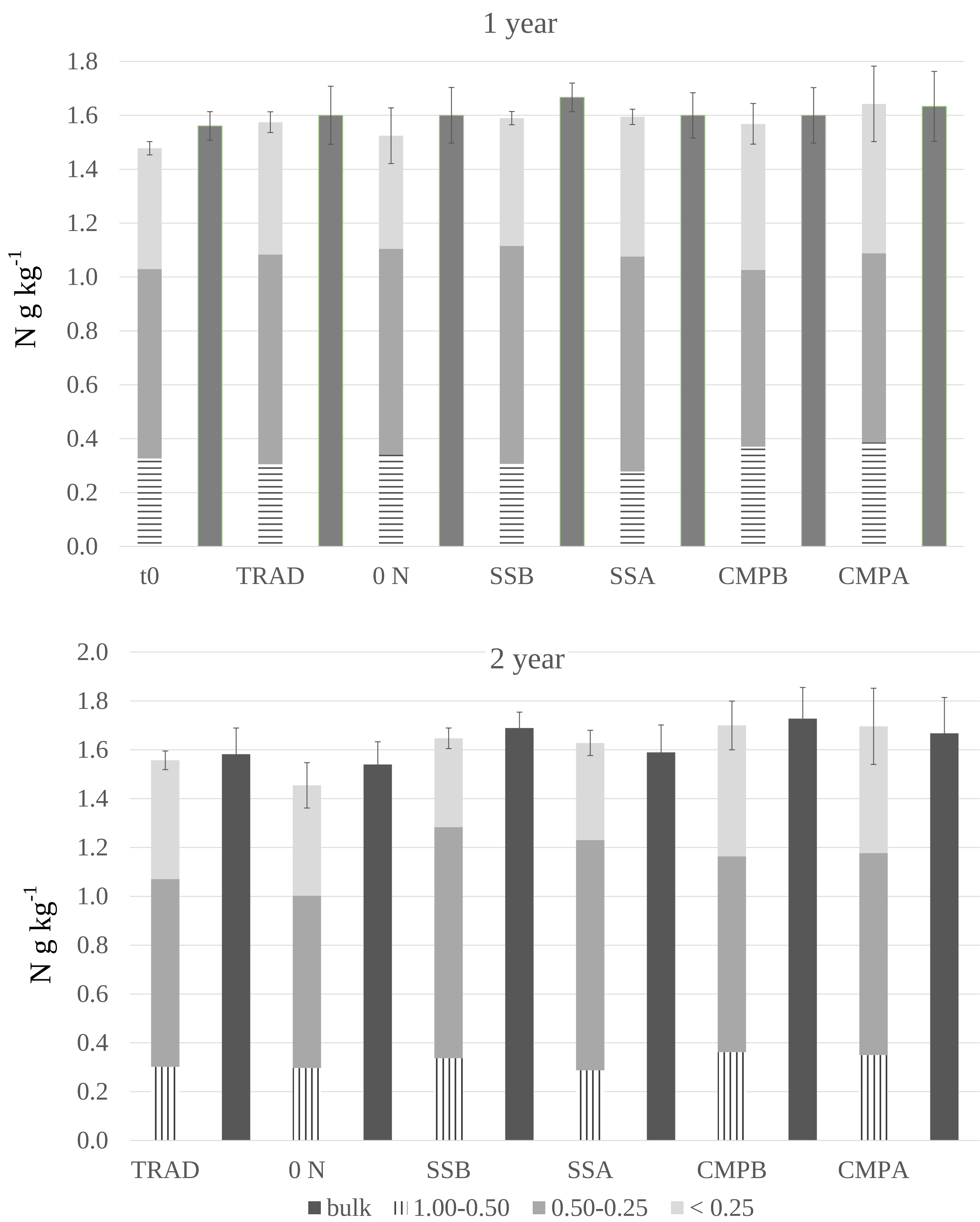
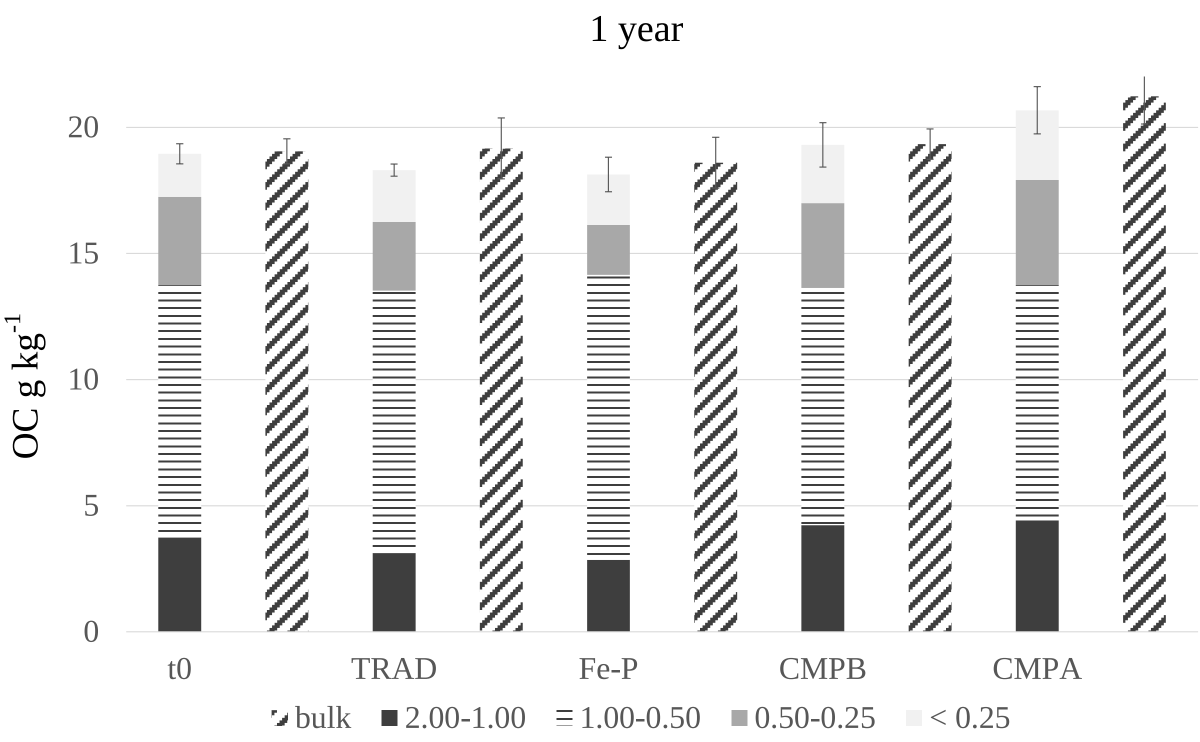


Figure 10b Grandi: total N content (g kg-1) in bulk soil and aggregates in different treatments for 1st and 2nd year of SOM management



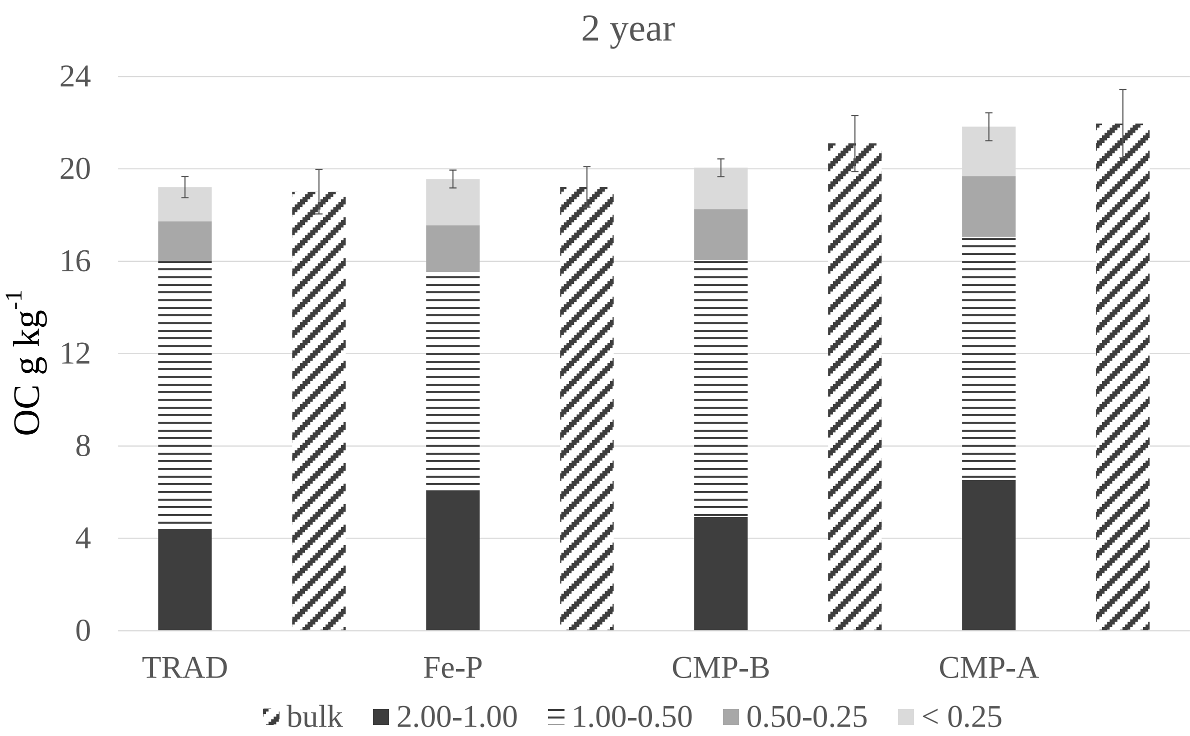
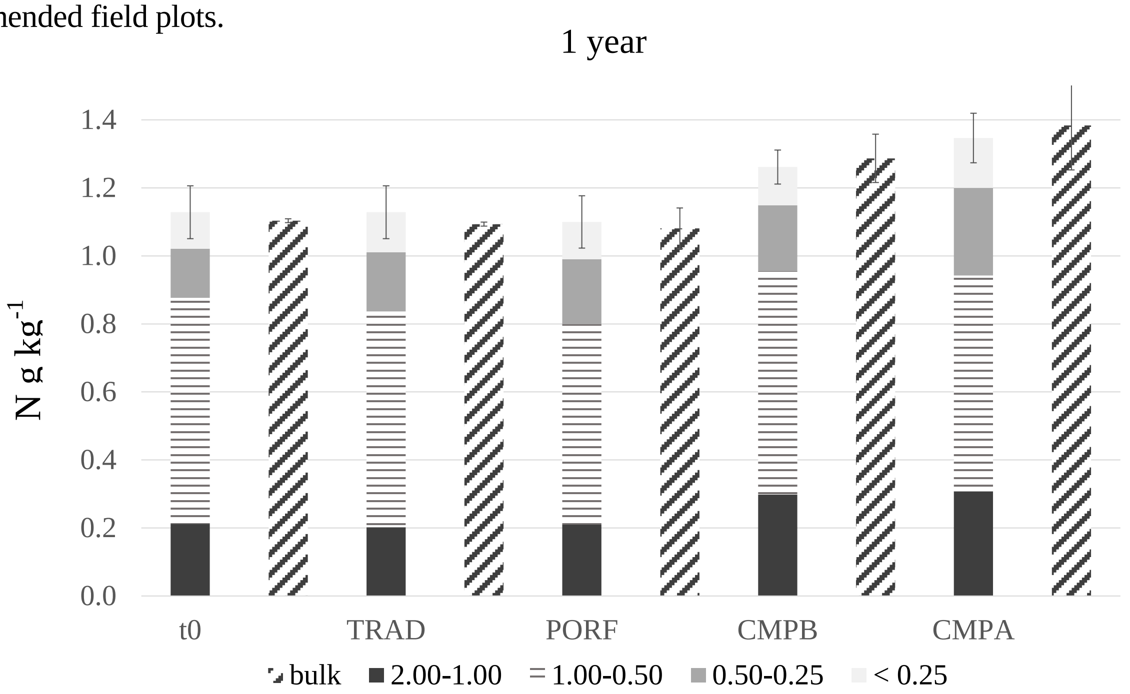


Figure 11a Castel Volturno: total SOC content (g kg-1) in bulk soil and aggregates in different treatments for 1st and 2nd year of SOM management

In the project site of Castel Volturno the TOC content of field plots at the second year, indicated a significant incorporation of exogenous organic materials in soil treatments with on-farm manure compost with an increase of about 2.1 and 2.8 g kg^{-1} for CMPB and CMPA (Fig. 11a). This finding may be related to the large content finest soil fraction (clay) and the larger aggregation capacity which may have exerted an additional physical protection on the exogenous OM inputs.

As noted for the other soils, slight changes were found in the relative distribution of TOC among soil aggregates, thereby supporting the major influence of textural components in the aggregation processes. Although also the yields of total N content showed a comparable trend (Fig. 11b), both soil treatments with either conventional management and biomimetic catalyst addition underwent to an unusual significant increase in respect to the first year, thereby reducing the gap with compost amended field plots.



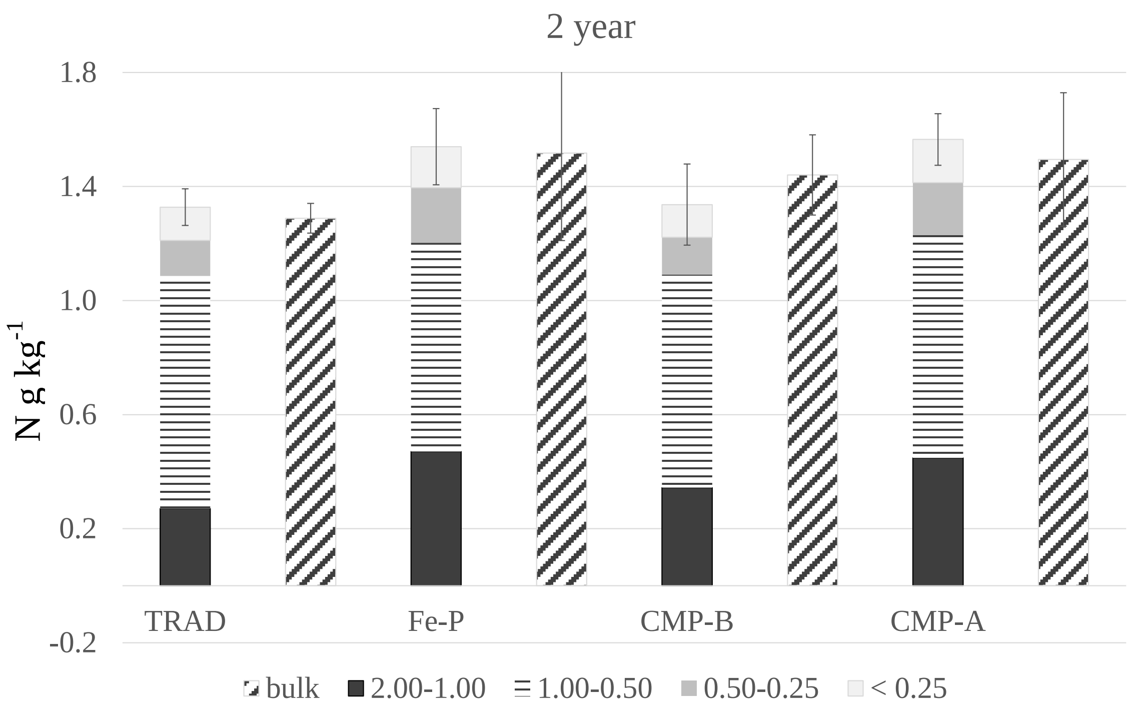
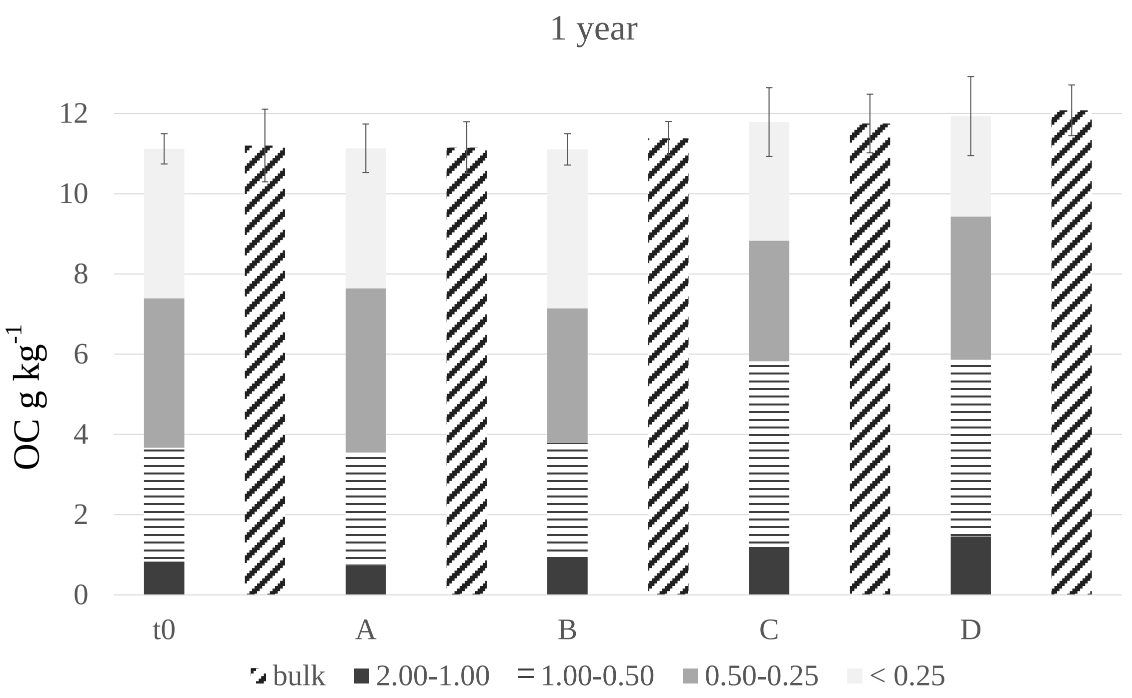


Figure 11b Castel Volturno: total N content (g kg-1) in bulk soil and aggregates in different treatments for 1st and 2nd year of SOM management



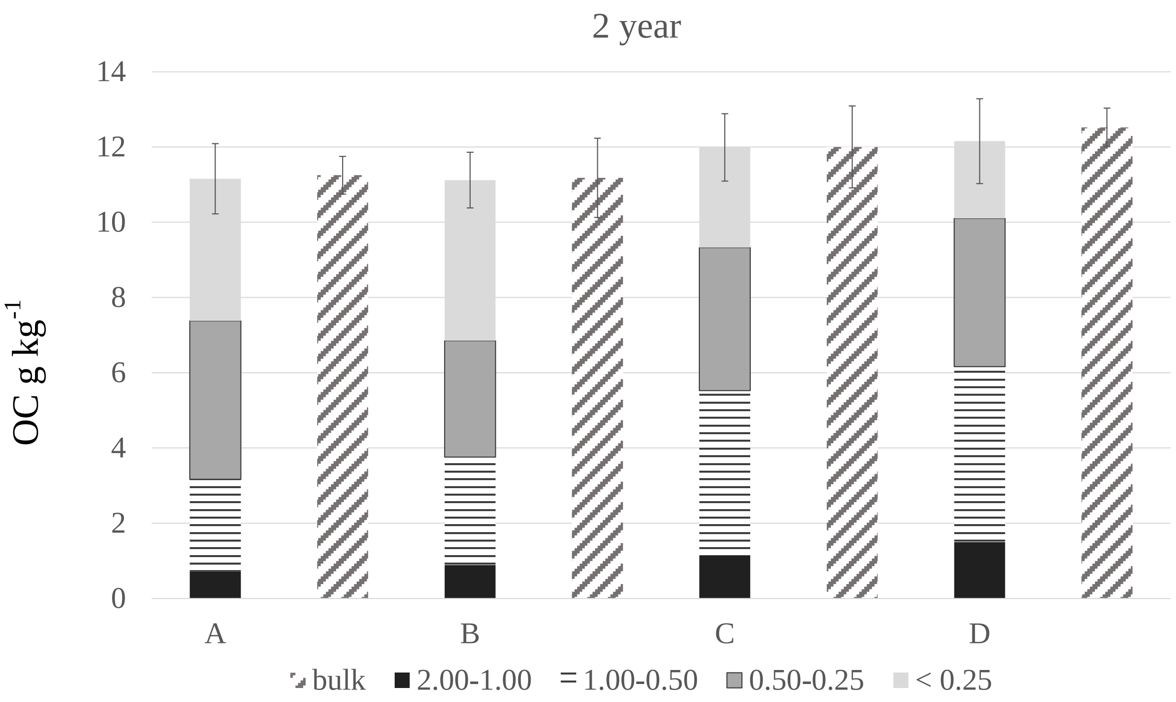


Figure 12a Prima Luce: total SOC content (g kg-1) in bulk soil and aggregates in different treatments for 1st and 2nd year of SOM management

An improvement of SOM content was found in both bulk soils and size-aggregates in field plots amended with on farm compost at the project sites of Prima Luce (Figs 8), which were characterized by an increase of 0.8-1.2 g kg-1 in respect to traditional samples. The incorporation of exogenous OM revealed a combined increase of OC concentration in the lower aggregate classes (< 0.50 mm) and a progressive accumulation of global TOC in larger size fractions of C and D treatments thereby confirming the positive effect of OM addition on the aggregation process.

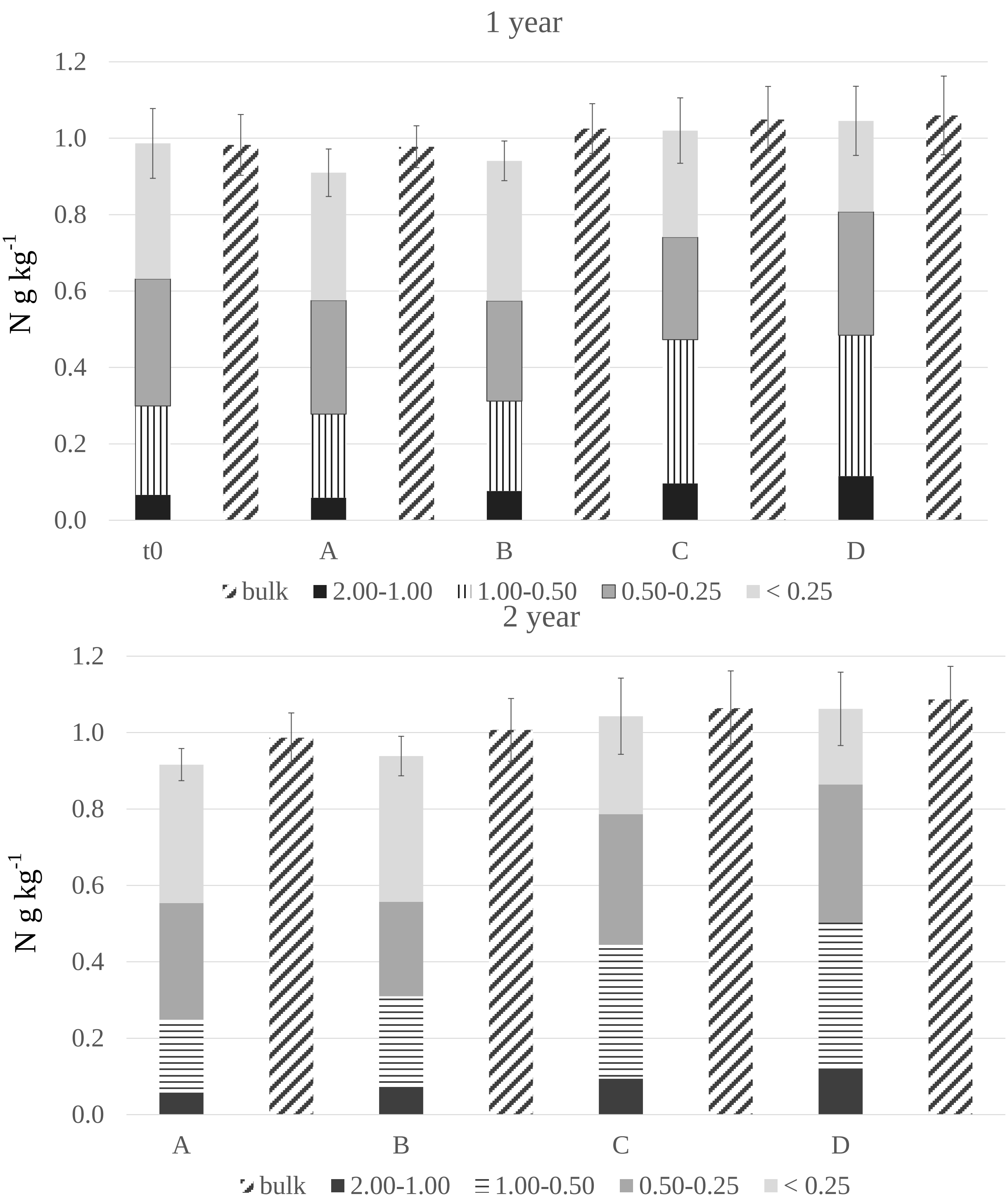
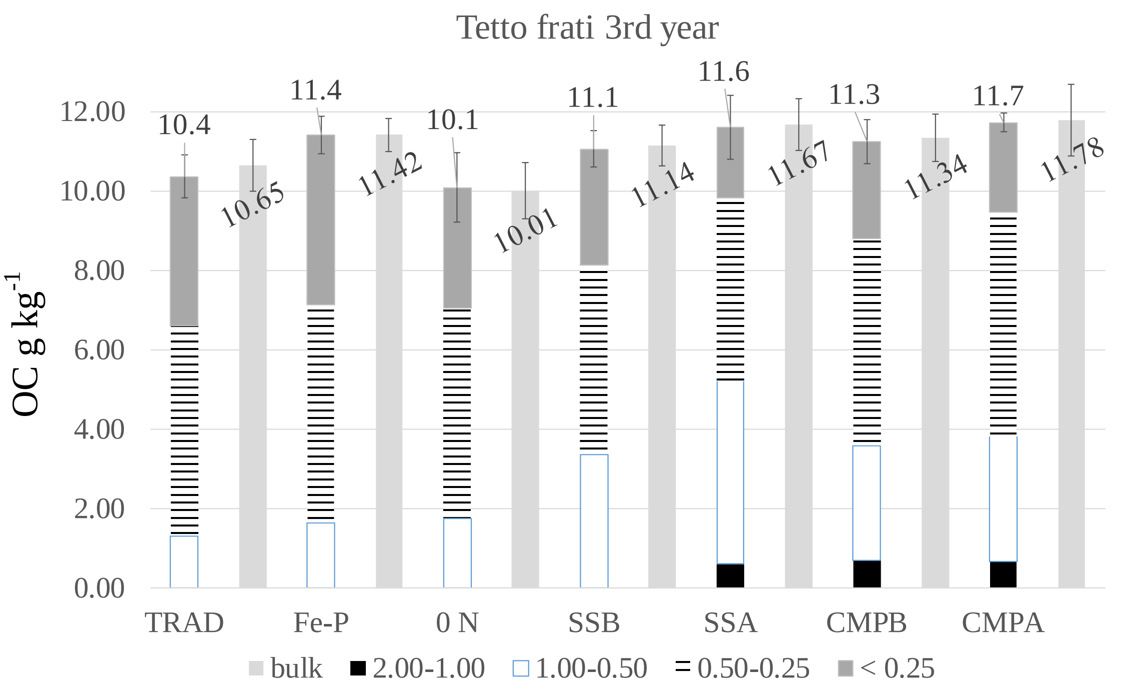


Figure 12b Prima Luce: total N content (g kg-1) in bulk soil and aggregates in different treatments for 1st and 2nd year of SOM management

In the second half of project activities the response of SOC content on application of SOM managements confirmed the indications obtained in the previous years, for all project sites. The evaluation of TOC content in both bulk soils and size aggregates of various fields and treatments, showed an increase of OC provided by soil addition with exogenous organic materials (Figs. 13, 14, 15, 16), in respect to conventional managed plots. The stable incorporation of OM inputs was confirmed by the OC distribution found in aggregate size fractions which stacked amounts revealed the steady larger OC content in compost amended plots of each field site.



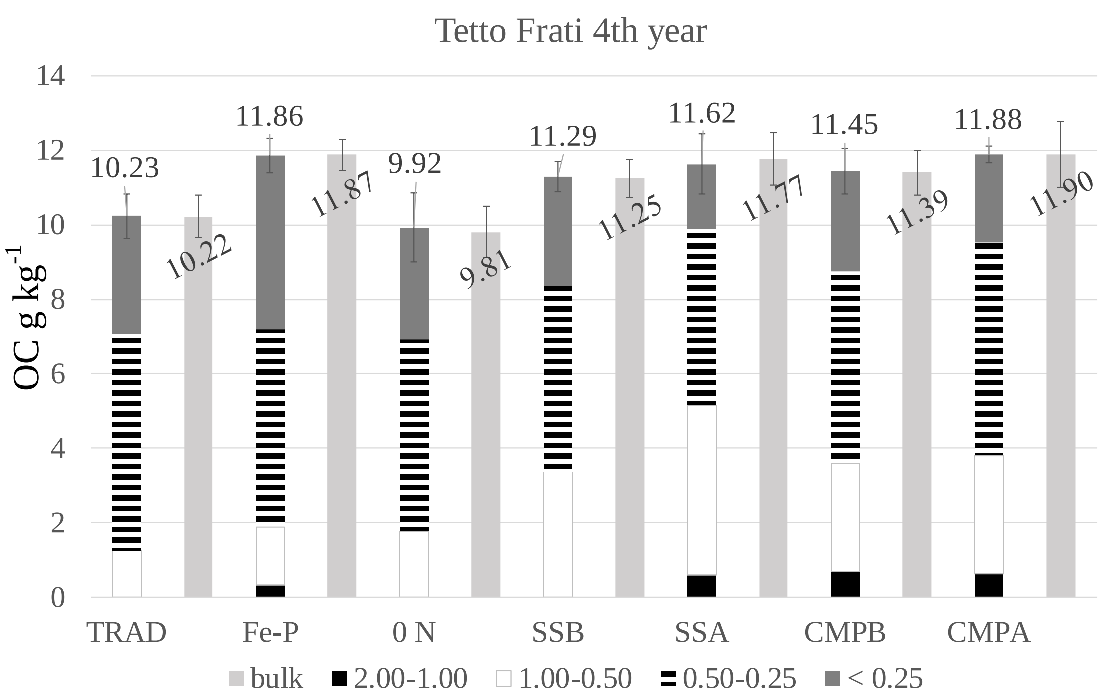


Figure 13a Tetto Frati: total SOC content (g kg-1) in bulk soil and aggregates in different treatments for 3rd and 4th year of SOM management

For the light textured soil Tetto Frati project site, a larger OC content was found in soil plots amended with mature compost in respect to soil treatments with fresh digestate (Fig. 13a) after three years of soil management; moreover, the compost addition promoted a slight incorporation of TOC in the larger aggregate sizes (2.0-1.0 mm), thereby confirming the positive effect noticed on the aggregation capacity. Small variations were found at last sampling time, with the field plots amended with the larger OM doses that maintained the higher OC content followed by the SSB and CMPB treatments. A significant annual increase (+0.4 gOC kg^{-1}) was found for soil treatment with higher dose of compost and biomimetic catalyst (FeP) which a final TOC slightly exceeded that of CMPA samples, thereby suggesting a possible additional OC stabilization effect promoted by the metal-porphyrin (Fig. 13a). The N concentraton of bulk soil and size aggregates were nearly specular to the result of C content, thus indicating an OM distribution mainly determined by the textural composition and soil management (Fig. 13b).

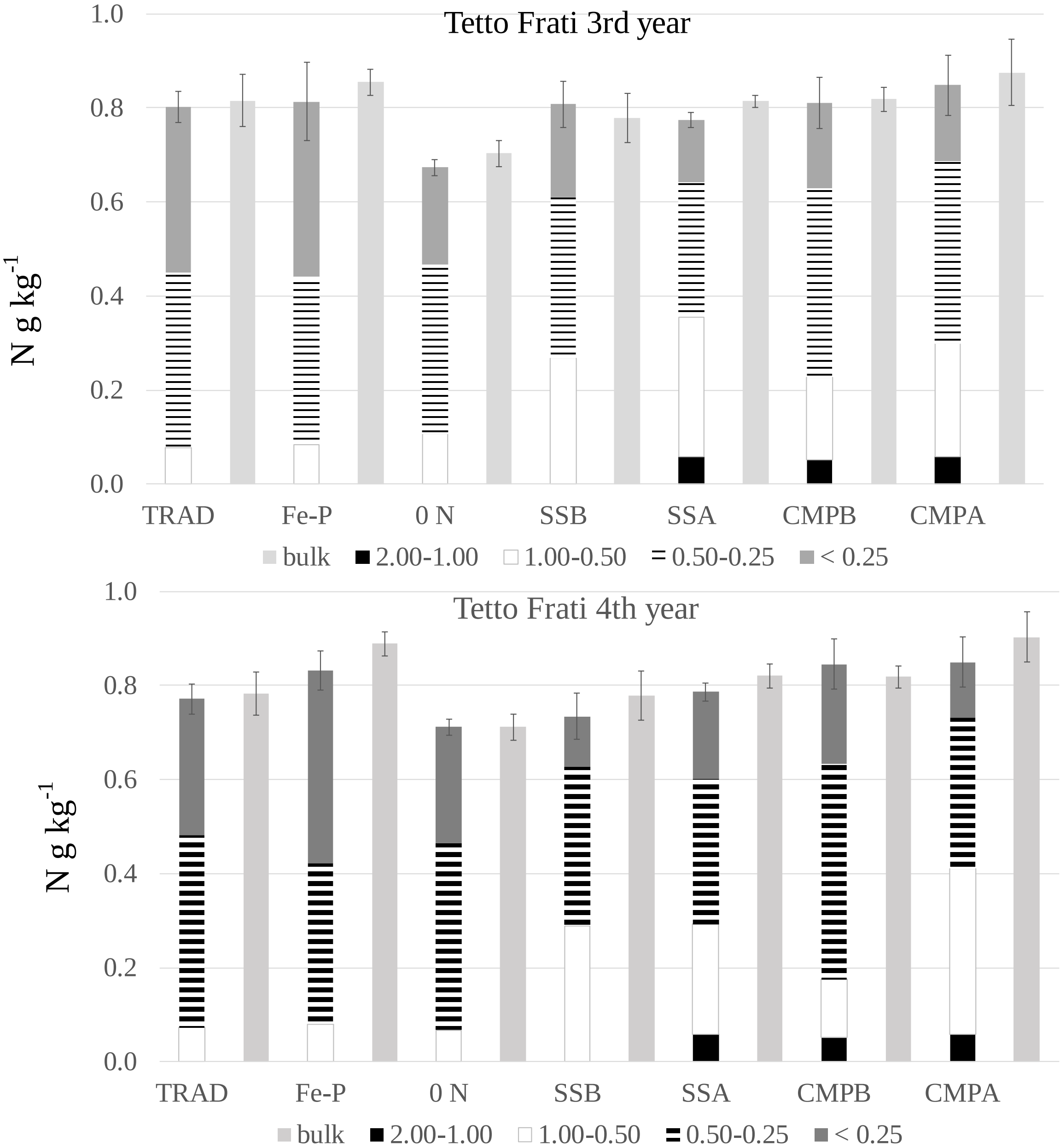


Figure 13b Tetto Frati: total N content (g kg-1) in bulk soil and aggregates in different treatments for 3rd and 4th year of SOM management

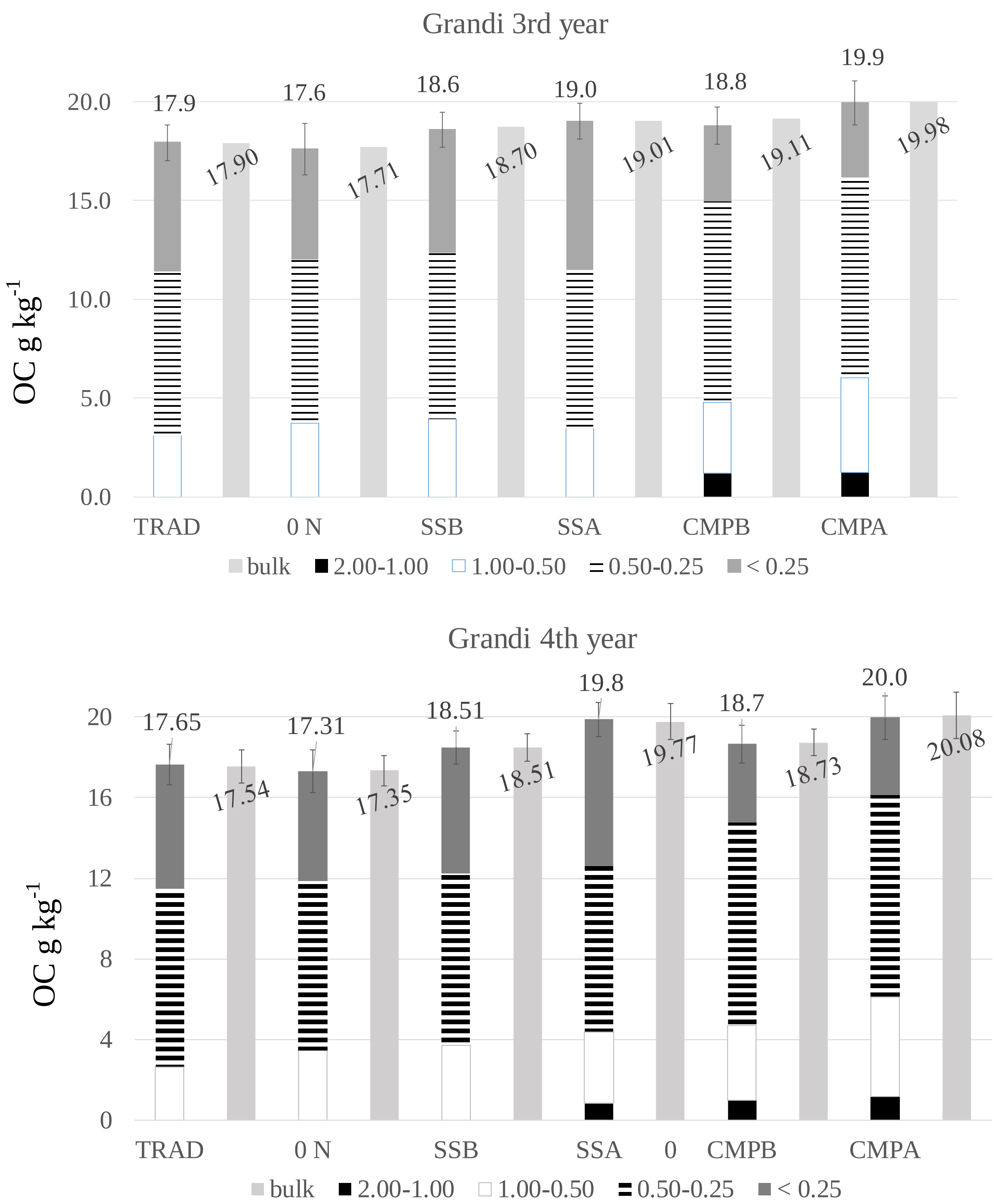


Figure 14a Grandi: total SOC content (g kg-1) in bulk soil and aggregates in different treatments for 3rd and 4th year of SOM management

Also the analyses of TOC content in soil treatments of Grandi indicated an increasing retention of organic molecules in both bulk samples and soil aggregates after the subsequent addition with both fresh digestate and compost (Fig. 14a). In respect to Tetto Frati a steady larger N content was shown by soil treatments of Grandi site (14b). The improved incorporation on Nitrogen (24-27%) in the larger aggregate sizes (> 0.5 mm), found in soil addition with mature compost, may indicate a more amount of available forms of nutrients, with lower fixation of P, thereby allowing a easily uptake from horticultural crops.

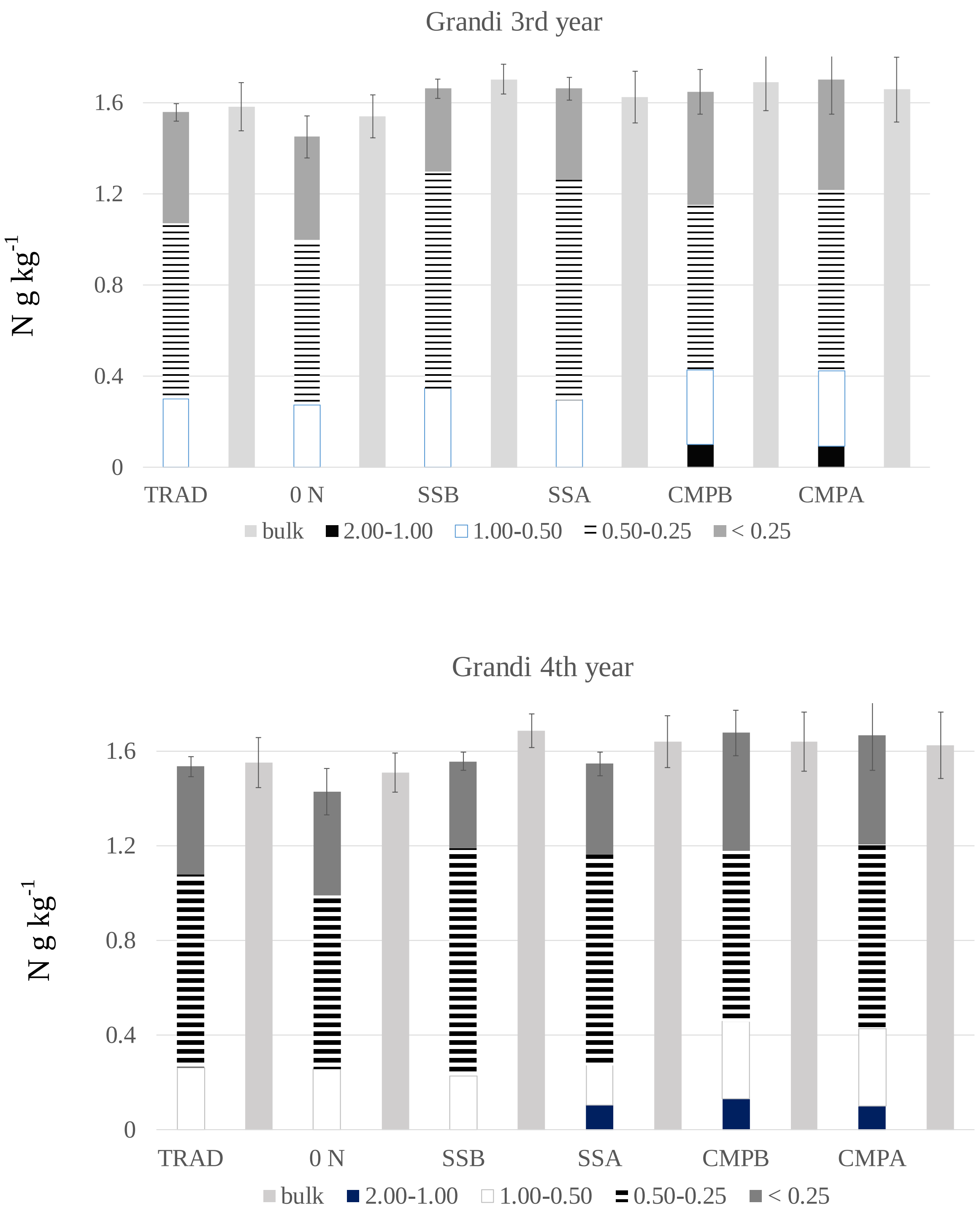


Figure 14b Grandi: total N content (g kg-1) in bulk soil and aggregates in different treatments for 3rd and 4th year of SOM management

A steady improvement of SOC content after OM addition was found in both the heavier textured soils of Castel Volturno and Prima Luce as revealed by the OC yields of bulk samples and size-aggregates in field plots amended with on farm compost at both project sites (Figs. 15, 16), in respect to traditional managed samples. The incorporation of exogenous OM revealed a combined increase of OC concentration in the lower aggregate classes (< 0.50 mm) and a progressive accumulation of global TOC in larger size fractions of compost treatments thereby confirming the positive effect of OM addition on the aggregation process.

At Castel Volturno in the last two years the soil treatment with biomimetic catalyst promoted a progressive stabilization of SOC, with a final increase of about 0.8 g kg-1 on respect to the control sample (Fig. 15a), corresponding to about 1.6 - 3.2 tons of OC ha-1 for a ploughing depth of 0.13 or 0.30 m, respectively.

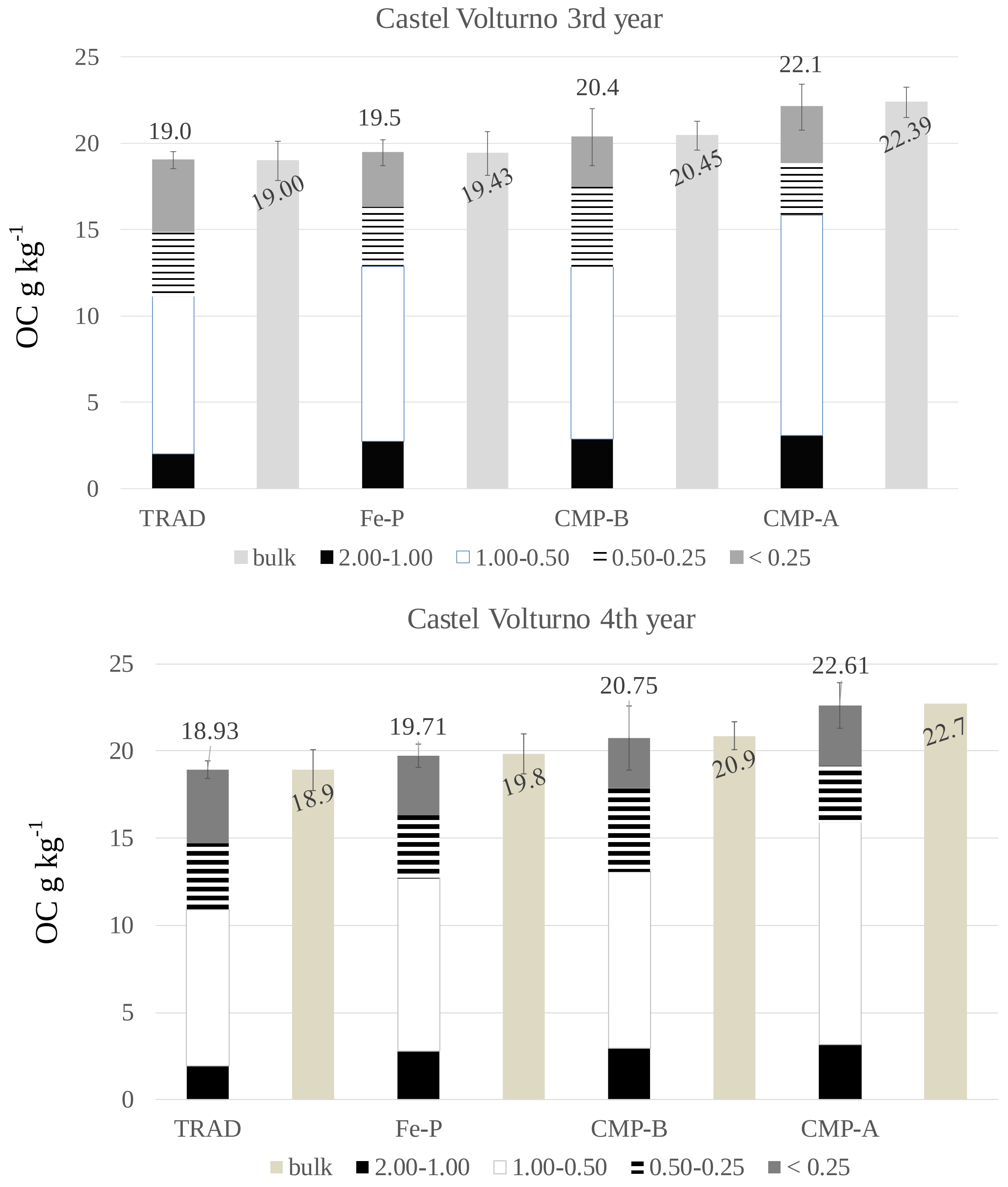
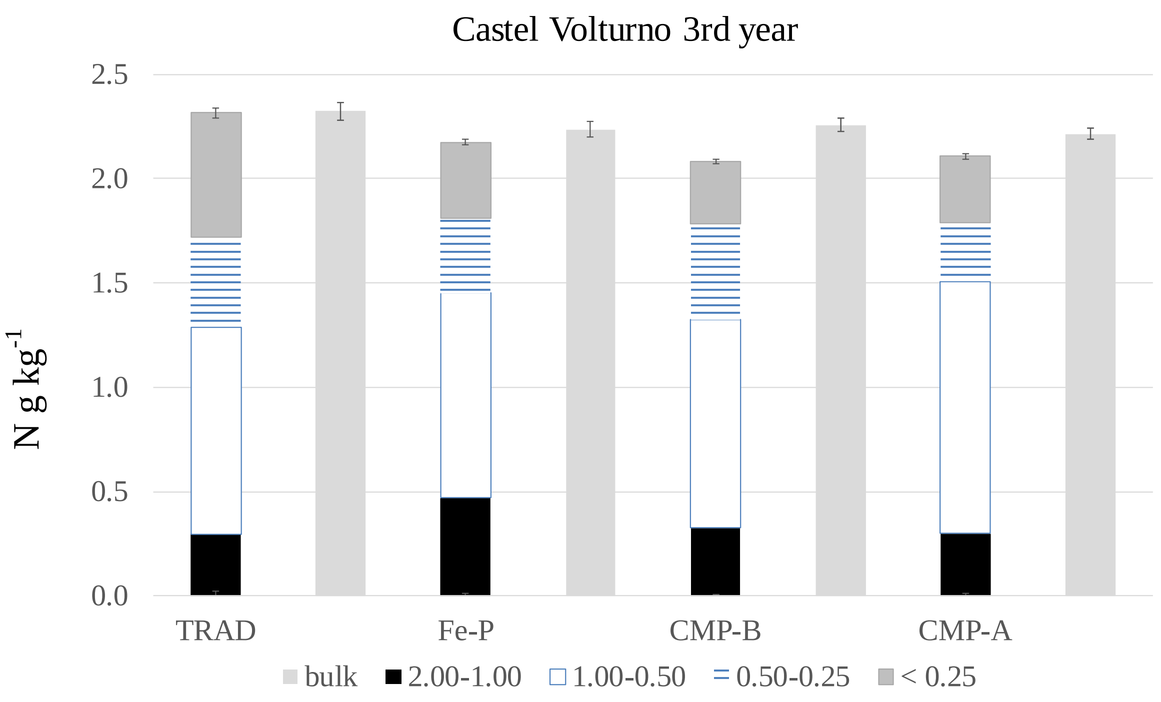


Figure 15a Castel Volturno: total SOC content (g kg-1) in bulk soil and aggregates in different treatments for 3rd and 4th year of SOM management



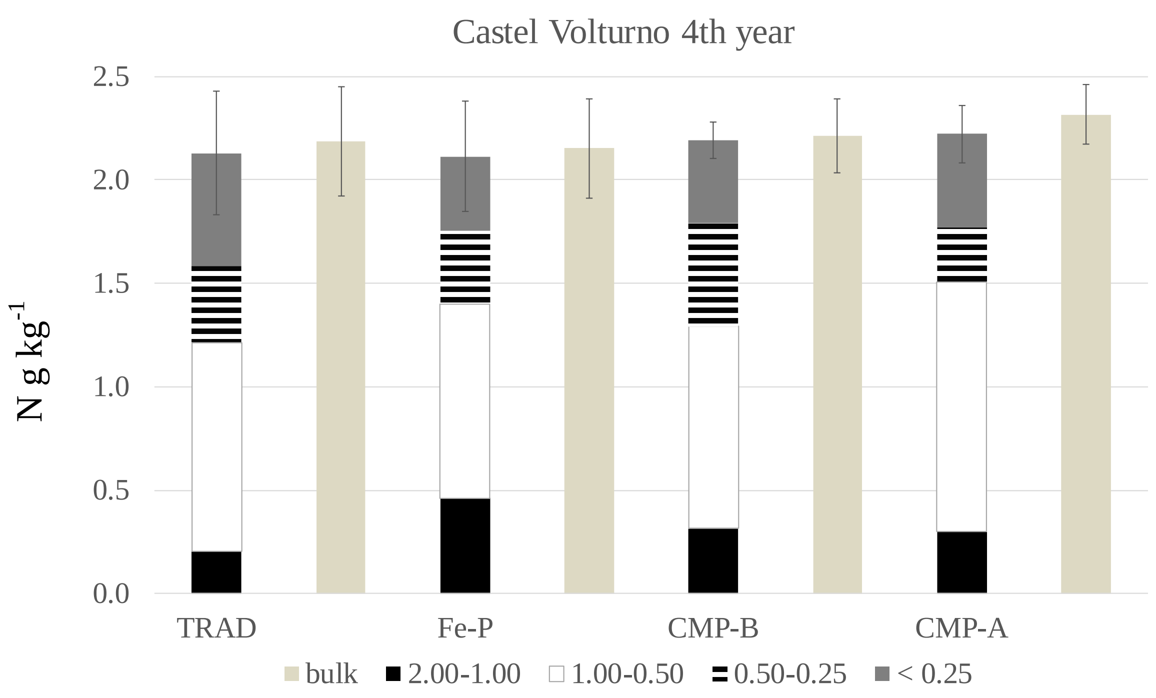
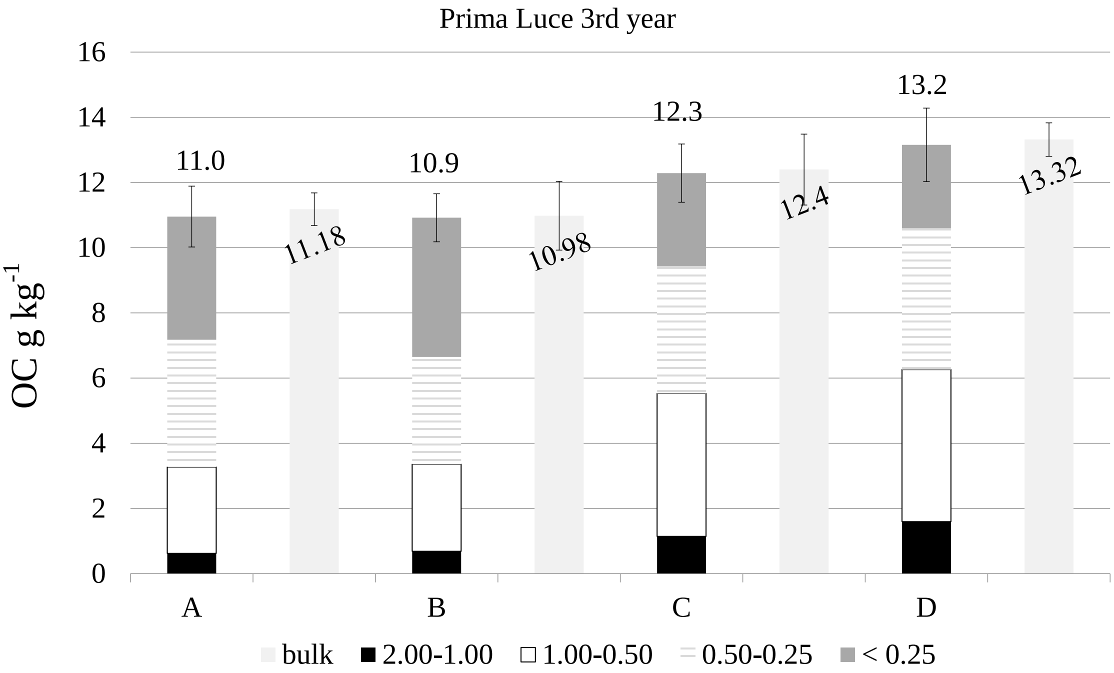


Figure 15b Castel Volturno: total N content (g kg-1) in bulk soil and aggregates in different treatments for 3rd and 4th year of SOM management



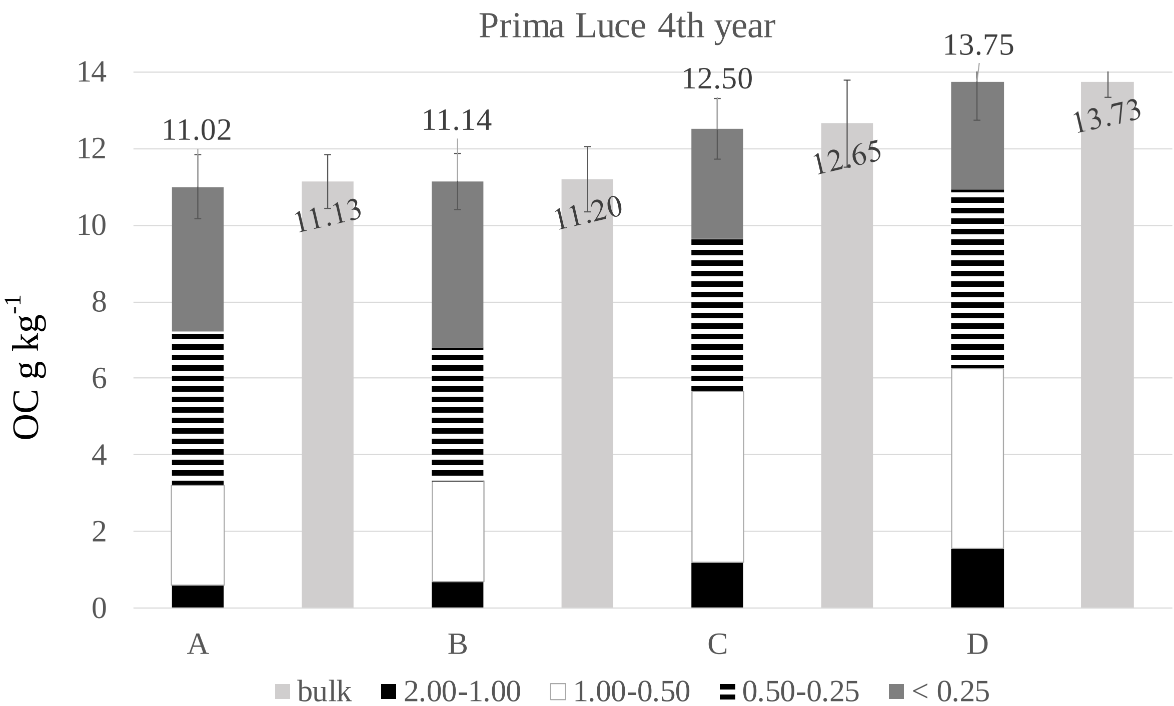
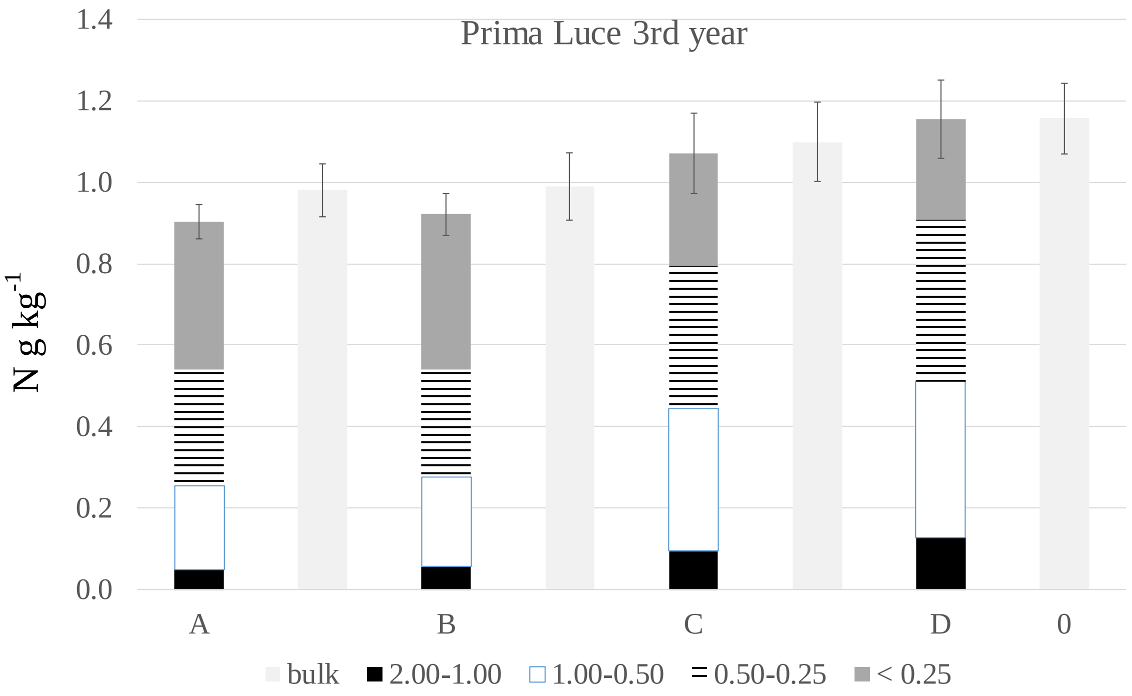


Figure 15a Castel Volturno: total SOC content (g kg-1) in bulk soil and aggregates in different treatments for 3rd and 4th year of SOM management



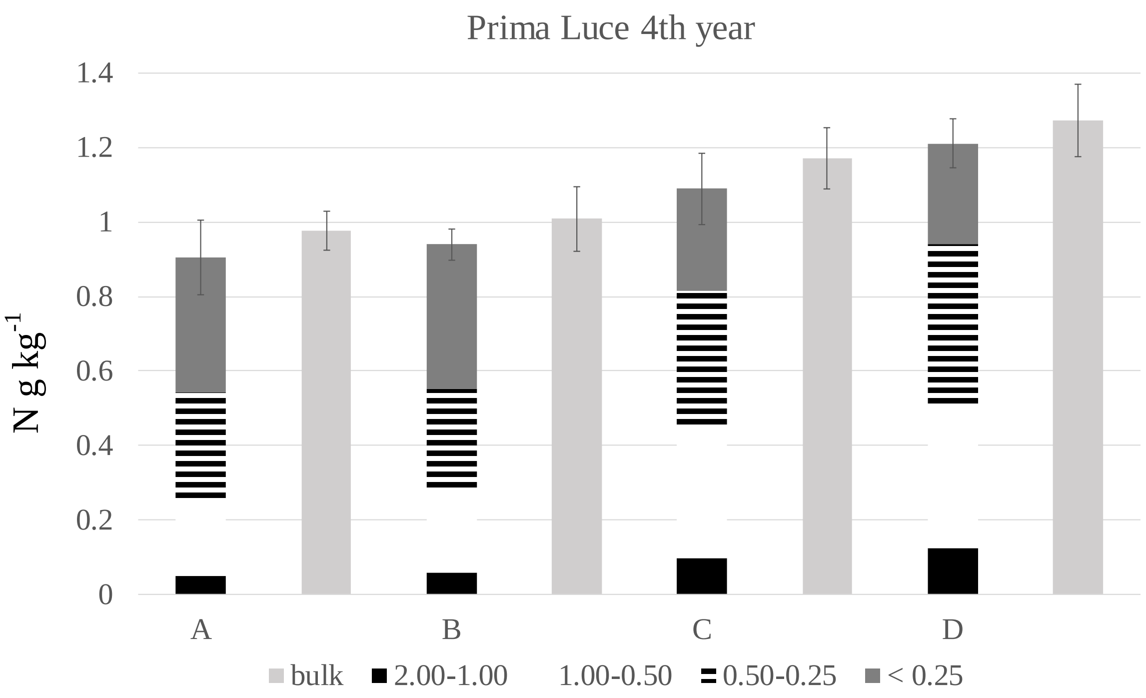


Figure 16b Castel Volturno: total N content (g kg-1) in bulk soil and aggregates in different treatments for 3rd and 4th year of SOM management

The data of TOC distribution in different project sites suggested that in Tetto Frati and Grandi the soil treatments with fresh digestate and compost underwent to a fast increase of organic matter, thereby reaching at the third year an almost new steady state. The incorporation of organic materials in all particle-size fractions allowed also a slight improvement of soil aggregate stability. Conversely an a slower trend was observed in the soil treatments of Castel Volturno, with a no significant effect on soil aggregates, which however showed an increase of OC retention also in the last SOM management period. An intermediate behaviour was revealed by the addition of on-farm green compost on the field plots of Prima Luce, that showed both an increase of aggregate stability and of OC content. Besides the molecular composition and biochemical recalcitrance of the different organic materials, this behaviour may be also related to the different textural composition and the consequent larger OC saturation capacity and the more effective physical protection of heavy textured soil of Castel Volturno, that may favour a slower but time extended incorporation of added compost derivatives.

After four years of SOM managements the evaluation of OC distribution in bulk soils and aggregate fractions at the different project sites revealed an overall increase in TOC in amended plots with exogenous OM with an effective stabilization of SOM, and a large preservation of the added OC.

As example, the amount of OC added with either solid digestate and mature compost in the ploughed horizon of the project sites located in Piemonte may be estimated as follow:

Lower dose (SSB/CMPB) 1000 kg OC yr-1; Higher dose (SSA/CMPA) 2000 kg OC yr-1
Mass of soil held in 1 hectare of the ploughed horizons:
Grandi: 10000 (m2) x 0.20 (m) x 1.5 (tons/m3) = 3000 tons
Tetto frati: 10000 (m2) x 0.30 (m) x 1.45 (tons/m3) = 4350 tons

Lower dose (SSB/CMPB) 1000 kg OC yr-1; Higher dose (SS-A/CMP-A) 2000 kg OC yr-1
Mass of soil held in 1 hectare of the ploughed horizons:
Grandi: 10000 (m2) x 0.20 (m) x 1.5 (tons/m3) = 3000 tons
Tetto frati: 10000 (m2) x 0.30 (m) x 1.45 (tons/m3) = 4350 tons

Theoretical increase of TOC after four years of OM additions:
Grandi: SSB/CMPB 1000 (kg OC) x 4 (years) / 3000 tons = 1.3 kg OC /ton
SSA/CMPA 2000 (kg OC) x 4 (years) / 3000 tons = 2.7 kg OC /ton

Tetto Frati: SSB/CMPB 1000 (kg OC) x 4 (years) / 4350 tons = 0.92 kg OC /ton
SSA/CMPA 2000 (kg OC) x 4 (years) / 4350 tons = 1.84 kg OC /ton

From the data of TOC incorporated in the size aggregates of OM managed plots, compared to the traditional soil it results that from the 65 to about the 90% of added OC was retained in the soil fraction. Similar calculation for the project sites of Castel Volturno and Prima Luce (Fig. 15, 16) result in a overall maintenance of OC that ranged from the 70 to 80%

Although the previous calculation represents only a rough indirect estimation of SOC, these data suggest that the soil addition with humified mature compost have effectively promoted a progressive and fast stabilization of SOC pools

Despite the SOM managements were based on soil addition with humified materials, which are usually characterized by a large C/N ratios, the evaluation of N content revealed an overall maintenance of total nitrogen in the compost amended plots for the project sites of Grandi and Tetto Frati, in respect to soil samples added with mineral fertilizers, while an even slight larger yields were revealed by the N distribution in the SSA and SSB fields; this finding may be related with the inclusion in fresh digestate of a larger amount of bio-labile components, such as peptide moieties, as compared to mature compost

3.3 13C isotopic content

The analyses of isotopic content were performed in the first year of field activities and after the third and fourth distribution of organic materials, for the project sites of Tetto Frati, Grandi and Castel Volturno (Tabs, 1, 2, 3)

The higher isotopic values found in control and organic amended samples in bulk soils and soil aggregates of Tetto Frati (Table 1) highlighted the previous incorporation of 13C labelled OC derived from the long term cultivation of C4 plants (maize) which has provided a natural labelling of SOM pools.

Table 1 13C isotopic ratio (δ) measured in organic materials of control and organic amended field plots of different project sites at 1st year of project activities

Organic materials	Solid digestate -26.7		Digestate Compost -25.3		Manure compost -23.7
Treatments[a]	Bulk soil	Soil aggregates			
		2.00-1.00	1.00-0.50	0.50-0.25	<0.25
TF-Trad	-20.252	/	-19.619	-20.199	-20.694
TF-SSB	-20.406	/	-20.218	-20.218	-20.698
TF-SSA	-20.550	/	-20.220	-20.644	-20.676
TF-CMPB	-20.380	/	-19.857	-20.364	-20.560
TF-CMPA	-20.512	/	-20.061	-20.472	-20.727
G-Trad	-24.650	/	-24.836	-24.609	-24.566
G-SSB	-24.705	/	-25.042	-24.587	-24.603
G-SSA	-24.615	/	-24.804	-24.541	-24.548
G-CMPB	-24.626	/	-24.810	-24.552	-24.563
G-CMPA	-24.598	/	-24.793	-24.518	-24.534
CV-Trad	-25.079	-25.176	-25.099	-25.123	-25.080
CV-CMPB	-25.029	-25.128	-25.043	-25.037	-25.068
CV-CMPA	-24.979	-25.023	-25.019	-25.013	-25.007

a(TF: tetto Frati, G: Grandi, CV: Castel Volturno)

The yield of 13C- OC (% of TOC) deriving from exogenous organic materials in bulk soils, calculated from the isotopic measurements, mostly match the theoretical amounts added with soil treatments (Figs. 17, 18, 19).

In the project site of Tetto Frati the added organic carbon was initially incorporated in soil macro-aggregates for both soil amendments with fresh digestate and compost (Fig. 17). Conversely a more even distribution was found in the project sites of Grandi and Castel Volturno with a fraction of new organic inputs transferred also in the micro-aggregates, even if the13C content of macro-aggregates represented more than the 75% of added organic carbon (Figs 18, 19)

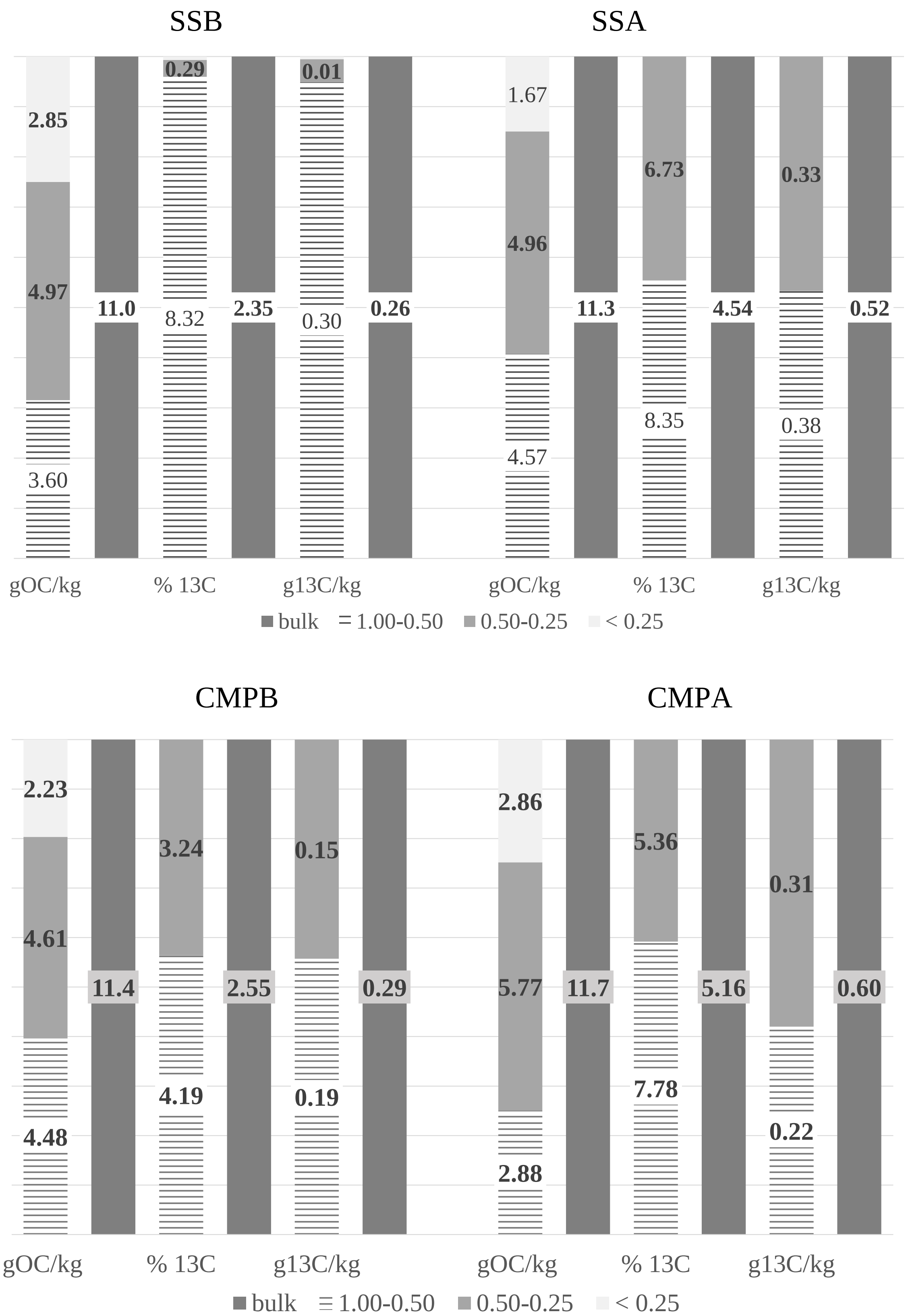


Figure 17 Tetto Frati: 13C-OC content (% and absolute) in bulk soils and aggregates of field amended plots, compared to TOC content (1st year)

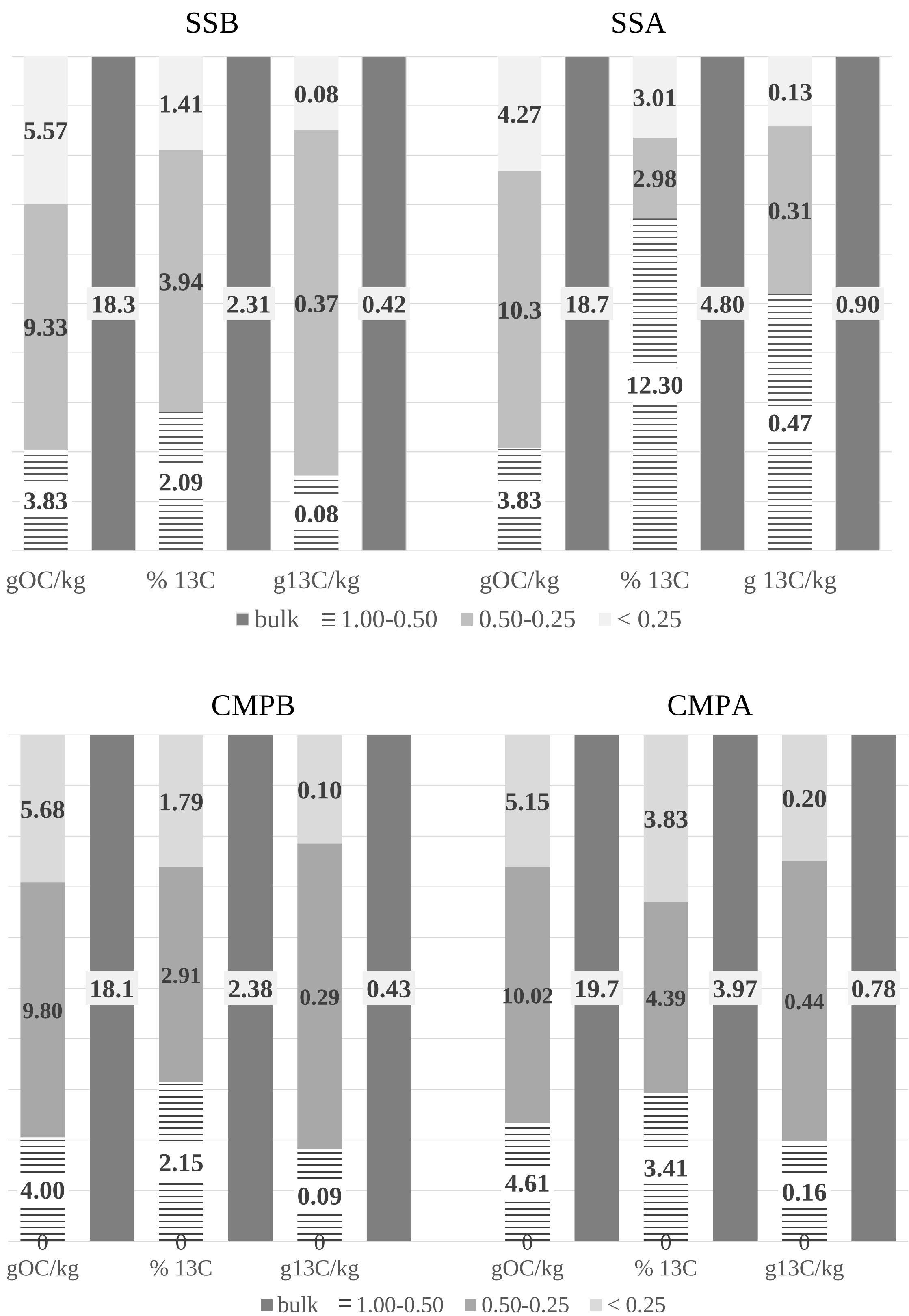


Figure 18 Grandi: 13C-OC content (% and absolute) in bulk soils and aggregates of field amended plots, as compared to TOC content (1st year)

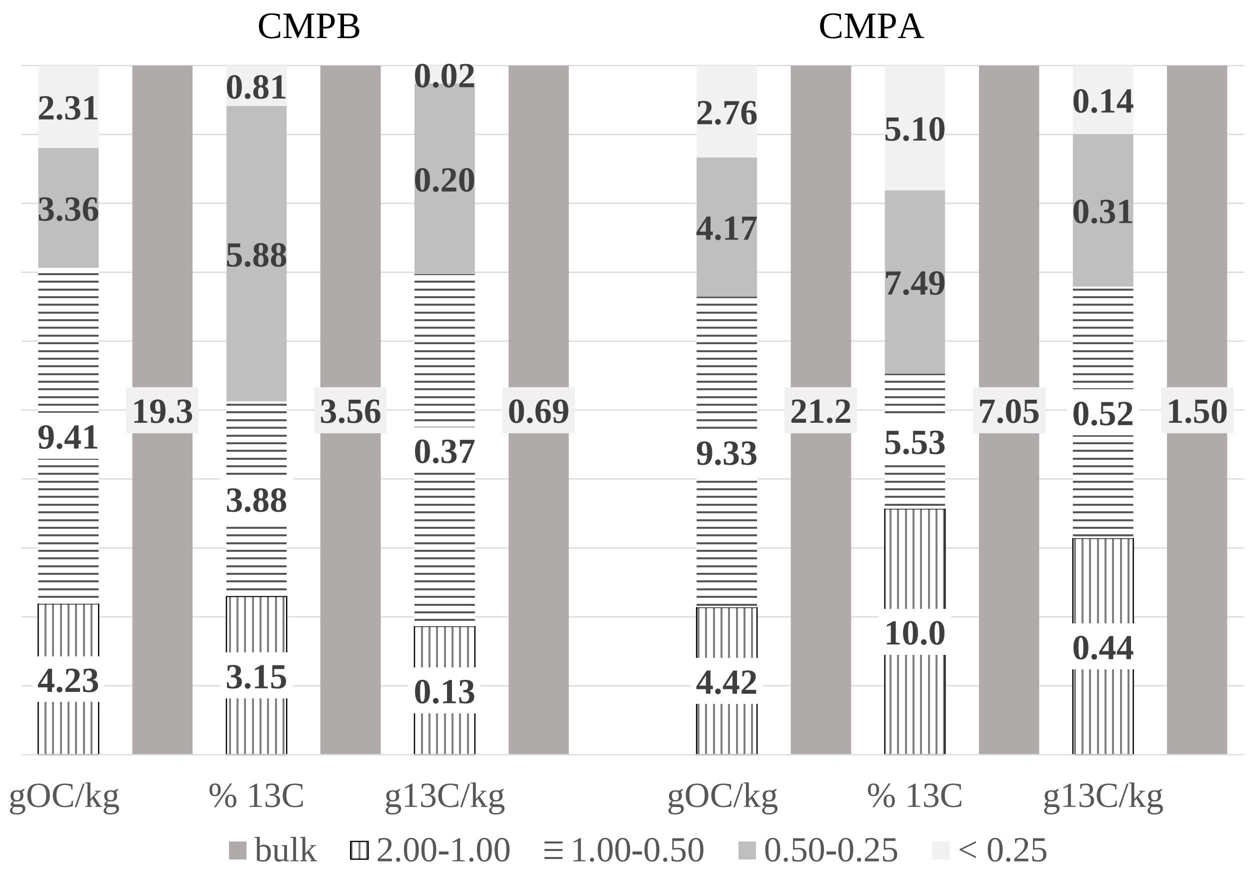


Figure 19 Castel Volturno: 13C-OC content (% and absolute) in bulk soils and aggregates of field amended plots, compared to TOC content (1st year)

The data of 13C-OC isotopic dilution of the last two years of SOM managements (Tables 2, 3, 6) confirmed the progressive incorporation of exogenous materials, in the soil organic compartments. The calculation related to the different project sites revealed a different dynamic of the added materials; the results of Tetto Frati and Grandi (Tables 5, 6) showed that only the 75-85% of OC increase found in compost amended samples (CMPB and CMPA) may be associated to the incorporation of organic derivatives from added composts. In SSB and SSA treatments the fraction of OC derived directly from solid digestate was equal to about the 92-95% of the total OC increase

This difference suggested that the addition of humified organic molecules inherited from compost exerted an additional protection on the annual organic inputs deriving from both crop residues and microbial biomasses, thereby providing a stabilization of biolabile soil organic matter pool, as well as on the original SOM fractions

Table 2 13C isotopic dilution (δ) of soil treatments at Tetto Frati at 3rd and 4th year of SOM managements

Organic materials	Solid digestate -26.7		Digestate Compost -25.3		
Treatments[a]	Bulk soil	Soil aggregates			
		2.00-1.00	1.00-0.50	0.50-0.25	<0.25
3rd year					
TF-Trad	-20.24	/	-19.63	-20.18	-20.67
TF-SSB	-20.77	/	-20.27	-20.49	-21.12
TF-SSA	-20.98	/	-20.55	-20.74	-20.92
TF-CMPB	-20.54	/	-20.12	-20.59	-20.63
TF-CMPA	-20.69	/	-20.11	-20.75	-20.81
4th year					
TF-Trad	-20.25	-20.25	-19.65	-20.20	-20.69
TF-SSB	-20.85	/	-20.35	-20.68	-21.25
TF-SSA	-21.05	-21.22	-20.72	-20.84	-21.12
TF-CMPB	-20.68	-20.70	-20.32	-20.68	-20.85
TF-CMPA	-20.83	-20.77	-20.24	-20.90	-20.90

Table3 13C isotopic dilution (δ) of soil treatments at Grandi at 3rd and 4th year of SOM managements

Organic materials	Solid digestate -26.7		Digestate Compost -25.3		
Treatments[a]	Bulk soil	Soil aggregates			
		2.00-1.00	1.00-0.50	0.50-0.25	<0.25
3rd year					
G-Trad	24.58	-24.61	-24.57	-24.59	-24.49
G-SSB	-24.70	/	-24.64	-24.65	-24.65
G-SSA	-24.68	-24.67	-24.68	-24.62	-24.79
G-CMPB	-24.62	-24.63	-24.60	-24.62	-24.58
G-CMPA	-24.63	-24.65	-24.61	-24.62	-24.61
4th year					
G-Trad	-24.60	-24.62	-24.58	-24.61	-24.58
G-SSB	-24.70	/	-24.69	-24.72	-24.69
G-SSA	-24.78	-24.75	-24.72	-24.65	-24.92
G-CMPB	-24.63	-24.63	-24.62	-24.64	-24.62
G-CMPA	-24.66	-24.67	-24.65	-24.66	-24.66

Table 4 13C-OC distribution in bulk samples and size aggregates in soil treatments of Tetto Frati at 4th year of SOM managements

	SSB			SSA		
	gOC/kg	% ^{13}C	g13C/kg	gOC/kg	% ^{13}C	g13C/kg
bulk	11.25	9.12	1.03	11.77	12.16	1.43
2.00-1.00	/	/	/	0.60	14.75	0.09
1.00-0.50	3.34	10.15	0.34	4.56	15.30	0.70
0.50-0.25	5.00	7.27	0.36	4.74	9.69	0.46
< 0.25	2.94	9.08	0.27	1.73	6.95	0.12
	CMPB			CMPA		
	gOC/kg	% ^{13}C	g13C/kg	gOC/kg	% ^{13}C	g13C/kg
bulk	11.40	8.50	0.97	11.90	11.47	1.37
2.00-1.00	0.69	8.89	0.06	0.65	10.28	0.07
1.00-0.50	2.91	12.35	0.36	3.16	10.94	0.35
0.50-0.25	5.15	9.45	0.49	5.72	13.77	0.79
< 0.25	2.70	3.38	0.09	2.35	4.47	0.11

Table 5 13C-OC distribution in bulk samples and size aggregates in soil treatments of Grandi at 4th year of SOM managements

	SSB			SSA		
	gOC/kg	% ^{13}C	g13C/kg	gOC/kg	% ^{13}C	g13C/kg
bulk	18.51	4.59	0.85	19.77	10.00	1.98
2.00-1.00	/	/	/	0.85	10.48	0.09
1.00-0.50	3.73	4.92	0.18	3.53	8.95	0.32
0.50-0.25	8.49	4.99	0.42	8.23	9.98	0.82
< 0.25	6.27	4.92	0.31	7.27	10.74	0.78
	CMPB			CMPA		
	gOC/kg	% ^{13}C	g13C/kg	gOC/kg	% ^{13}C	g13C/kg
bulk	18.73	6.01	1.13	20.08	10.33	2.08
2.00-1.00	1.01	8.92	0.09	1.16	8.92	0.10
1.00-0.50	3.71	5.61	0.21	4.96	15.43	0.77
0.50-0.25	10.08	5.86	0.59	10.00	7.32	0.73
< 0.25	3.85	5.61	0.22	3.84	11.22	0.43

The 13C-OC analyses of field treatments of Castel Volturno highlighted the effective incorporation of compos-derived organic matter in bulk soil and size aggregates already stressed by the evaluation of TOC content (Table 6). As pointed out for the other project sites, also the data of Castel Volturno indicated that about the 80-85 %the final increase of TOC found in CMPB and CMPA samples was inherited from compost materials (Table 7), thereby further supporting the stabilization effect promoted by the added humified molecules on either original SOM fraction and additional inputs from crop residues

Table 6 13C isotopic dilution (δ) of soil treatments at Castel Volturno at 3rd and 4th year of SOM managements

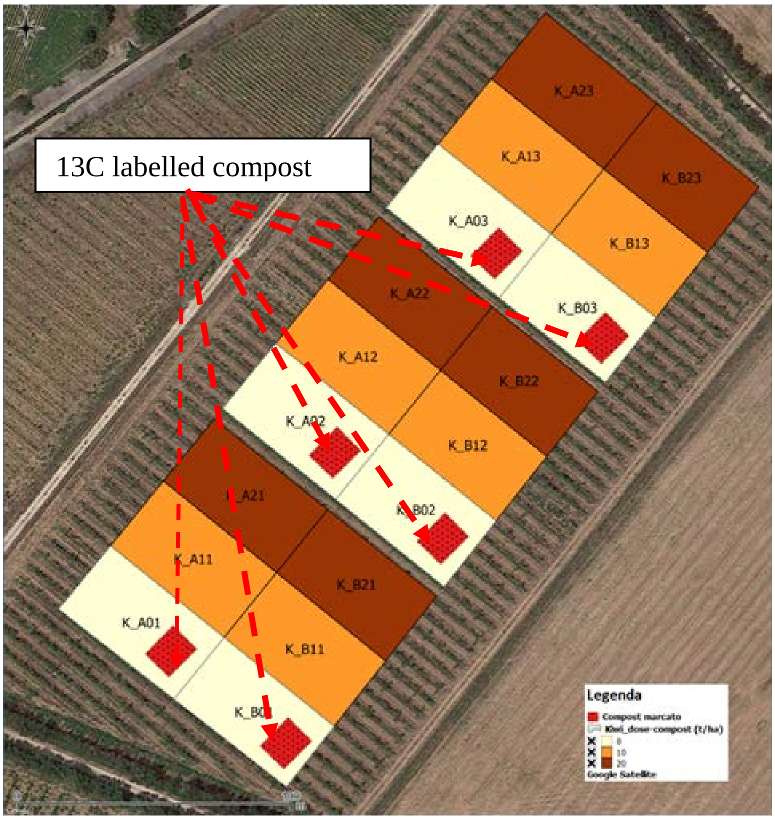
ON farm manure compost -23.7					
Treatments	Bulk soil	Soil aggregates			
		2.00-1.00	1.00-0.50	0.50-0.25	<0.25
3rd year					
CV-Trad	-25.09	-25.20	-25.10	-25.11	-25.10
CV-CMPB	25.00	-25.10	-25.00	-25.02	-25.03
TF-CMPA	24.92	-24.96	-24.97	-24.90	-24.93
4th year					
CV-Trad	-25.08	-25.18	-25.10	-25.12	-25.08
CV-CMPB	-24.97	-25.08	-24.97	-25.00	-25.01
CV-CMPA	-24.87	-24.91	-24.92	-24.84	-24.88

Table 7 13C-OC distribution in bulk samples and size aggregates in soil treatments of Castel Volturno at 4th year of SOM managements

	CMPB			CMPA		
	gOC/kg	% ^{13}C	g13C/kg	gOC/kg	% ^{13}C	g13C/kg
bulk	20.86	7.66	1.60	22.72	14.66	3.33
2.00-1.00	2.95	6.32	0.19	3.14	17.46	0.55
1.00-0.50	10.13	8.91	0.90	12.77	12.36	1.58
0.50-0.25	4.77	8.36	0.40	3.22	19.23	0.62
< 0.25	2.90	4.89	0.14	3.49	13.99	0.49

Mellone

The Figure 20 show the field treatments on Kiwi orchard, while the initial OC content are shown in in Tables 8 and 9



Kiwi	Area m2	Compost t/ha
K_A01	909	0
K_A02	944	0
K_A03	917	0
K_A11	943	10
K_A12	924	10
K_A13	936	10
K_A21	908	20
K_A22	908	20
K_A23	945	20
K_B01	860	0
K_B02	899	0
K_B03	859	0
K_B11	910	10
K_B12	874	10
K_B13	865	10
K_B21	884	20
K_B22	867	20
K_B23	861	20

Figure 20 Filed treatments in kiwi orchard: In each control plots (e.g. A0 and B0) a sub-plot was used for the addition of 13C labelled on farm compost obtained by sorghum (red square in picture)

Table 8 TOC content (%) in initial field orchards*

kiwi 1	1.65	peach 1	1.57
kiwi 2	1.74	peach 2	1.52
kiwi 3	1.66	peach 3	1.57
kiwi 4	1.59	peach 4	1.54
kiwi 5	1.76	peach 5	1.55
kiwi 6	1.81	peach 6	1.68
kiwi 7	1.76	peach 7	1.70
kiwi 8	1.70	peach 8	1.63
kiwi 9	1.78	peach 9	1.61

*The data are referred to the sampling points selected by initial EMI Maps

The date of OC content analysed after two and three year of SOM managements for peach and kiwi fields, respectively, showed that from the 57 to thee 80 % of OC added with on-farm green compost was retained in the bulk soils (Table 9). No differences were found between the two types of compost. This finding suggests that, irrespective with the starting biomasses and the final yields on mature compost, the on-farm composting process provide an effective stabilization of organic components. The extrapolation of the data of TOC content correspond to an increase that ranged from 7 to 16 tons OC ha-1 and for 4 to 10 tons OC ha-1, for kiwi and peach in the order.
Besides the confirmation of the indication obtained on the other project sites, these results are even more reliable and significative since they were related to a large plot extension (about 900m2 for each plot) and on a soil which underwent for a more than 10 years to a sustainable SOM management based on green manure.

Table 9 TOC content (%) in soil treatments of kiwi and peach orchards, after three and two years of SOM managements

Kiwi	Compost ton/ha	C %		Peach	Compost ton/ha	C %
K_A01	0	1.59		P_A01	0	1.78
K_A02	0	1.66		P_A02	0	1.7
K_A03	0	1.69		P_A03	0	1.59
K_A11	10	1.75		P_A11	10	1.885
K_A12	10	1.84		P_A12	10	1.8
K_A13	10	1.82		P_A13	10	1.73
K_A21	20	1.98		P_A21	20	1.97
K_A22	20	1.88		P_A22	20	1.95
K_A23	20	2.01		P_A23	20	1.88
K_B01	0	1.69		P_B01	0	1.65
K_B02	0	1.7		P_B02	0	1.59
K_B03	0	1.75		P_B03	0	1.72
K_B11	10	1.91		P_B11	10	1.86
K_B12	10	1.87		P_B12	10	1.83
K_B13	10	1.96		P_B13	10	1.71
K_B21	20	2.07		P_B21	20	2.00
K_B22	20	1.99		P_B22	20	1.87
K_B23	20	2.02		P_B23	20	1.9

The soil samples amended with on-farm green compost labelled with the addition of sorghum residues, allowed the evaluation of SOC dynamics through the analyses of 13C-OC isotopic dilution (Tables 10, 11). The data of both kiwi and peach orchards indicated that almost the 60 to 80% of added OC was retained within the sup-plots, again without differences between *summer* or *winter* composts. The isotopic analyses revealed that form the 75 to the 85% of total OC increase may be directly inherited from the labelled compost, with a significative preservation of original SOM and/or incorporation of plant residues, root exudates and microbial derivatives. This result further highlights the stabilization potential provided by the humified molecules of on farm green compost.

Table 10 Isotopic dilution (δ13C-OC) of control soil and 13C amended plots after three and two years of soil treatment with sorghum labelled compost on kiwi and peach orchards

	K_A01	K_A02	K_A03	K_B01	K_B02	K_B03
Control	-25.74	-25.82	-25.54	-25.67	-25.5982	-25.78
13C amended sub plots	-25.43	-25.49	-25.24	-25.41	-25.36	-25.44
	P_A01	P_A02	P_A03	P_B01	P_B02	P_B03
Control	-25.87	-25.72	-25.76	-25.8	-25.68	-25.73
13C amended sub plots	-25.59	-25.51	-25.58	-25.61	-25.44	-25.53

Table 11 13C-OC distribution in soil treatments of kiwi and peach orchards (δ13C compost= -22.29)

TOC (%) content control soils A0 =1.69, B0= 1.65						δ13C compost= -22.29		
	K_A01			K_A02			K_A03	
gOC/100g	% 13C	g13C/kg	gOC/100g	% 13C	g13C/kg	gOC/100g	% 13C	g13C/kg
1.84	7.83	0.14	1.81	6.13	0.11	1.79	5.19	0.09
	K_B01			K_B02			K_B03	
gOC/100g	% 13C	g13C/kg	gOC/100g	% 13C	g13C/kg	gOC/100g	% 13C	g13C/kg
1.76	5.42	0.10	1.79	7.09	0.13	1.77	5.82	0.10
TOC (%) content control soils A0 =1.65, B0= 1.71						δ13C compost= -22.29		
	P_A01			P_A02			P_A03	
gOC/100g	% 13C	g13C/kg	gOC/100g	% 13C	g13C/kg	gOC/100g	% 13C	g13C/kg
1.84	7.83	0.14	1.81	6.13	0.11	1.79	5.19	0.09
	P _B01			P_B02			P_B03	
gOC/100g	% 13C	g13C/kg	gOC/100g	% 13C	g13C/kg	gOC/100g	% 13C	g13C/kg
1.76	5.42	0.10	1.79	7.09	0.13	1.77	5.82	0.10

3.4 off line THM-GC-MS

Representative total ion chromatograms (TIC) derived from the thermochemolysis of soil samples from the different project sites of Tetto Frati, Grugliasco, Castel Volturno and Prima Luce are shown in Figure 21 while the compounds identified in the pyrograms are listed in Table 12.

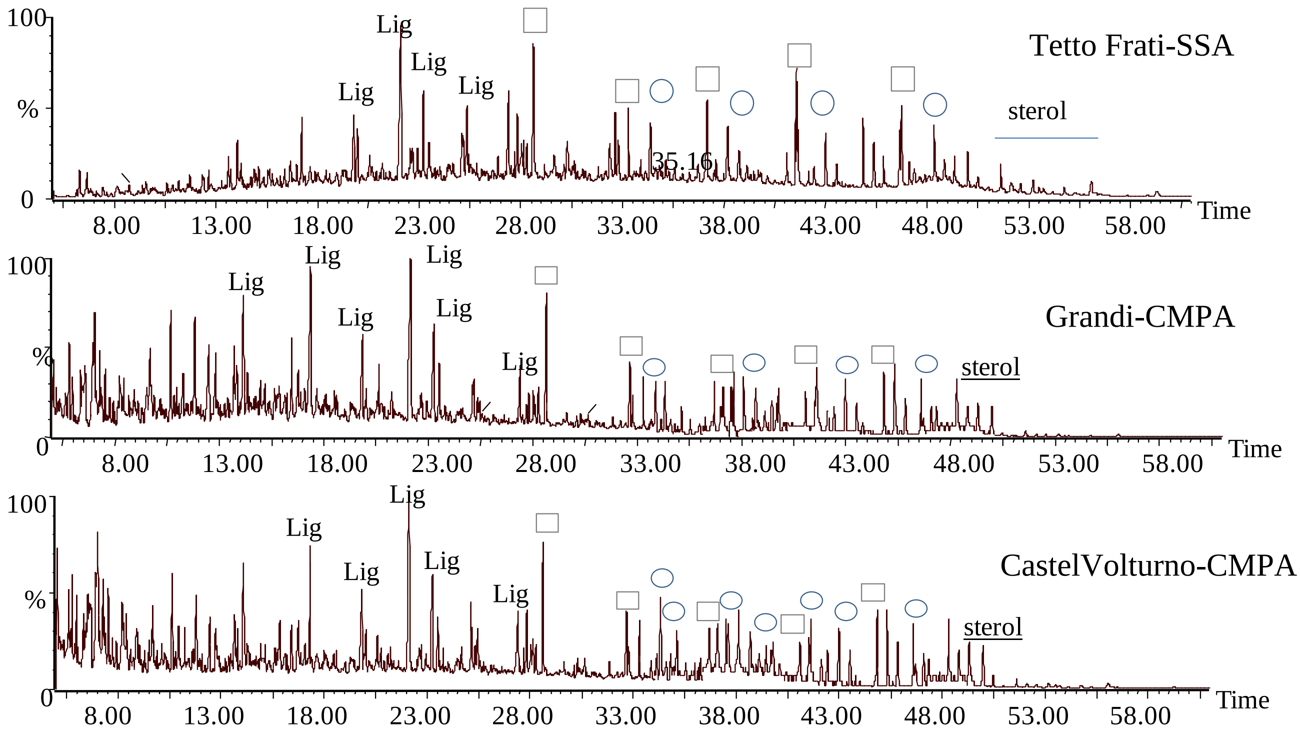


Figure 21 Total ions chromatograms of thermochemolysis products released from soils samples: Lg Lignin, □ Fatty acids, ○ Biopolyesters (alkyl-dioic acids, hydroxyl acids)

The thermochemolysis released more than hundred recognizable different molecules, which were identified as methyl ethers and esters of natural compounds (Table 8). The majority of these compounds originated from higher plants and microbial by-products and was represented by lignin components, fatty acids, aliphatic biopolymers, hydrocarbons and alcohols. The large yield of TAHM GC-MS products enabled a feasible quantitative determination of the organic compounds (Tables 9-12). The lignin monomers released by the field plots are inherited from the structural components which build up the lignified tissues of higher plants. The specific compounds have been determined by the main fragmentation pattern and were associated to the current symbols used to distinguish the different structural units: P=p-hydroxyphenyl, G=guaiacyl (3-methoxy, 4-hydroxyphenyl), and = syringyl (3,5-dimethoxy, 4-hydroxyphenyl). The lignin molecules found in soil samples (Table 12) indicated the presence of, both, fresh decaying plant residues and that of microbial processed organic materials. The latter derivatives included the oxidized products of both di- and tri-methoxy phenylpropane molecules, with the aldehydic (G4, S4), ketonic (G5, S5) and benzoic-acid (G6, S6) forms as main components.

Table 12 List of thermochemolysis products[a] released from soil treatments

RT[a]	assignment [b]	characteristic ions m/z
5.4	1-Methyl 4-CH_3O Benzene *Lg P2*	77, 91, 107 M^+122
8.5	Benzene, 1,2-di CH_3O *Lg G1*	77, 95, 123 M^+138
8.8	Carbohydrate derivative	88, 101, 130 M^+nd
11.9	Carbohydrate derivative	88, 101, 130 M^+nd
12.5	Carbohydrate derivative	101, 129, 161 M^+nd
12.7	1,3,5-tri CH_3O Benzene	125, 153 M^+168
13.1	C10 Fame	74, 87, 155 M^+ 186
13.6	N Compound	98 M^+nd
14.5	1,2,3-tri CH_3O Benzene *Lg S1*	110, 125, 153 M^+168
14.6	Benzoic Acid, 4- CH_3O ME *Lg P6*	77, 92, 135 M^+166
14.9	Carbohydrate derivative	88, 101, 143, 175 M^+nd
15.1	Carbohydrate derivative	88, 101, 143, 175, 188 M^+nd
17.2	Carbohydrate derivative	88, 101, 130 M^+nd
17.6	Benzaldehyde, 3,4-di CH_3O *Lg G4*	151, 165 M^+166
17.7	Carbohydrate derivative	88, 101, 175 M^+nd
18.2	Carbohydrate derivative	101, 129, 161 M^+nd
18.5	Carbohydrate derivative	101, 129, 161 M^+nd
18.8	C12 FAME	74, 87, 183 M^+ 214
19.9	3,4 di CH_3O Acetophenone *Lg G5*	137, 165, M^+180
20.7	Benzoic Acid, 3,4-di CH_3O, ME *Lg G6*	165, 181, M^+196
20.9	Benzaldehyde, 3,4,5-tri CH_3O *Lg S4*	125, 181, M^+196
21.6	cis -2-(3,4-di CH_3O phenyl)-1-CH_3O ethylene *Lg G7*	151, 179 M^+194
21.9	trans -2-(3,4-di CH_3O phenyl)-1- CH_3O ethylene *Lg G8*	151, 179, M^+194
22.2	cis-1-Methoxy-1-(3,4-di CH_3O phenyl)-1-Propene *Lg G10*	165, 193 M^+208
23.0	trans -3-(4-CH_3O phenyl)-3-Propenoic acid ME *Lg P18*	133, 161 M^+ 192
23.0	trans-1-Methoxy-1-(3,4-di CH_3O phenyl)-1-Propene *Lg G11*	165, 193 M^+208
23.9	N compound	98 M^+nd
24.1	Benzoic Acid, 3,4,5-Trimethoxy ME *Lg S6*	195, 211 M^+226
24.3	C14 FAME	74, 87, 211 M^+ 242
24.6	trans-3- CH_3O -1-(3,4-di CH_3O phenyl)-1-Propene *Lg G13*	91, 177 M^+208
25.5	cis-1-(3,4,5-tri CH_3O phenyl)-1- CH_3O ethylene *Lg S10*	195, 223 M^+238
25.9	Thr/Eryth-1-(3,4-di CH_3O phenyl) -1,2,3-tri CH_3O propane *Lg G14*	166, 181 M^+270
25.9	C15 *iso* FAME *Mic-PLFA*	74, 87, 213 M^+256
26.0	trans-1-(3,4,5-tri CH_3O phenyl)-1- CH_3O ethylene *Lg S11*	195, 223 M^+238
26.1	C15 *anteiso* FAME *Mic-PLFA*	74, 87, 213 M^+256
26.2	Thr/Eryth.-1-(3,4-di CH_3O phenyl) -1,2,3-tri CH_3O propane *Lg G15*	166, 181 M^+270
26.5	cis-1- CH_3O-1-(3,4,5-tri CH_3O phenyl)-1-Propene *Lg S13*	91, 207 M^+238
26.8	C15 *n*-FAME	74, 87, 225 M^+256
28.3	2-Propenoic Acid, 3-(3,4-di phenyl)-, ME *Lg G18*	191, 207 M^+222
28.4	C16 *iso* FAME *Mic-PLFA*	74, 87, 227 M^+270
28.6	Thr./Eryth-1-(3,4,5-tri CH_3O phenyl) -1,2,3-tri CH_3O propane *Lg S14*	181, 211 M^+300
28.8	C16:1 FAME *Mic-PLFA*	55, 74, 236 M^+268

Table 12 continue

RT[a]	assignment[b]	characteristic ions m/z
28.9	Thr./Eryth-1-(3,4,5-tri CH_3O phenyl) -1,2,3-tri CH_3O propane *Lg S15*	181, 211,M^+300
29.0	C16:1 FAME	55, 74, 236 M^+268
29.4	C16 FAME	55, 74, 239 M^+270
30.4	7 methyl C16FAME *Mic-PLFA*	74, 87, 157 M^+284
30.6	Carbohydrates derivative	88, 101, 115, 175, 235 M^+nd
30.7	cis-3-(3,4,5-tri CH_3O phenyl-3-Propenoic acid ME *Lg S18*	221, 237 M^+252
30.8	C17 *iso* FAME *Mic-PLFA*	74, 87, 241 M^+284
31.0	C17 *anteiso* FAME *Mic-PLFA*	74, 87, 227 M^+284
31.2	trans-3-(3,4,5-tri CH_3O phenyl-3-Propenoic acid ME *Lg S18*	221, 237 M^+252
31.4	C17 cy FAME *Mic-PLFA*	55, 69, 250 M^+282
31.6	cis-1-(3,4,5-tri CH_3O phenyl) -1,3-di CH_3O prop-1-ene *Lg S16*	206, 237 M^+268
31.8	C17 *n*-FAME	74, 87, 253 M^+284
33.4	C18 *iso* FAME *Mic-PLFA*	74, 87, 253 M^+298
33.5	C18:1 FAME	55, 69 264 M^+296
34.0	C18 FAME	74, 87 267 M^+298
34.6	16, 16-CH_3O, FAME	55, 74, 268, 285 M^+300
34.9	C18, 10 CH_3O, FAME	55, 69, 183, 215, 297 M^+328
36.3	Carbohydrates derivative	88, 101, 187, 219, 423 M^+nd
36.7	C16 Dioic Acid DIME	74, 98, 241, 283 M^+314
37.4	C16, 8(9,10)-16 diCH_3O, FAME	71, 95, 87, 201, 215 M^+330
37.9	Carbohydrates derivative	88, 101, 187, 279, 423 M^+nd
38.1	C18:1, 18 CH3O, FAME	55, 67, 81, 262, 294 M^+326
38.3	C20 FAME	74, 87, 295 M^+326
38.8	Carbohydrates derivative	88, 101, 187, 219, 359 M^+nd
39.8	C24 Alkane	57, 71, 85, M^+338
40.2	Carbohydrates derivative	88, 101, 111, 187, 391 M^+nd
40.3	C21 FAME	74, 87, 309 M^+340
40.6	C22-CH_3O	57, 69, 83, 318, M^+340
40.8	C18 Dioic acid DIME	74, 98, 241, 311 M^+342
41.1	Carbohydrates derivative	88, 101, 145, 187, 279 M^+nd M^+nd
41.7	C25 Alkane	57, 71, 85, M^+352
42.3	C22 FAME	74, 87, 323 M^+354
42.5	Carbohydrate derivative	88, 101, 187, 219, 279, 423 M^+nd
42.6	Carbohydrate derivative	88, 101, 111, 187, 391 M^+nd
42.8	C20, 20 CH_3O, FAME	55, 74, 292, 324, M^+356
43.1	C18 9, 10, 18 tri CH_3O FAME	71, 81, 169, 187, 201 M^+388
43.6	C26 Alkane	57, 71, 85, M^+366
44.2	C23 FAME	74, 87, 337 M^+368
44.4	C24-CH_3O	57, 69, 83, 346, M^+368
44.6	C22, 2 CH_3O, FAME *Mic*	57, 71, 97, 325 M^+384
45.0	C20 Dioic Acid DIME	74, 98, 241, 339 M^+370

Table 12 continue

RT[a]	assignment[b]	characterisitc ions m/z
45.4	C27 Alkane	57, 71, 85, M^+380
46.0	C24 FAME	74, 87, 351 M^+382
46.2	C23, 2 CH_3O, FAME *Mic*	57, 71, 97, 339 M^+398
46.5	C22, 22 CH_3O, FAME	55, 74, 320, 352 M^+384
47.2	C28 Alkane	57, 71, 85, M^+394
47.4	squalene	69, 81, 136, 341 M^+410
47.7	C25 FAME	74, 87, 365 M^+396
47.9	C26-CH_3O	57, 69, 83, 364, M^+396
48.0	C24, 2 CH_3O, FAME *Mic*	57, 71, 97, 353 M^+412
48.2	C22 Dioic acid DIME	74, 98, 241, 367 M^+398
48.9	C29 Alkane	57, 71, 85, M^+408
49.4	C26 FAME	74, 87, 379 M^+410
49.6	C25, 2 CH_3O, FAME *Mic*	57, 71, 97, 367 M^+426
49.9	C24, 24 CH3O, FAME	55, 74, 348, 380 M^+412
50.5	C30 Alkane	57, 71, 85, M^+422
51.0	C28-CH_3O	57, 69, 83, 392, M^+424
51.2	C26, 2 CH_3O, FAME *Mic*	57, 71, 97, 381 M^+440
51.5	C24 Dioic acid DIME	74, 98, 241, 395 M^+426
51.7	Phytosterol (tetracyclic)	213, 255, 289, 382 M^+414
52.1	C31 Alkane	57, 71, 85, M^+426
52.4	Phytosterol (tetracyclic)	213, 255, 329, 396 M^+428
52.6	C28 FAME	74, 87, 407 M^+438
52.8	Phytosterol (tetracyclic)	213, 273, 329, 396 M^+428
53.1	C26, 26 CH3O, FAME	55, 74, 376, 408 M^+440
53.4	Phytosterol (tetracyclic)	215, 233, 257, 398 M^+430
53.7	Triterpenol (pentacyclcic)	189, 203, 262 M^+nd
54.0	C30-CH_3O	57, 69, 83, 420, M^+452
54.2	Triterpenol (pentacyclcic)	5, 218, 275, 410 M^+442
54.5	Triterpenol (pentacyclcic)	204, 218, 301, 316 M^+440
55.0	Triterpenol (pentacyclcic)	204, 248, 394 M^+454

a. RT = Retention Time (minutes)

b. cy=cyclopropane; CH_3O = Methoxy; DIME = dimethyl ester; FAME= fatty acid methyl ester; Lg=lignin; ME = methyl ester; Mic=microbial; nd=not determined

Conversely the concomitant release from the thermochemolysis of soil samples, of 1-(3,4-dimethoxyphenyl)-1(3)-methoxy-propene (G10/11, G13) and 1-(3,4,5-trimethoxyphenyl)- 1(3)-methoxy-propene (S10/11, S13), as either cis or trans isomers (Table 8), may be related to the incorporation on SOM of slightly decomposed plant debris.

Moreover, the identification of the enantiomers of 1-(3,4-dimethoxyphenyl)-1,2,3-trimethoxypropane (G14 and G15) and 1-(3,4,5- trimethoxyphenyl)-1,2,3-trimethoxypropane (S14 and S15), confirmed the persistence of not decomposed lignified plant tissues. The aldehydic and acidic forms of guaiacyl and syringyl structures result from the progressive oxidation of lignin monomers, while the corresponding homologues holding methoxylated side chains are indicative of unaltered lignin components, which retain the propyl ether intermolecular linkages. The Ad/Al index is the ratio of peak areas of acidic structures over those of the corresponding aldehydes (G6/G4, S6/S4), while the Γ index is the ratio of peak areas of acidic structures over the sum of peak areas for the threo/erythro isomers (Γ_G = G6/[G14+G15]; Γ_S = S6/[S14+S15]). Both these indices are considered useful indicators of the bio-oxidative transformation of lignin components. The overall larger values found in the initial samples for the majority of the structural indexes (Tab. 3) indicated the prevalence of decomposed lignin monomers. Among the last eluted lignin monomers, the 3-(4,5-dimethoxyphenyl)-2-propenoic (G18) and the 3-(3,4,5-trimethoxyphenyl)-2-propenoic (S18) acid forms, may have originated from either the side chain oxidation of guaiacyl and siringyl units or from the partial decomposition of aromatic domains of suberin biopolymers in plant tissues.

The various alkyl molecules found in the pyrograms, were mainly composed by aliphatic and alicyclic lipid compounds of plant and microbial origin (Table 12). The most abundant compounds were the methyl ester of linear fatty acids, dominated by the hexadecanoic and octadecanoic saturated and unsaturated homologues. Notwithstanding the multiple possible origins of the C16 and C18 acids, the predominance of even carbon atoms, indicated the plant waxes as prevalent source of the straight chain aliphatic acids. These compounds may derive from the breakdown of long chain ester as well as from the terminal oxidation of other components such as linear hydrocarbons and aliphatic alcohols. The prevailing role of plant input in soil lipid composition was also suggested by the detection of the C24, C26 and C28 aliphatic alcohols (Table 12), which are common components of wax layer of non-lignified tissues. This finding was confirmed by the observed distribution of long-chain hydrocarbons (Table 12), marked by the peculiar prevalence of heavier odd-numbered alkanes. The off-line pyrolysis, produced also a notable yield of the methylated form of ω-hydroxy alkanoic acids and alkan-dioic acids (Tables 13, 14, 15, 16).

These molecules are the main constituents of the external protective barriers of fresh and lignified plant tissues, namely cutin and suberin. No clear predominance of particular monomer was revealed by both of these compound classes, which instead showed an almost uniform distribution of even carbon-numbered long chain components (Table 12). The 9,16-/10,16-dihydroxyhexadecanoic isomers, and the 9,10 epoxide 18 hydroxy-octadecanoic (Table 12) acid were the most abundant representative monomers of mid-chain hydroxyl acids, structural units of plant cuticles, frequently used also as plant biomarkers. The relatively least abundant lipid compounds were the high molecular weight tetra- and pentacyclic triterpenes. The sterol and triterpenol molecules have been tentatively identified as methyl ethers and esters of both methyl/ethyl cholesten-3-ol structures, and of ursane, lupeane and oleanane derivatives that are characteristic lipid components of aerial and root plant tissues.

The contribution of microbial input to soil lipids was shown by the inclusion of various structural components of microbial cells, such as phospho-lipid fatty acids (PLFA) and 2-hydroxy aliphatic acids (Table 8). The most representative PLFA monomers were, in order of elution, the 12- and 13-methyl tetradecanoic (iso/anteiso pentadecanoic), the 14- and 15-methyl hexadecanoic (iso/anteiso heptadecanoic) acids and the cyclopropane-(2-hexyl)-octanoic acid (C17 cy FAME), which are common microbial constituents of natural organic matter in soil and sediments.

A relative lower amount of carbohydrates derivatives was found among the pyrolysis products of the field management from Torino soil. This finding has been related to the lower efficiency of off-line pyrolysis techniques to detect carbohydrate units of polysaccharides in complex matrices. The thermal behaviour and pyrolitic rearrangement of poly-hydroxy compounds combined with the basic reaction condition of TMAH reagent solution, are believed to negatively interfere in the release of polysaccharides. However, despite the expected low response of carbohydrates, various methylated forms of mono- and oligo-saccharides components were still found among thermochemolysis products. These compounds may be mainly associated to xylans and cellulose moieties of coarse ligno-cellulosic debris of plant residues.

The soil addition with organic materials produced an overall increase in the yields of both aliphatic and lignin components in all the project sites, thereby confirming the incorporation of exogenous OC in soil samples (Tables 13-16). Moreover, the inclusion of stabilized OM was indicated by the progressive decrease of structural indices (Ad/Al, Γ) associated with lignin monomers in compost amended plots. This finding further highlights the modification of SOM quality promoted by the soil treatments

Table 13 Composition[a] and yields (μg g^{-1}) of main TAHM products released from the field plots of Tetto Frati project site

	Compounds	t0	Trad	0N	FeP	SSB	SSA	CMPB	CMPA
					1st year				
Lipids	Fatty acids C_{12}÷C_{28}	3063	3040	2970	3110	3150	3250	3190	3210
	Microbial (%)	7	6	6	5	8	9	8	10
	Mid-chain hydroxy acids	172	164	148	154	190	215	210	220
	ω-Hydroxy acids C_{16}÷C_{22}	263	255	266	247	282	291	270	314
	Alkanes C_{25}÷C_{31}	89	78	65	95	85	95	110	100
	Alcohols C_{16}-C_{26}	120	125	115	119	125	125	132	135
	Phytosterols	42	35	40	50	60	65	70	80
	Total lipids	3749	3692	3598	3768	3890	4040	3982	4055
Lignin	Guaiacyl	127	130	125	140	150	180	195	210
	$(Ad/Al)_G$[b]	4.4	4.3	4.5	4.7	4.0	4.1	4.1	4.0
	(Γ_G)[b]	3.0	3.1	2.8	3.1	2.8	2.8	2.8	2.7
	p-Hydroxyphenyl	64	55	67	60	70	58	65	72
	Syringyl	174	190	185	170	164	190	200	210
	$(Ad/Al)_S$[b]	3.5	3.4	3.1	3.6	3.0	3.2	3.2	3.1
	(Γ_S)[b]	3.8	3.7	4.0	3.9	3.6	3.5	4.0	3.7
	Total lignin	365	375	377	370	384	428	460	492
					4th year				
Lipids	Fatty acids		2850	2980	3650	3375	3413.2	3246	3876
	Microbial (%)		6	7	11	11	14	12	13
	Mid-chain hydroxy acids		130	115	210	179	235	228	275
	ω-Hydroxy acids		205	195	228	280	352	298	360
	Alkanes C_{25}÷C_{31}		85	34	110	98	112	105	145
	Alcohols C_{16}-C_{26}		110	80	135	118	150	128	149
	Phytosterols		28	25	69	50	75	78	90

Lignin	Total lipids	3408	3429	4402	4100.0	4337	4083	4895
	Guaiacyl	115	98	205	148	187	195	320
	$(Ad/Al)_G$[b]	4.3	4.4	3.7	3.6	3.5	3.2	3.2
	(Γ_G)[b]	3.7	4.1	3.1	2.9	2.7	3.0	2.7
	p-OH phenyl	60	58	92	85	115	75	98
	Syringyl	175	128	210	198	224	235	253
	$(Ad/Al)_S$[b]	3.4	3.7	3.0	2.9	2.9	3.0	2.7
	(Γ_S)[b]	3.9	4.0	3.2	3.2	3.3	3.1	3.0
	Total lignin	350	284	507	431	526	505	671

[a] Total range varying from Ci to Cj; Structural indexes: $(Ad/Al)_G$=G6/G4; $(Ad/Al)_S$=S6/S4; (Γ_G)=G6/(G14+G15); (Γs)=S6/(S14+S15).

Table 14 Composition[a] and yields (μg g^{-1}) of main TAHM products released from the field plots of Grandi project site

	Compounds	t0	Trad	0N	SSB	SSA	CMPB	CMPA
				1st year				
Lipids	Fatty acids	4465	4574	4481	4520	4583	4496	4523
	Microbial (%)	9.5	9	8	8.5	9	10	9
	Mid-chain hydroxy	351	344	352	365	359	372	362
	ω-Hydroxy acids $(C_{16}\div C_{22})$	288	274	285	310	304	324	335
	Alkanes$(C_{25}\div C_{31})$	95	85	87	92	102	87	93
	Alcohols C_{16}-	75	70	68	79	82	72	75
	Phytosterols	92	85	87	95	103	110	114
	Total lipids	5366	5432	5360	5461	5533	5461	5502
Lignin	Guaiacyl	195	207	175	200	210	214	205
	$(Ad/Al)_G$[b]	3.2	3.3	3.4	3.5	3.1	3.3	3.4
	(Γ_G)[b]	3.4	4.0	3.7	3.4	3.2	3.3	3.4
	p-Hydroxyphenyl	181	154	163	199	212	214	226
	Syringyl	164	171	175	181	189	190	195
	$(Ad/Al)_S$[b]	4.0	3.9	3.8	3.9	3.8	4.1	3.9
	(Γ_S)[b]	3.2	3.1	3.3	3.1	3.0	3.3	2.9
	Total lignin	540	532	513	580	611	618	626
				4th year				
Lipids	Fatty acids		4135.	3955	5036.419	5385.804	5078.733	5561.16
	Microbial (%)		7	6	12	13	15	13
	Mid-chain		278	265	408.4174	450.8772	434.0798	490.05
	ω-Hydroxy acids		274	245	338.5278	357.8764	360.6201	405.35
	Alkanes$(C_{25}\div C_{31})$		82	59	103.7424	114.1908	120.2067	133.1
	Alcohols C_{16}-		65	64	92.82213	94.178	102.3983	127.05
	Phytosterols		80	75	103.7424	123.6086	127.9979	133.1
	Total lipids		4914.	4663	6083.671	6526.535	6224.036	6849.81

Lignin	Guaiacyl	190	165	227	265	253	281
	$(Ad/Al)_G$[b]	3.4	3.6	3.2	3.1	3.2	3.1
	(Γ_G)[b]	3.9	3.8	3.3	3.2	3.1	3.2
	p-Hydroxyphenyl	176	152	225	273	273	310
	Syringyl	165	155	206	231	216	248
	$(Ad/Al)_S$[b]	3.6	3.7	3.2	3.0	2.9	3.1
	(Γ_S)[b]	3.8	3.7	3.1	2.8	2.7	3.2
	Total lignin	531	472	658	769	741	839

Total range varying from Ci to Cj; Structural indexes: $(Ad/Al)_G$=G6/G4; $(Ad/Al)_S$=S6/S4; (Γ_G)=G6/(G14+G15); (Γs)=S6/(S14+S15).

Table 15 Composition[a] and yields ($\mu g\ g^{-1}$) of main TAHM products released from the field plots of Castel Volturno project site

	Compounds	t0	Trad	FeP	CMPB	CMPA
	1st year					
Lipids	Fatty acids $C_{12}÷C_{28}$	5475	5165	5395	5585	5546
	Microbial (%)	11	10	10.5	11	10.5
	Mid-chain hydroxy acids(C_{16}, C_{18})	236	302	286	284	302
	ω-Hydroxy acids ($C_{16}÷C_{22}$)	410	394	422	411	425
	Alkanes($C_{25}÷C_{31}$)	148	152	133	158	163
	Alcohols C_{16}-C_{26})	230	190	247	212	225
	Phytosterols	105	98	95	125	142
	Total lipids	6604	6301	6578	6775	6803
Lignin	Guaiacyl	220	212	192	254	267
	$(Ad/Al)_G$[b]	4.1	3.9	3.9	4.0	3.9
	(Γ_G)[b]	3.6	3.7	3.8	3.5	3.7
	p-Hydroxyphenyl	222	234	215	235	247
	Syringyl	240	211	197	251	265
	$(Ad/Al)_S$[b]	4.0	4.0	3.9	3.9	3.9
	(Γ_S)[b]	3.4	3.3	3.5	3.3	32
	Total lignin	682	657	604	740	779
	4th year					
Lipids	Fatty acids $C_{12}÷C_{28}$		4948	5198	5988	63191
	Microbial (%)		7	9	12	14
	Mid-chain hydroxy		284	309	347	357
	ω-Hydroxy acids		299	435	423	472
	Alkanes($C_{25}÷C_{31}$)		128	179	160	185
	Alcohols C_{16}-C_{26})		148	207	247	267
	Phytosterols		74	100	134	164

	Total lipids	5889	6435	7311	7779
Lignin	Guaiacyl	170	221	292	326
	$(Ad/Al)_G$[b]	3.9	3.7	3.4	3.3
	(Γ_G)[b]	3.8	3.5	3.2	3.2
	p-Hydroxyphenyl	189	215	267	317
	Syringyl	164	221	302	343
	$(Ad/Al)_S$[b]	4.0	3.7	3.5	3.4
	(Γ_S)[b]	3.8	3.6	3.1	2.9
	Total lignin	523	656	861	986

Total range varying from Ci to Cj; Structural indexes: $(Ad/Al)_G$=G6/G4; $(Ad/Al)_S$=S6/S4; (Γ_G)=G6/(G14+G15); (Γs)=S6/(S14+S15).

Table 16 Composition[a] and yields (μg g^{-1}) of main TAHM products released from the field plots of Prima Luce project site

	Compounds	t0	A	B	C	D
		1 year				
Lipids	Fatty acids C_{12}÷C_{28}	2468	2797	2820	3210	3228
	Microbial (%)	8	9.2	9.2	8.9	9.3
	Mid-chain hydroxy	118	96	95	196	244
	ω-Hydroxy acids (C_{16}÷C_{22})	210	158	142	285	297
	Alkanes(C_{25}÷C_{31})	84	102	98	172	194
	Alcohols C_{16}-	125	118	114	174	195
	Phytosterols	95	89	108	115	134
	Total lipids	3100	3360	3377	4152	4292
Lignin	Guaiacyl	185	198	175	227	218
	$(Ad/Al)_G$[b]	5.1	4.9	4.8	4.6	4.7
	(Γ_G)[b]	4.2	4.3	4.0	4.1	4.0
	p-Hydroxyphenyl	148	175	164	192	210
	Syringyl	140	177	157	235	248
	$(Ad/Al)_S$[b]	4.1	3.9	4.1	4.0	3.8
	(Γ_S)[b]	3.7	3.8	4.0	3.8	3.6
	Total lignin	473	550	496	654	676
		4th year				
Lipids	Fatty acids		2297	2417	3528	3916
	Microbial (%)		8	9	11	13
	Mid-chain		88	77	228	296
	ω-Hydroxy acids		130	120	314	376
	Alkanes(C_{25}÷C_{31})		88	100	186	236
	Alcohols C_{16}-		110	88	191	285

	Phytosterols	103	91	138	169
	Total lipids	2823	2901	4596	5290
Lignin	Guaiacyl	164	175	270	334
	$(Ad/Al)_G$[b]	4.2	4.4	3.8	4.0
	(Γ_G)[b]	4.0	4.3	3.7	3.6
	p-Hydroxyphenyl	180	152	244	301
	Syringyl	173	158	258	323
	$(Ad/Al)_S$[b]	4.8	4.0	3.4	3.2
	(Γ_S)[b]	4.2	3.9	3.3	3.4
	Total lignin	517	485	771	958

Total range varying from Ci to Cj; Structural indexes: $(Ad/Al)_G$=G6/G4; $(Ad/Al)_S$=S6/S4; (Γ_G)=G6/(G14+G15); (Γs)=S6/(S14+S15)

3.4 Biological analyses

3.4.1 PLFA: Project sites Tetto Frati, Grandi, Castel Volturno

The Table 17 report the main PLFA associated with the different microbial communities

Table 17 Main PLFA biomarkers associated with different microbial groups

Microbial	Fatty acid
GRAM+ bacteria	a15:0
GRAM+ bacteria	15:00
GRAM+ bacteria	i16:0
GRAM+ bacteria	a16:0
GRAM- bacteria/FUNGI	16:1w7c
ARBUSCULAR MYCORRHYZA	16:1w5c
GRAM- bacteria	cy16:0
ACTINOMYCES	10Me16:0
GRAM+ bacteria	i17:0
GRAM+ bacteria	a17:0
GRAM- bacteria	cy 17:0
GRAM+ bacteria	17:00
FUNGI	18:2 w 6,9
FUNGI	18:1w9c

Following the SOM managements, the released PLFA showed significant increase of total biomass in all field plots added with different organic materials (Figs 22, 23, 24). A large initial improvement of total biomass was found for the project site Grandi in the soil treatments with fresh solid digestate (SSB, SSA). This result may be related to the larger presence of bioavailable components (e.g. peptides, carbohydrates) in fresh organic residues in respect to stable humified composts, which can easily support the development of microbial communities. Although the soil addition with decomposable materials may have negative effect of the overall SOC stability (priming effect) the results of SOC analyses on SSB and SSA treatments did not show any evidence of detrimental response on SOC thereby suggesting the mutual beneficial effect on the different SOC pools. A steady improvement of microbial biomass was shown in the three project sites with the additional amendment of organic exogenous materials (Fig, 22, 23, 24). Moreover, the soil treatments with both digestate and mature compost promoted also an increase of the NLFA associated with the arbuscular mycorrhyza fungi (AMF) that represent important symbiotic organisms for the mobilisation of the available fraction of soil phosphorus. In particular a favourable integration of AMF with plant roots have been related with the larger content of stable molecular component of SOM. This finding on AMF development may hence be partially associated with the increase on alkyl and lignin hydrophobic molecules highlighted by SOM characterization for the different project sites following SOM managements.

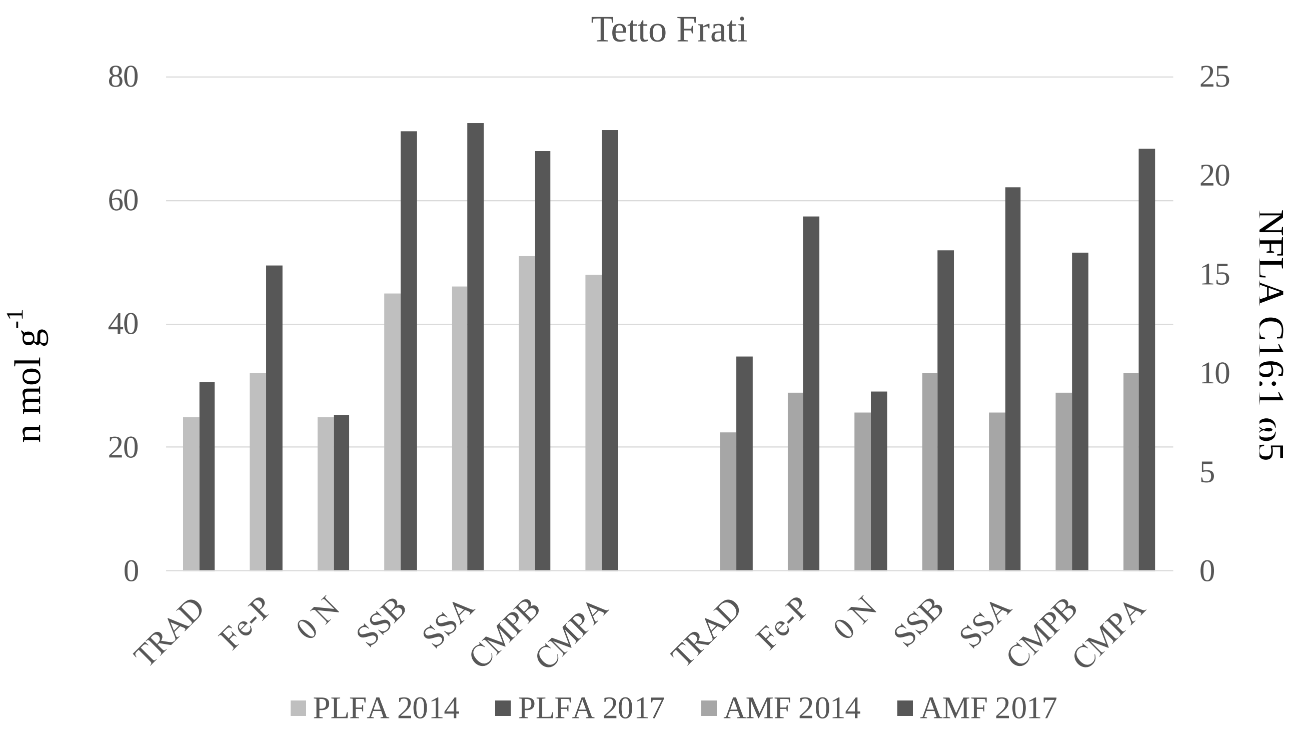


Figure 22 Total PLFA and AMF-NLFA released by soil treatments of Tetto Frati

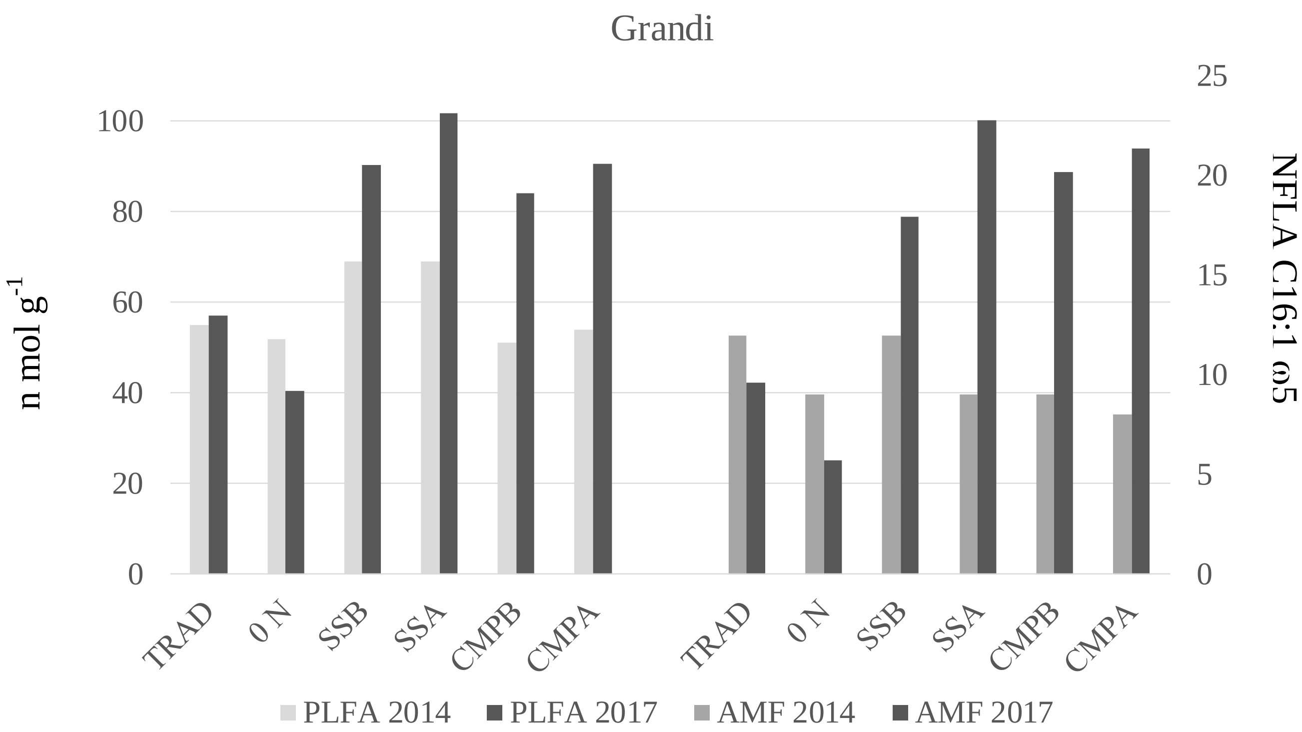


Figure 23 Total PLFA and AMF-NLFA released by soil treatments of Grandi

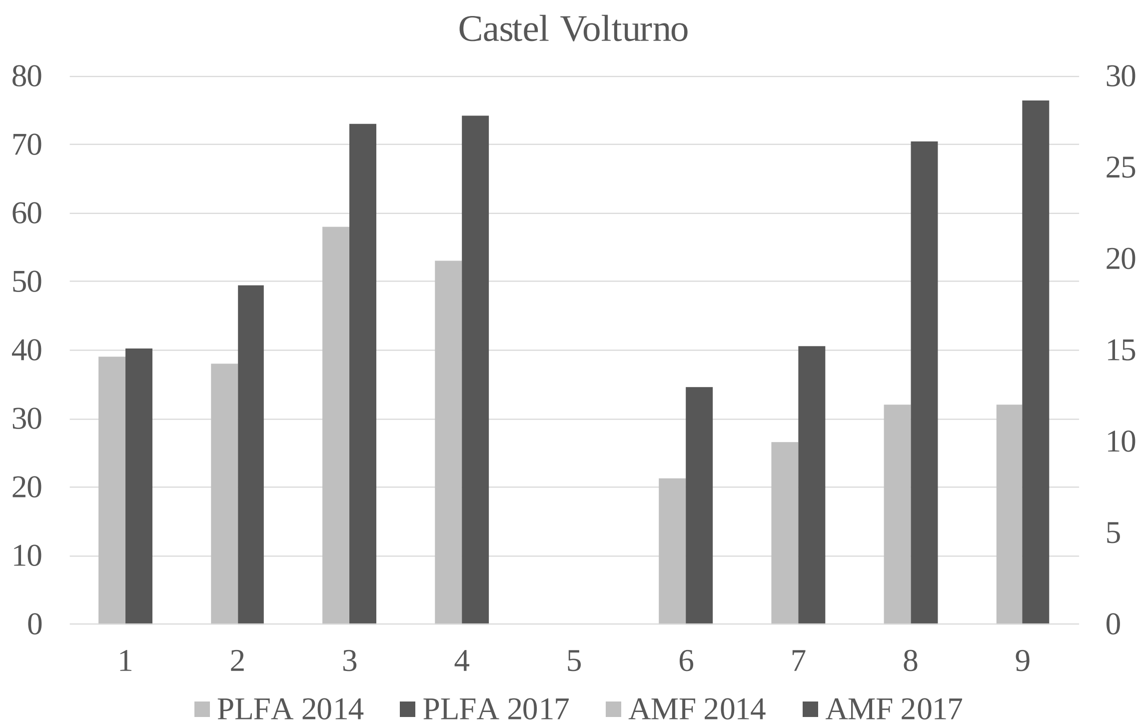


Figure 24 Total PLFA and AMF-NLFA released by soil treatments of Castel Volturno

3.4.2 Project site Prima Luce

- Enzimatic activity

On September 2015, after one year from the first amendment, soil samples for monitoring of biological fertility were collected and characterized for the main biological and biochemical soil properties. In particular, will be monitored generic enzymatic activities involved in the degradation of organic matter, as dehydrogenase and hydrolase, but also specific enzymes involved in carbon cycle (β-glucosidase, β-galactosidase, glucosaminidase and invertase), in phosphorus and sulphur cycles (phosphomonoestarase and arylsulphatase) or in nitrogen cycle (urease, nitrate reductase and protease). Moreover, to evaluate the effects of compost on soil microbial community, carbon and nitrogen biomass and microbial respiration will be monitored. Influence of the compost on functional biodiversity and catabolic profiling of soil microorganisms, were studied by Biolog Ecoplate, that allows measurement of catabolic activity against 31 different carbon substrates. Finally, soil microbial community will be analyzed by API-ZYM strips too.

Most of the analysis are still ongoing, but the firsts characterization by catabolic profiling are completed and available. The effect of soil organic amendment on the utilization of different carbon and nitrogen sources by soil microbial community, determined by BIOLOG Ecoplates ☐, was evaluated by analysis of variance (one-way ANOVA). Results showed that the catabolic activity of soil microorganisms was significantly affected by organic treatment only for 4 of the 31 analyzed substrates: D-Xylose, L-Phenylalanine, L-Threonine and D-Cellobiose (Table 18), that are both easily degradable substrates, such as amino acids, and more complex carbon compound as carbohydrates. Moreover, Biolog catabolic profile was used to determine the AWCD and the Shannon’s biodiversity index (Fig 25). The AWCD showed differences between the soil treatments after 48 h of incubation, with the higher values in the plots under 20 Mg ha-1 of compost (Fig. 25A). Although the differences of the Shannon’s biodiversity index values of the soils under different organic amendment were not very strongly, also in this case plots under the highest dose of compost (20 + 20 Mg ha-1) showed the highest value (Fig. 25 B). After one year and two amendments, the BIOLOG method did not revealed deep changes in soil microbial community catabolic characteristics, resulting in a different utilization of carbon sources. Only the catabolic activity of the plots under the highest dose of compost, was affected by organic amendments and able to metabolize carbon substrates in different way. The low rate of used compost and the short period of observation, could be taken into account to explain this results. Anyway, the other soil biological and biochemical characterization, useful to understand this phenomena, are still ongoing.

- Ergosterol content in soil.

Preliminary results showed average values of 0.88 µg of ergosterol per gram of soil (d.w.) in non fertilized (control) plots and 2.51 µg g-1 d.w. in the plots amended with 20 + 20 t/ha of compost. This indicates that 20 + 20 t/ha of compost stimulates the growth of the fungal biomass, whose amount were almost triple than control after two amendments.

However, the peculiar texture of the studied soil made difficult the separation of cyclohexane from the hydrophilic phase, since most soil debris placed at the interface during the centrifugation step. This problem was firstly minimized increasing the amount of cyclohexane. However, further tests are necessary in order to optimize the extraction procedure to this kind of soil, before reaching a standardized method applicable to all the samples to obtain definitive and complete results.

Table 18 ANOVA one way for all carbon substrates utilized by microorganisms in Biolog Ecoplates (n = 12 samples) and their numbering. Type of treatment (3 d.f.) was the independent variable

			Type of treatment	
Type	Number	Substrate	F	P value
Carbohydrates	A2	□-Methyl-D Glucoside	2.9155	0.1005
Carbohydrates	A3	D-Galactonic Acid □-Lactone	2.4808	0.1353
Amino acids	A4	L-Arginine	0.5122	0.6851
Caboxylic acid	B1	Pyruvic Acid Methyl Ester	0.7781	0.5384
Carbohydrates	B2	D-Xylose	5.4867	0.0242*
Caboxylic acid	B3	D-Galacturonic Acid	2.8363	0.1060
Amino acids	B4	L-Asparagine	0.4846	0.7022
Polymers	C1	Tween 40	0.4773	0.7069
Carbohydrates	C2	i-Erythritol	0.7293	0.5628
Phenols	C3	2-Hidroxy Benzoic Acid	1.0279	0.4303
Amino acids	C4	L-Phenylalanine	8.0414	0.0085*
Polymers	D1	Tween 80	0.3471	0.7924
Carbohydrates	D2	D-Mannitol	1.4929	0.2886
Phenols	D3	4-Hydroxy Benzoic Acid	1.1825	0.3757
Amino acids	D4	L-Serine	0.1507	0.9263
Polymers	E1	□-Cyclodextrine	1.7225	0.2393
Carbohydrates	E2	N-Acetil-Dglucosamine	0.3132	0.8156
Caboxylic acid	E3	g-Hydroxybutyric Acid	1.1593	0.3833
Amino acids	E4	L-Threonine	5.7462	0.0215*
Polymers	F1	Glycogen	1.8859	0.2104
Caboxylic acid	F2	D-Glucosaminic Acid	0.0636	0.9776
Caboxylic acid	F3	Itaconic Acid	0.7766	0.5392
Amino acids	F4	Glycil-L-Glutamic Acid	1.8515	0.2161
Carbohydrates	G1	D-Cellobiose	8.1247	0.0082*
Carbohydrates	G2	Glucose-1-Phosphate	0.7896	0.5329
Caboxylic acid	G3	□-Ketobutyric Acid	2.5685	0.1272
Amines/amides	G4	Phenyletyl-amine	1.0365	0.4270
Carbohydrates	H1	□-D-Lactose	0.6386	0.6111
Carbohydrates	H2	D,L-□-Glycerol Phosphate	2.0060	0.1244
Caboxylic acid	H3	D-Malic Acid	0.6072	0.6287
Amines/amides	H4	Putrescine	0.6340	0.6137

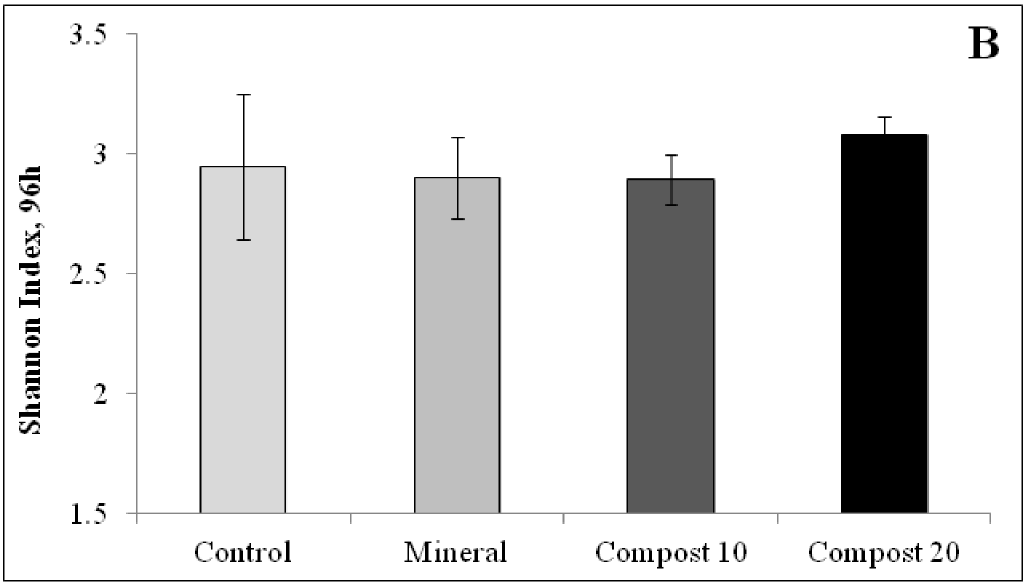


Figure 25. Average well-color development (AWCD, panel A) and Shannon–Weaver index (panel B) of metabolized substrates in BIOLOG EcoPlate™ by the microbial communities of soil under different organic amendment. Control: control soil no treated; Mineral: organic-mineral amendment, 100 kg N ha^{-1}; Compost 10 and 20: compost amendment, 10 and 20 Mg ha^{-1}, respectively.

3.5 Humic acids

The 13C NMR spectra of humic acids obtained from the different project sites at initial time are shown in Figure 26 while the Table 19 indicate the relative C distribution among the main functional groups.

The signals found in the alkyl-C region (0–45 ppm) regions, revealed the large incorporation of alkyl chains pertaining to different components. The peaks at 16, 23 and 30 ppm may be derived mainly from CH3- and CH2- groups of various lipid compounds, such as waxes, polyesters, and phospholipids. In addition to the peaks between 0 and 30 ppm, distinct resonances were shown in the broad alkyl-C region around 30–45 ppm, which indicate the simultaneous presence of different alkyl chains from branched and ciclyc compounds (e.g. sterol) and peptide derivatives. The signal at 56 ppm may be associated with either the methoxyl substituent on the aromatic rings of guaiacyl and syringyl units in lignin structures. Besides the lignin compounds, the the 45–60 ppm chemical shift range may also include the C–N bonds in amino acid moieties.

The different resonances in the O-alkyl-C region (60–110 ppm) are currently assigned to monomeric units in oligo and polysaccharide chains of plant tissue. The intense signal around 72 ppm corresponds to the overlapping resonances of carbons 2, 3, and 5 in the pyranoside structure in cellulose and some hemicelluloses, whereas the signal at 104 ppm is assigned to the anomeric carbon 1 of the glucose unit in cellulose.

The shoulders localized around 65 and 88 ppm result from carbons 6 and 4 of monomeric units, respectively. Besides the signals usually assigned to cellulose, the spectra of different HAs revealed additional resonances around 94 98 ppm. These signals may be related, espectively, to the di-O-alkyl-C of monomeric units of simple carbohydrates and to the C1 of either hemicellulose or pectic polysaccharides chains contained in cell walls of plants, such as 1,5 arabinan, 1,4 galactan, and 1,4 galacturonan.

In the aromatic/olefinic-C region (110–145 ppm), the different resonances around 120 and 130 ppm are related to unsubstituted and C-substituted aryl carbon pertaining to both lignin monomers and ring components of polyphenols. The evident resonances shown at 152 (sharp) and 158-ppm chemical shift range are usually assigned to carbons 3, 4, and 5 in the aromatic ring of lignin components, with carbon 3 and 5 being coupled to the corresponding methoxyl substituents. The attenuated signal intensity shown by the specific O-aromatic region (145–160 ppm) in HAs, confirms the lower incorporation of O-substituted ring carbon derived from different lignin structures. Finally, all humic acids were characterized by the intense signal in the carbonyl region (160–190 ppm) at 174 ppm suggesting the occurrence of intense oxidative processes.

The main properties of humic acids may be summarises by the calculation of dimensionless structural indexes. The hydrophobic index (HB) which is related to the overall biochemical stability of humic fractions, indicate that the HAs from Tetto Frati, Grandi and CastelVolturno has a large preservation of hydrophobic components, mainly attributable to aliphatic compounds, while the aromatic index (Ar) suggested the lower presence of plant derived lignin components in all humic materials. In this respect the comparison of signal intensity in the 45–60 ppm interval over that in the 140–160 ppm range, may be helpful for a more accurate assignment of methoxyl and phenolic resonances. This dimensionless index, hereby denoted as lignin ratio (LR), has been used to improve discrimination between signals from lignin units characteristic of other phenolic components or peptidic moieties. While a lower ratio around 1 is usually associated with the inclusion of tannin and polyphenol constituents to the global O-aromatic-C signals, the opposite prevalence of upper fractional part indicates the significant contribution of C–N bonds in the 45–60 ppm area. Therefore, the discrepancy between methoxyl-C and phenolic-C signals, summarized by the larger lignin ratio found in all humic extracts (Table 15), suggests the incorporation biolabile peptidic materials and a lesser preservation of lignin components in the stabilized organic fractions. Finally the larger amount of polysaccharides found in HA from Prima Luce (Fig. 26) revealed a steady maintenance of biolabile compounds showing a lower hydrophobic character (Table 19).

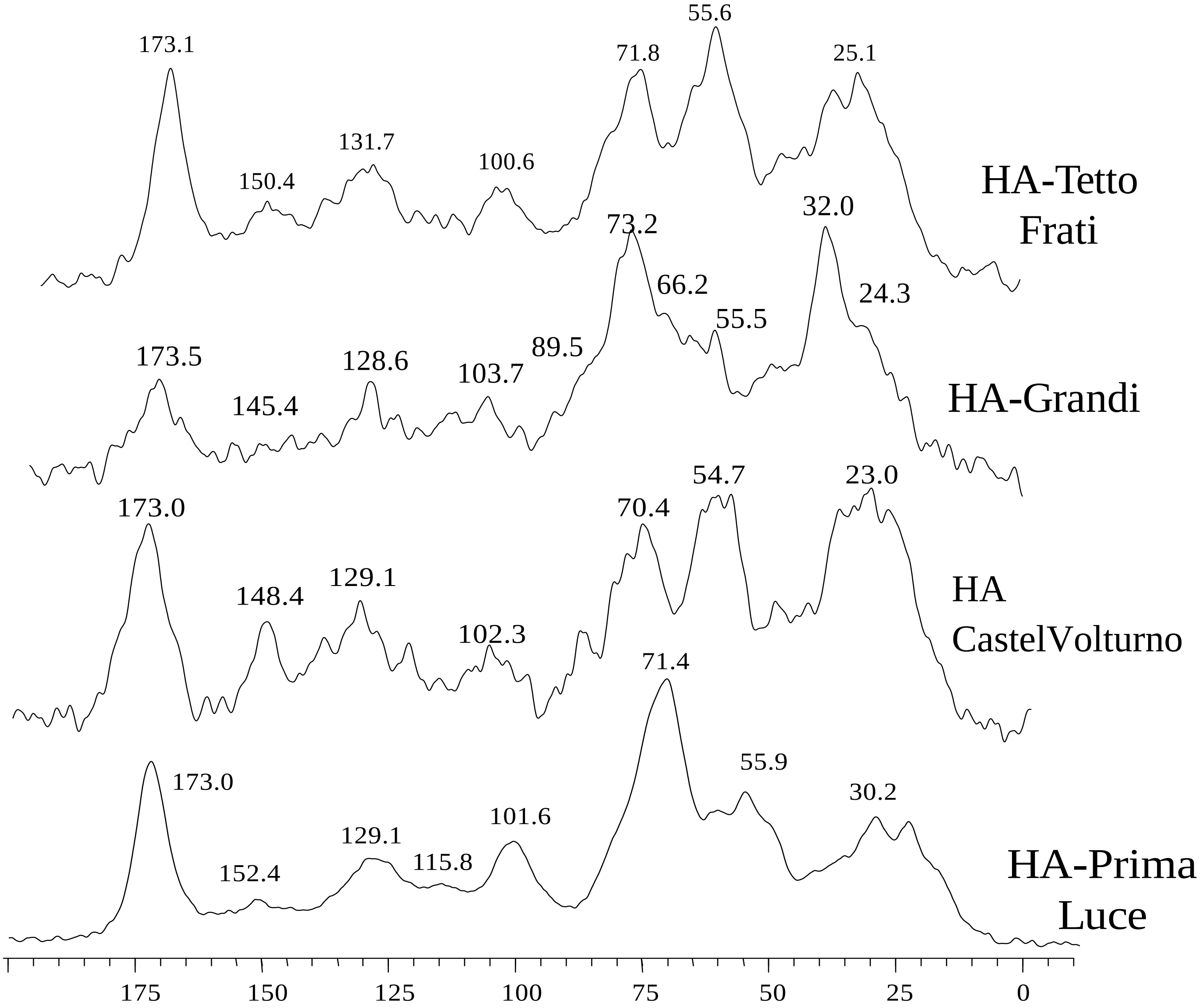


Figure 26 13C CPMAS NMR spectra of HA from control soils at initial experimental time

Table 19 Relative distribution (%) of signal area over the six main chemical shift regions (ppm) and structural indices of grouped C molecular types assessed by 13C CPMAS-NMR spectroscopy of the Humic acids extracted from initial soil samples (TF: Tetto Frati; GR. Grandi; CV. CastelVolturno; PL: Prima Luce)

Soil	Carboxyl	O-Aromatic	Aromatic	O-Alkyl	CH_3O/CN	Alkyl	Structural index [a]		
HAs	190-160	160-145	145-110	110-60	60-45	45-0	LR	HB	Ar
TF	10.9	4.4	13.3	29.3	15.8	26.3	3.6	1.08	17.7
GR	6.9	2.2	10.7	37.3	11.3	31.5	5.1	1.00	12.9
CV	12.4	4.6	13.2	23.3	18.0	28.5	3.9	1.24	17.7
PL	11.3	2.8	12.7	42.1	12.5	18.7	4.55	0.68	15.4

LR Lignin ratio = (60-45)/(145-110)

HB Hydrophobic index= Σ (45-0)+(60-45)/2+(110-160)/Σ (60-45)2+(60-110)+(160-190)

Representative NMR spectra of humic materials extracted at final sampling time at Tetto Frati and Castel Volturno are shown in Figures 27 and 28. The data of OC distribution (Tables 17-20), indicated significative differences related to the different SOM management at each project site. In fact, notwithstanding with the slight modifications of relative signal area, the soil samples with the addition of humified composts revealed a evident structural changes highlighted by the structural index. The steady lowering values of LR ratios found in humic samples from compost amended soils, suggested the progressive selective incorporation of lignin components form added organic materials. Conversely the higher LR values of traditional managed soils suggested the lowers amount of lignin derivatives in the corresponding humic materials. The same trend was revealed by the calculation of Aromatic index, (Ar) that highlighted a steady increase in soil plots with compost additions (Tables 17-20). An intermediate behaviour was shown by the NMR spectra of humic substances from field plots with fresh digestate in Tetto Frati and Grandi project sites, that were characterized by a LR ratio and Ar index that averaged those of Control (Trad) and compost (CMPB, CMPA) amended samples. Finally, the improvement of aromatic and aliphatic hydrophobic components deriving from exogenous organic materials, already indicated by the data of thermochemolysis, were confirmed by the results of hydrophobic index in all project sites (Table 17-20). The values found for the HB ratios, revealed the inclusion in soil humic substances of alkyl and aryl components for all the filed sites, with the largest increase for the soil treatments with composts

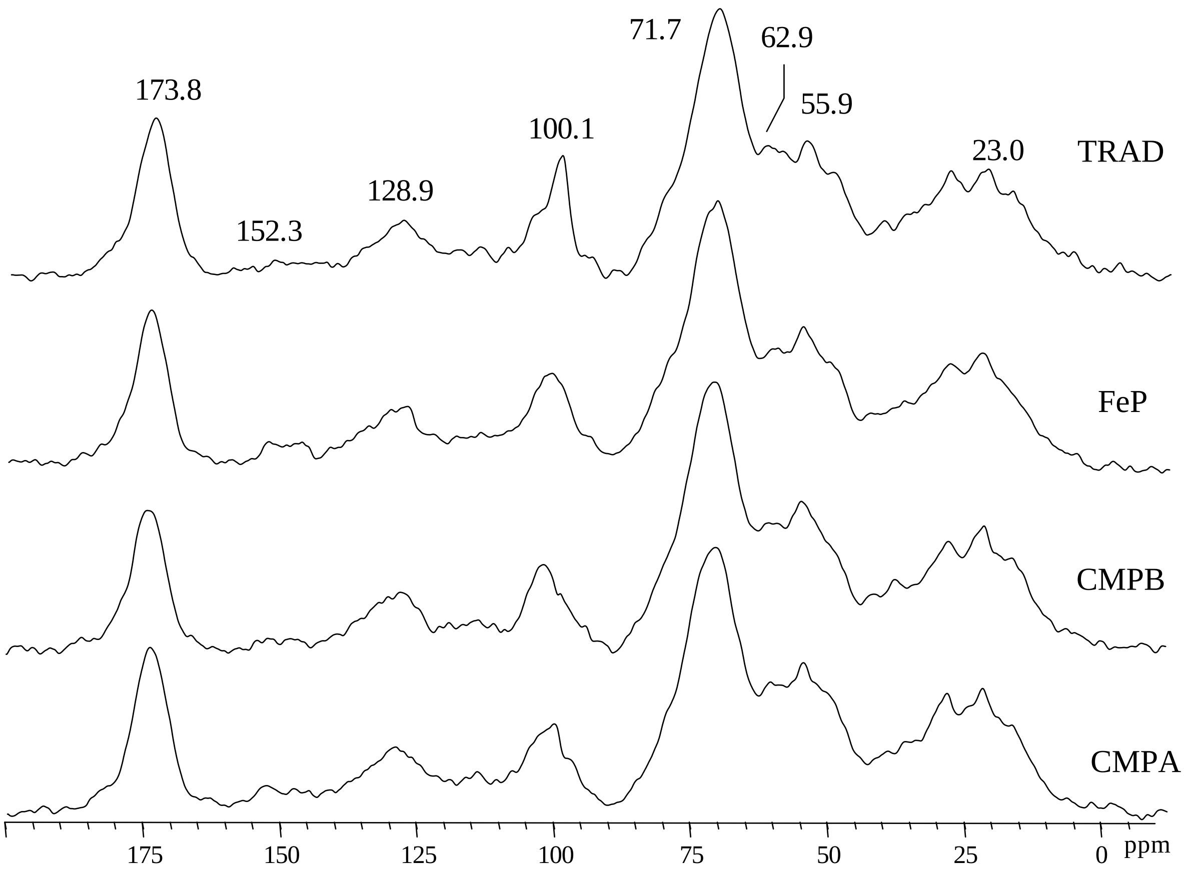


Figure 27 13C CPMAS NMR spectra of HA from the soil treatments of Castel Volturno at last sampling period

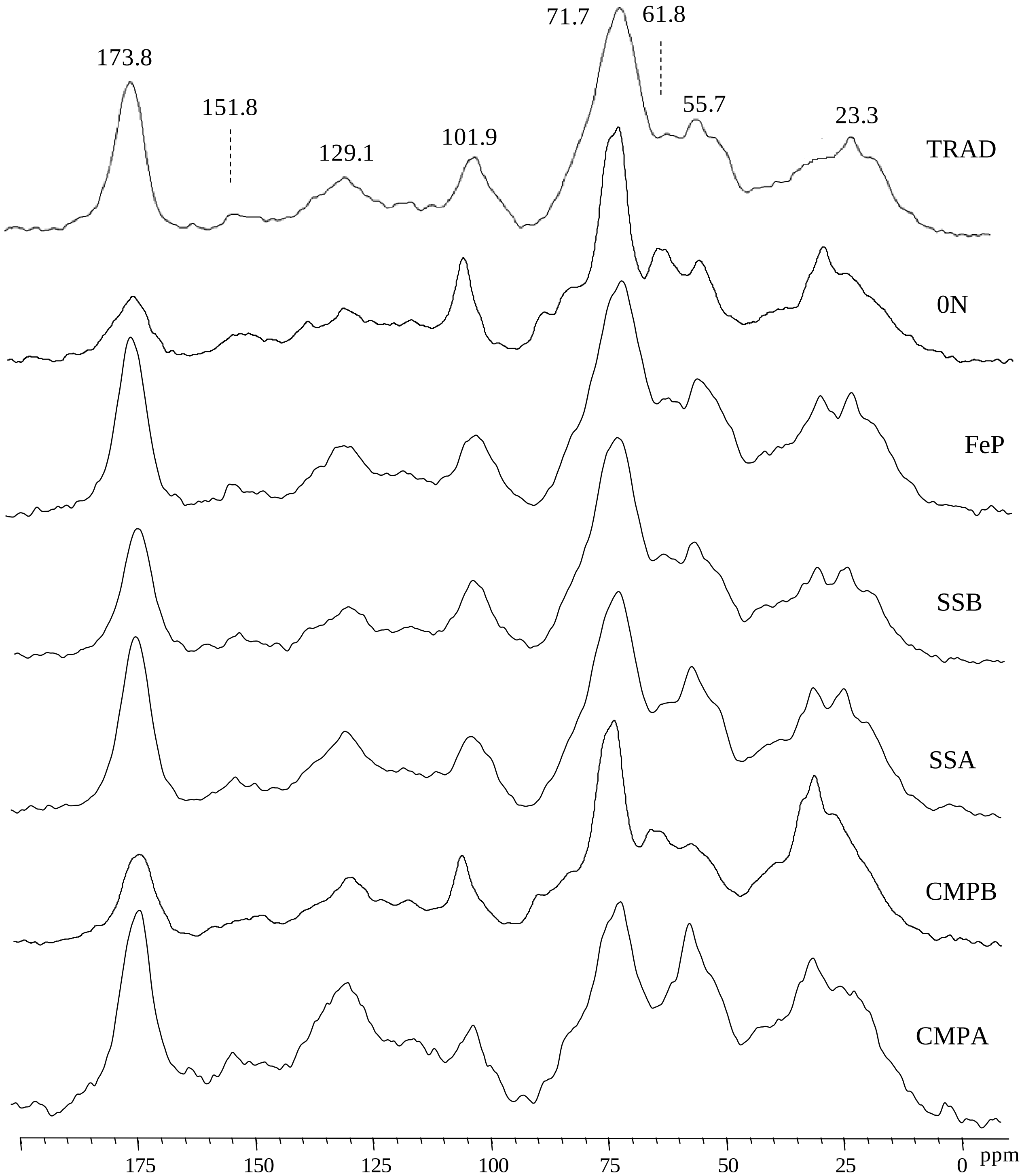


Figure 28 13C CPMAS NMR spectra of HA from the soil treatments of Tetto Frati at last sampling period

Table 17 Relative distribution (%) of signal area over the six main chemical shift regions (ppm), and structural indices of grouped C molecular types assessed by 13C CPMAS-NMR spectroscopy of the Humic acids extracted from final soil treatments of Tetto Frati

	Carboxyl	O-Aromatic	Aromatic	O-Alkyl	CH_3O/CN	Alkyl	Structural index [a]		
	190-160	160-145	145-110	110-60	60-45	45-0	LR	HB	Ar
Trad	12.9	3.9	11.3	31.3	14.3	26.3	3.67	0.95	15.2
FeP	9.7	6.5	13.3	30.5	14.3	25.7	2.20	1.11	19.8
0N	13.9	3.9	10.3	34.3	14.3	23.3	3.67	0.81	14.2
SSB	12.2	5.1	11.8	30.8	14.8	25.3	2.90	0.98	16.9
SSA	9.2	5.4	12.5	31.5	14.8	26.6	2.74	1.08	17.9
CMPB	7.9	5.9	12.3	30.8	13.8	29.3	2.34	1.19	18.2
CMPA	9.2	6.3	13.3	29.8	14.8	26.6	2.35	1.16	19.6

LR Lignin ratio = (60-45)/(145-110)

HB Hydrophobic index= Σ (45-0)+(60-45)/2+(110-160)/Σ (60-45)2+(60-110)+(160-190)

Table 18 Relative distribution (%) of signal area over the six main chemical shift regions (ppm), and structural indices of grouped C molecular types assessed by 13C CPMAS-NMR spectroscopy of the Humic acids extracted from final soil treatments of Grandi

	Carboxyl	O-Aromatic	Aromatic	O-Alkyl	CH_3O/CN	Alkyl	Structural index [a]		
	190-160	160-145	145-110	110-60	60-45	45-0	LR	HB	Ar
Trad	9.4	2.2	10.2	40.9	9.8	27.5	4.45	0.81	12.4
FeP	10.4	1.7	8.7	40.4	8.8	30	5.18	0.81	10.4
0N	7.3	3	10.7	37.3	11.8	29.9	3.93	0.98	13.7
SSB	6.9	2.8	11.1	37.7	11.3	30.2	4.04	0.99	13.9
SSA	7.7	3.4	11.9	35.3	10.8	29.9	3.18	1.05	15.5
CMPB	7.1	3.3	12.6	34.8	11.7	30.5	3.55	1.09	15.90
CMPA	9.4	2.2	10.2	40.9	9.8	27.5	4.45	0.81	12.4

LR Lignin ratio = (60-45)/(145-110)

HB Hydrophobic index= Σ (45-0)+(60-45)/2+(110-160)/Σ (60-45)2+(60-110)+(160-190)

Table 19 Relative distribution (%) of signal area over the six main chemical shift regions (ppm), and structural indices of grouped C molecular types assessed by 13C CPMAS-NMR spectroscopy of the Humic acids extracted from final soil treatments of Castel Volturno

	Carboxyl	O-Aromatic	Aromatic	O-Alkyl	CH_3O/CN	Alkyl	Structural index [a]		
	190-160	160-145	145-110	110-60	60-45	45-0	LR	HB	Ar
Trad	14.4	4.1	12.2	28.3	20	21	4.88	0.90	16.3
FeP	13.9	5.1	14.2	27.3	17	22.5	3.33	1.01	19.3
CMPB	10.4	6.6	16.2	21.3	15	30.5	2.27	1.55	22.8
CMPA	9.3	7.3	17.2	22.3	16	27.9	2.19	1.53	24.5

LR Lignin ratio = (60-45)/(145-110)

HB Hydrophobic index= Σ (45-0)+(60-45)/2+(110-160)/Σ (60-45)2+(60-110)+(160-190)

Table 20 Relative distribution (%) of signal area over the six main chemical shift regions (ppm), and structural indices of grouped C molecular types assessed by 13C CPMAS-NMR spectroscopy of the Humic acids extracted from final soil treatments of Prima Luce

	Carboxyl	O-Aromatic	Aromatic	O-Alkyl	CH_3O/CN	Alkyl	Structural index [a]		
	190-160	160-145	145-110	110-60	60-45	45-0	LR	HB	Ar
Trad	A	11.7	2.8	11.7	45.1	13	15.7	4.64	0.58
FeP	B	10.8	2.6	12.3	45.1	11.5	17.7	4.42	0.62
CMPB	C	9.3	3.8	13.9	40.1	14.7	18.2	3.87	0.76
CMPA	D	9.3	4	14.4	38.1	14.5	19.7	3.63	0.83

LR Lignin ratio = (60-45)/(145-110)

HB Hydrophobic index= Σ (45-0)+(60-45)/2+(110-160)/Σ (60-45)2+(60-110)+(160-190)

Annex 3 – Closing Report on C.3 Action

Monitoring of the agronomical, phytopathological and practical sustainability of proposed strategies

Project responsible: CREA-OF

Project sites and local responsibility: Piemonte (Tetto Frati, Grandi) AGROSELVIT; Campania CastelVolturno-CERMANU; Prima Luce: CREA-OF/PRIMA LUCE; Mellone UNIBAS

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1. Project sites and treatments
Piemonte Beneficiary responsible AGROSELVIT

1.1 University farm Tetto Frati: crop system maize (4 crop cycles)
7 soil treatments: Trad (chemical fertilizers); 0N no nitrogen addition; CMB-B and CMP-A: low (1000 kgOC/ha) and high (2000 kgOC/ha) doses of compost from bio-digestate; SS-B and SS-A: low (1000 kgOC/ha) and high (2000 kgOC/ha) doses of fresh solid digestate; FeP: like TRAD with the addition of biomimetic catalyst (5 kg/ha)

1.2 Commercial farm Grandi: open field horticultural crops (4 crop cycles lettuce, brassicaceae)
6 soil treatments: Trad (chemical fertilizers); 0N no nitrogen addition; CMB-B and CMP-A: low (1000 kgOC/ha) and high (2000 kgOC/ha) doses of compost from bio-digestate; SS-B and SS-A: low (1000 kgOC/ha) and high (2000 kgOC/ha) doses of fresh solid digestate

Campania
1.3 University farm Castel Volturno: crop system maize (4 crop cycles) Beneficiary responsible CERMANU
4 soil treatments: Trad (chemical fertilizers); CMB-B and CMP-A: low (10 tn/ha) and high (20 tn/ha) doses of on-farm compost; FeP: soil addition of biomimetic catalyst (5 kg/ha)

1.4 Commercial farm Prima Luce: open field horticultural crops (4 crop cycles: endive scarole, pumpkin, broccoli, bean) Beneficiary responsible CREA-ORT, PRIMA LUCE
4 soil treatments: **A**) Non-fertilized/Non-amended (control); **B**) Organic and mineral fertilization; **C**) On farm compost 10 t ha^{-1}; **D**) On farm compost 20 t ha^{-1}

1.5 Commercial farm Mellone: orchard systems (3 years - peach and kiwi) Beneficiary responsible UNIBAS
5 soil treatments for each orchard system: Trad (chemical fertilizers); A1 and A2 low(10 tn/ha) and high(20 tn/ha) dose of *light* on farm compost; B1 and B2 low (10 tn/ha) and high (20 tn/ha) dose of *heavy* on farm compost

2. Results

2.1 Tetto Frati

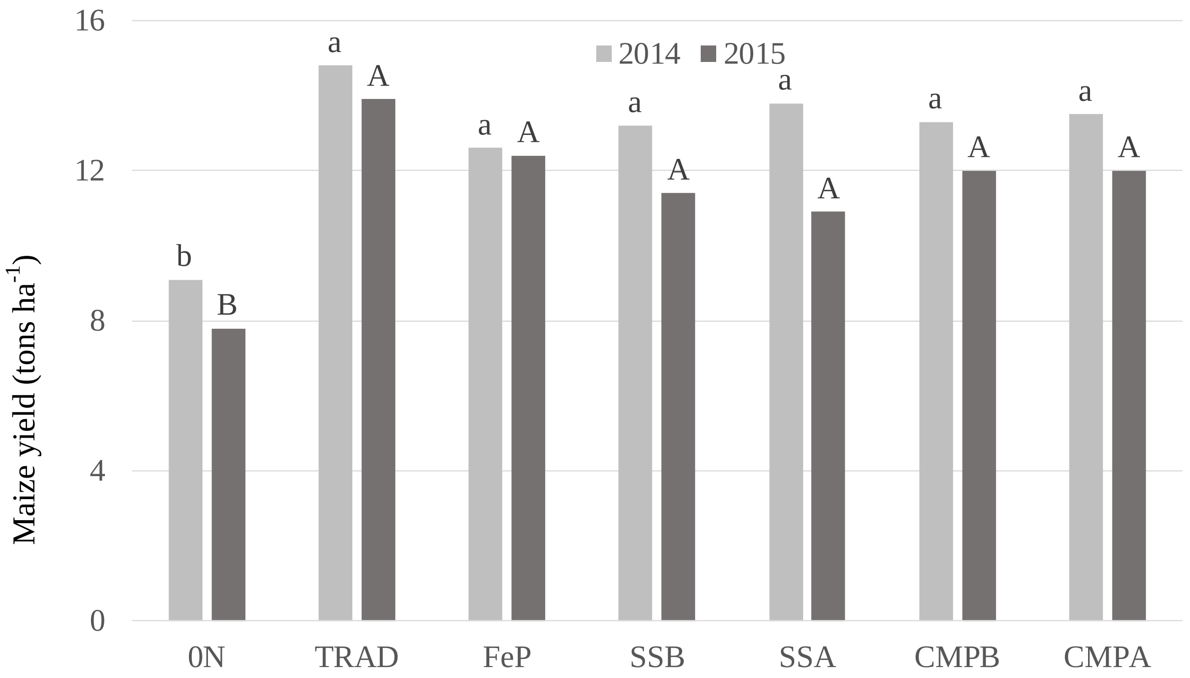


Figure 1. Grain yield of maize crop at Tetto Frati experimental trial, in 2014 and 2015 (different letters indicate significative differences for p=0.05 between treatments in the same crop cycle)

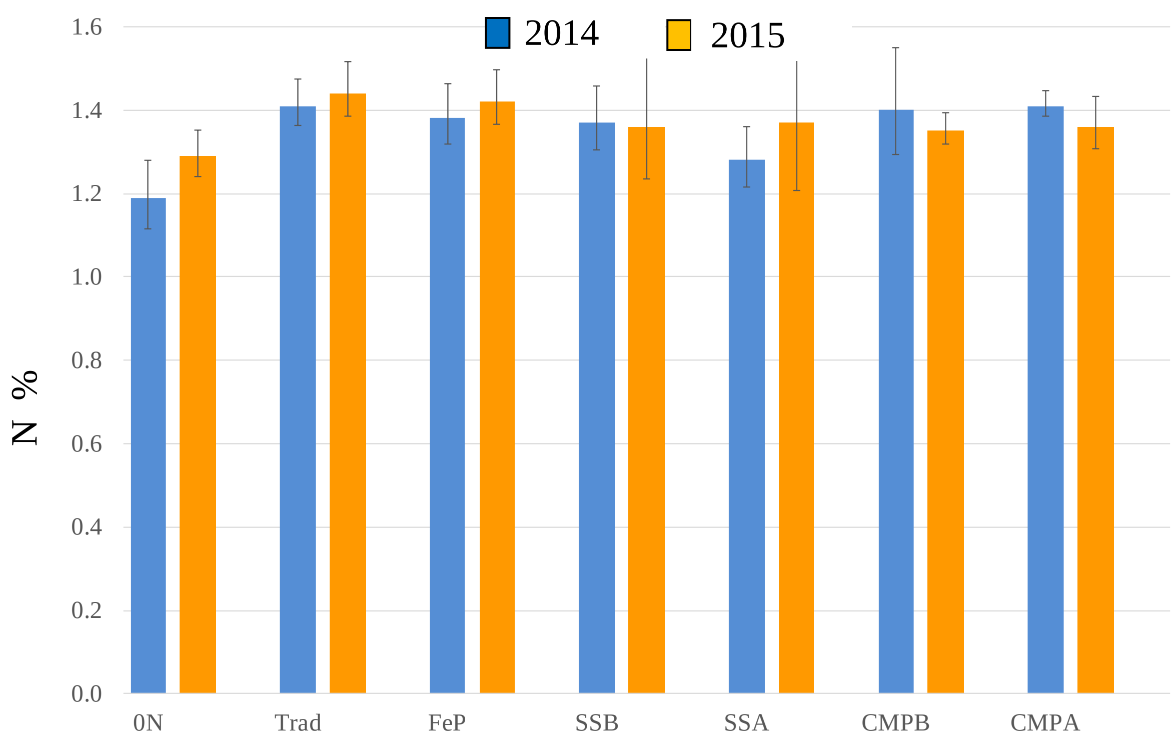


Figure 2. Nitrogen content in maize grain from Tetto Frati experimental trial, in 2014 and 2015

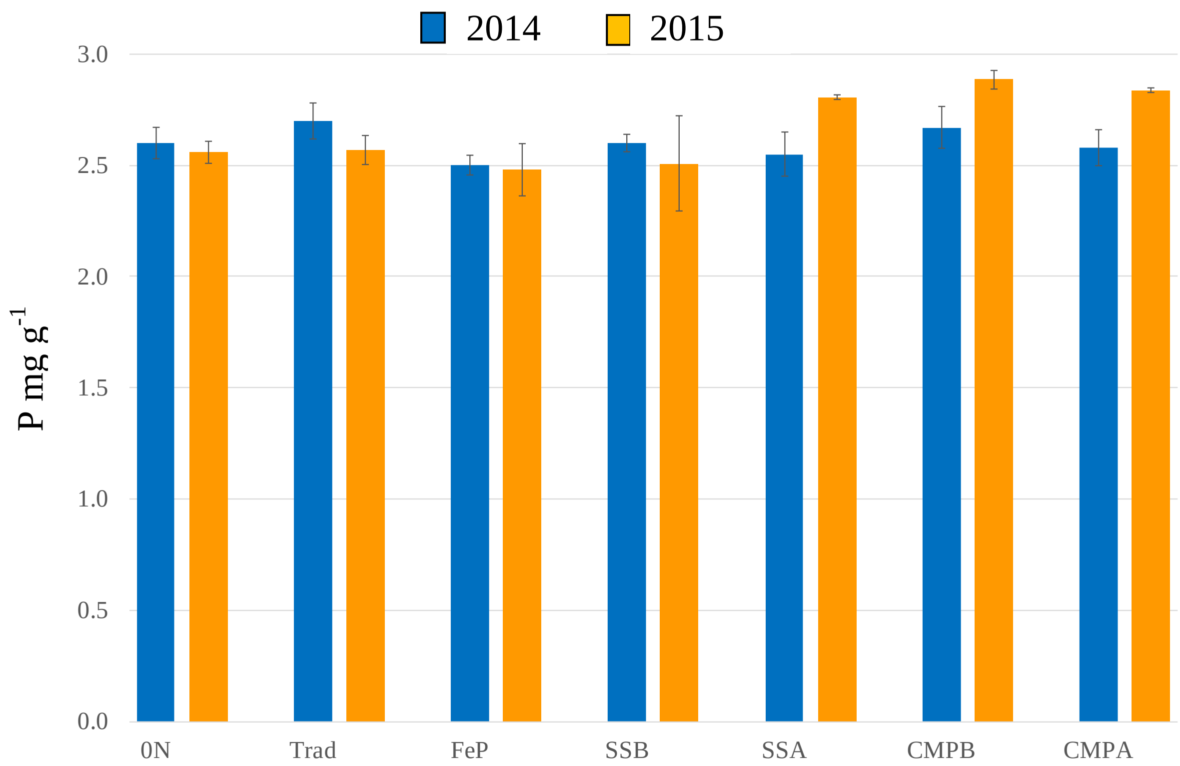


Figure 3. Phosphorus content in maize grain from Tetto Frati experimental trial, in 2014 and 2015

Table 1 NDVI (Normalizes Difference Vegetation Index) values for maize in 2014 and 2015

MAIZE	2014				2015					
Treatment	**20/06/2014**		**27/06/2014**		**29/05/2015**		**16/06/2015**		**26/06/2015**	
0N	794,0	c	756,2	b	98,0	b	691,6	b	706,5	b
CMPA	821,9	abc	837,8	a	129,1	a	742,1	ab	810,6	a
CMPB	830,6	ab	865,1	a	121,8	a	736,9	ab	787,1	a
PORF	824,4	abc	835,5	a	129,3	a	753,5	a	796,8	a
SSMPA	803,8	bc	836,6	a	119,5	a	758,3	a	809,8	a
SSMPB	807,8	bc	853,0	a	128,6	a	766,6	a	795,2	a
TRAD	851,4	a	871,3	a	132,3	a	755,6	a	824,0	a

After two year of soil management an overall maintenance of maize yield was shown by all treatments (Fig. 1). However, although no differences were highlighted by the statistical evaluation, a steady lower amount of maize grain was found in soil plots with organic matter addition. The decrease of dry grain ranged from – 13% for compost to about -20% for fresh solid digestate (Fig. 1), while the expected lower productivity was observed for the control treatment where no nitrogen was supplied (0N). Conversely, excluding the control treatment without N addition, minor differences were found for the nutrient status of maize crops, as shown by both N and P content in maize grains and for the similar photosynthetic status of analysed plants (Figs 2, 3; Table 1). This data suggest that the initial effect of the SOM managements produced a shortage in the available or soluble pool of macro nutrients in respect to plant requirements. In particular, the total N added in organic form in a unique initial solution may have failed to meet the plant uptake for a mismatch in timing availability in respect to physiological phases, as compared to the prompt response allowed to maize crops with the repeated conventional mineral fertilization.

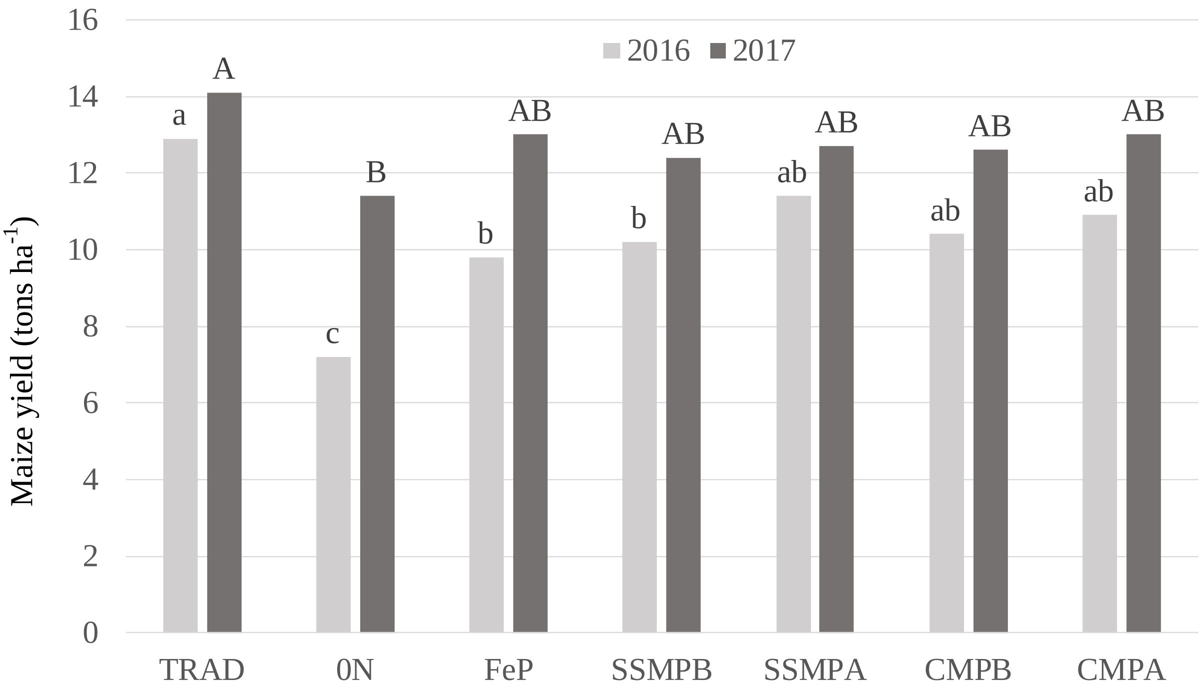


Figure 4. Grain yield of maize crop at Tetto Frati experimental trial, in 2014 and 2015 (different letters indicate significative differences for p=0.05 between treatments in the same crop cycle)

In the second half of project activities, although the SOM managed plots showed a steady lower performance in respect to the samples added with mineral fertilizer, after the last SOM management, the data of maize yield revealed an overall improvement for all treatments with organic matter addition (Fig. 4). The final data indicated a progressive approach to the result of conventional management, with a yield that ranged from -10% for fresh digestate to -9 and -7% for CMPB and CMPA treatments, respectively. The results of N content (Fig. 5) indicated a slight larger contend in maize grain of traditional plots followed by soil treatments with fresh digestate, while the lower N amount were found in CMPB and CMPA field plots. The data of N content further support the hypothesis that a limiting factor is represented by the time displacement in the availability of nitrogen forms added with organic materials. In this respect the less humified fresh digestate may provide a more available organic nitrogen forms, while a larger time interval may be necessary in the soil amendment with humified compost to reach a new steady state by which the soil biomass may become able to release the stable nitrogen components incorporated with mature composts. Differently from the results of N distribution, the analyses of P content revealed an overall maintenance of P content in SOM managed soils, in respect to traditional plots (Fig. 6). The different behaviour may be attributed by the characteristics that determine the P equilibrium in soil environments. This nutrient is, in fact, a representative limiting factor in Italian soils for the lower amount of soluble-available phosphates usually found in the circulating solution, the P forms being progressively chemically combined and retained within the less soluble inorganic calcium-phosphate forms. The soil treatments with organic materials may have hence promoted a shift towards an increase of available or not retained pool, which slow release from the specific organic molecules limited the fixation in the inorganic salts and allowed a suitable uptake by plant roots.

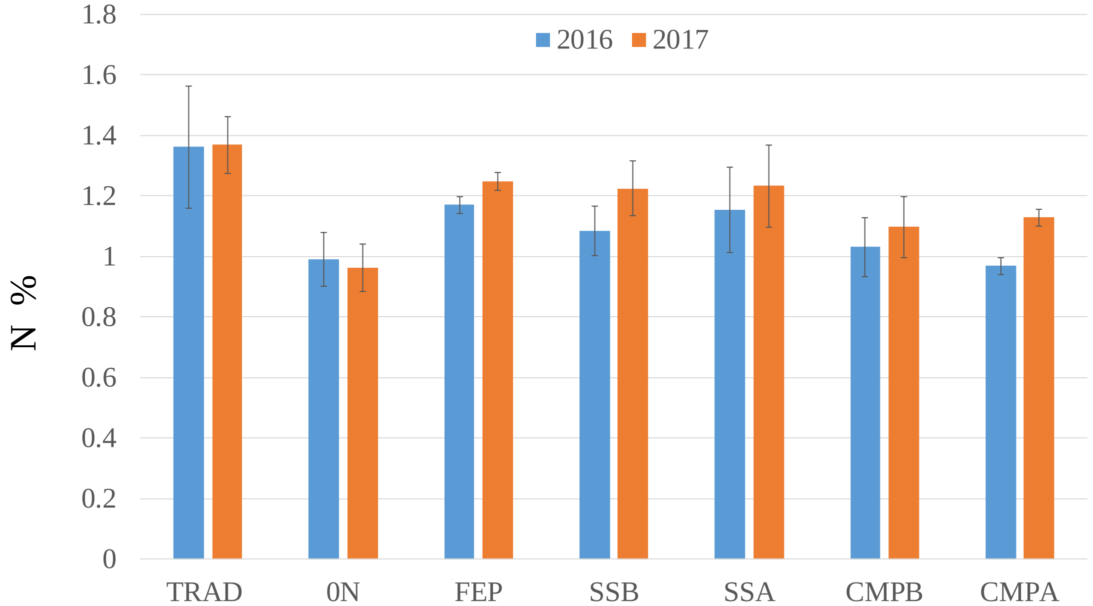


Figure 5. Nitrogen content in maize grain from Tetto Frati experimental trial, in 2016 and 2017

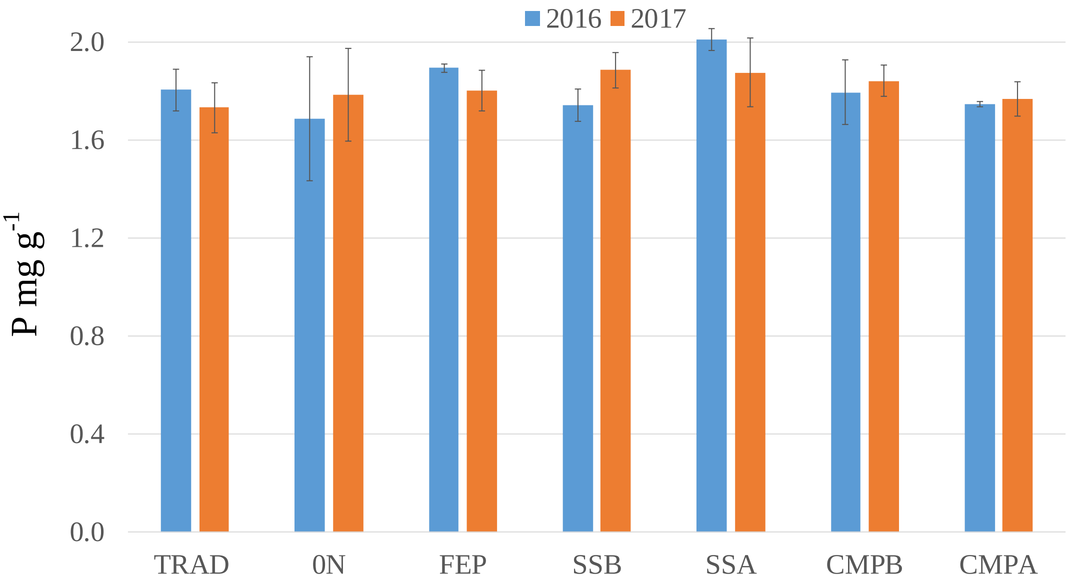


Figure 6. Phosphorus content in maize grain from Tetto Frati experimental trial, in 2016 and 2017

According with the differences found for total yields and N content in maize grains a slight improvement of photosynthetic status was found during the crop cycle for traditional managed plots in 2016 (Fig. 7). On the other hand, following the recovery of nutrient uptakes observed in the last crop cycle, the analyses of NDV index revealed an overall maintenance photosynthetic activity among treatments, with a slight final increase for soil managements with both fresh digestate and mature compost (Fig. 7)

Figure 7 NDVI (Normalizes Difference Vegetation Index) values for maize in 2016 and 2017

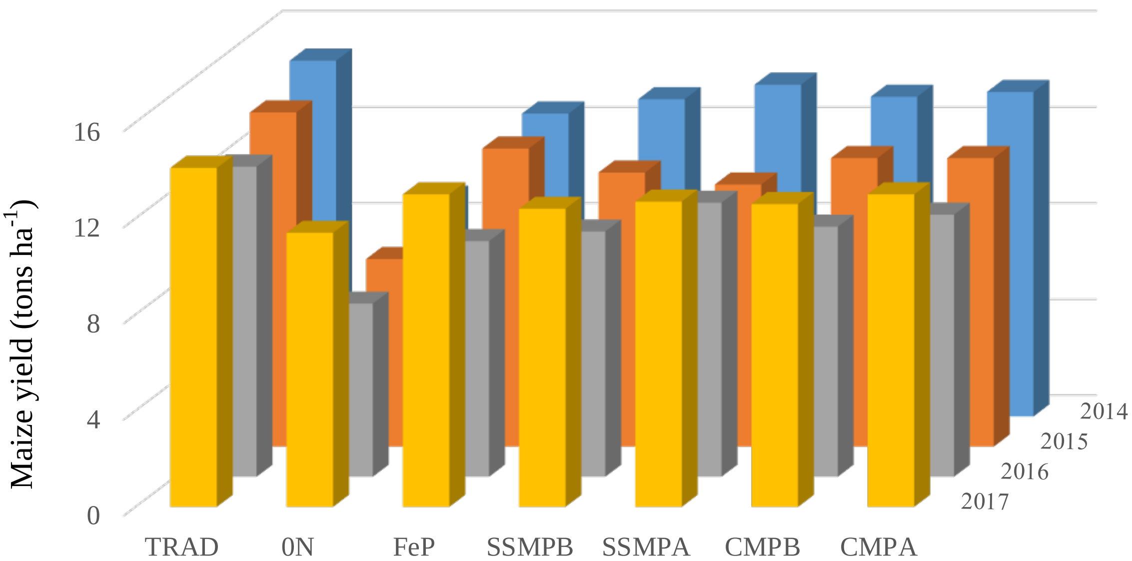


Figure 8 Resume of maize yield at Tetto Frati project site

2.2 Grandi

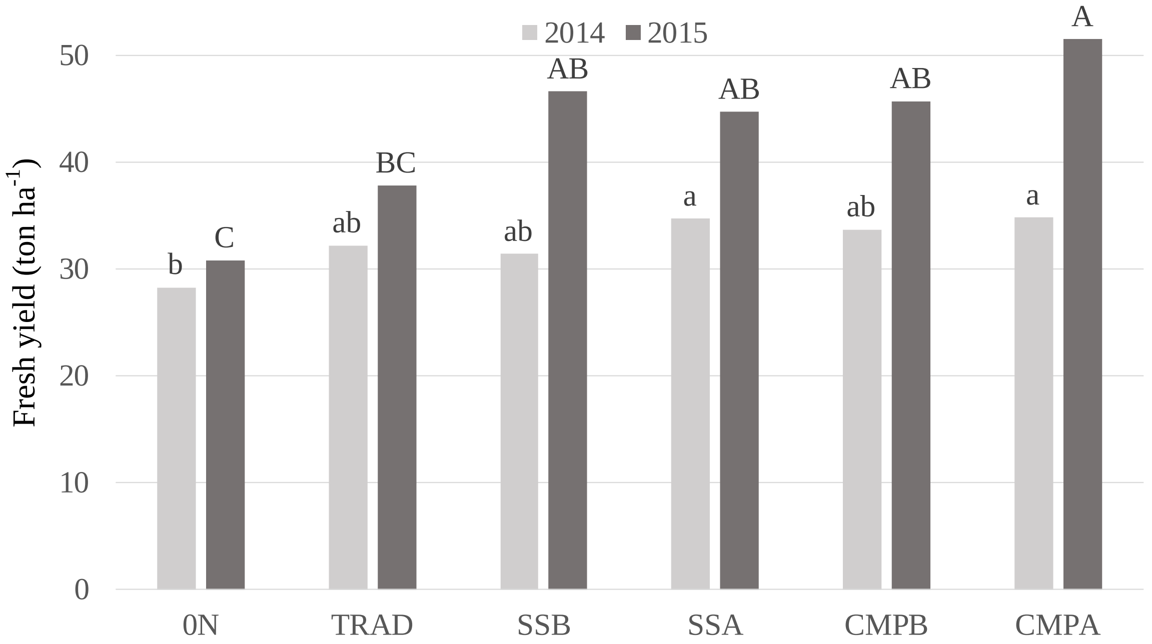


Figure 9. Fresh lettuce produced at the "Grandi" experimental trial in 2014 and 2015 8(different letters indicate significative differences for p=0.05 between treatments in the same crop cycle)

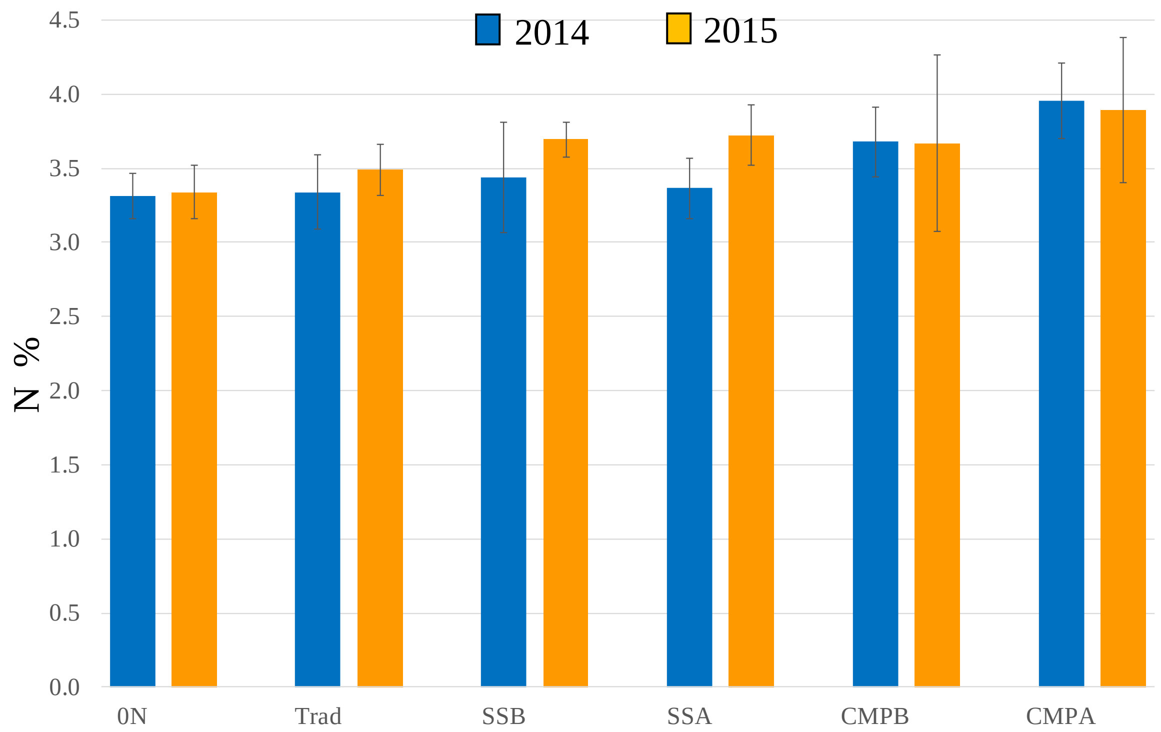


Figure 10. Nitrogen content in lettuce from Grandi experimental trial, in 2014 and 2015

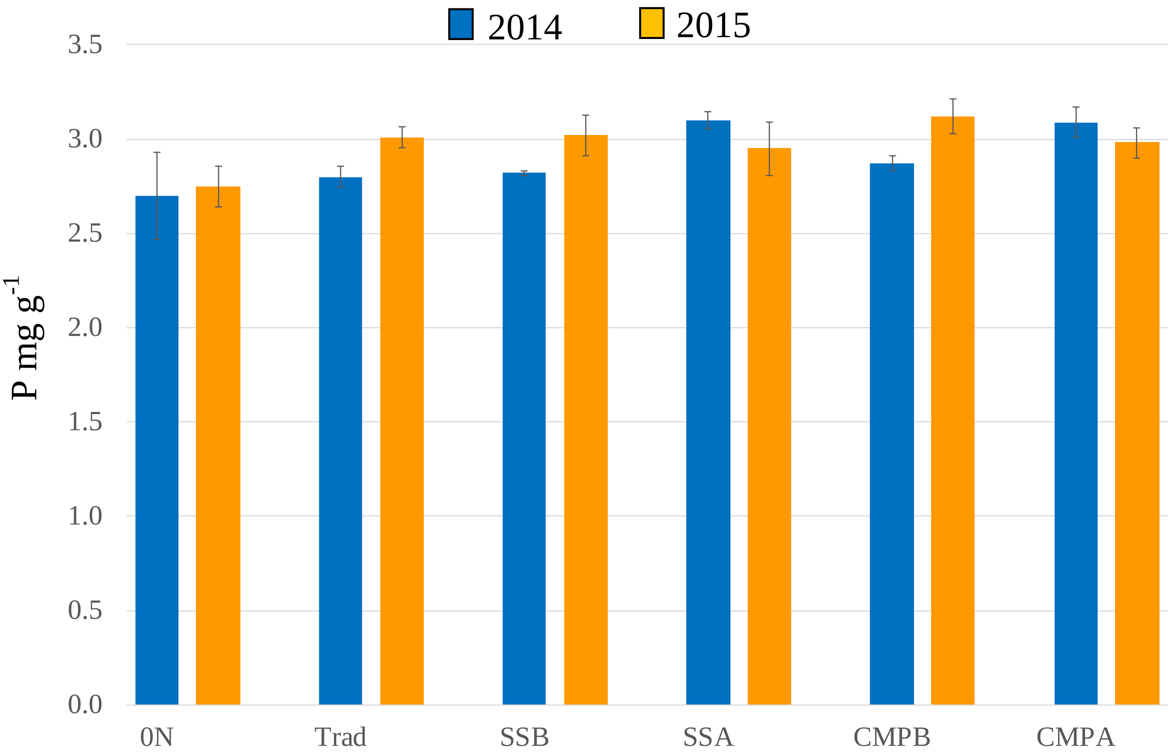


Figure 11. Phosphorus content in lettuce from Grandi experimental trial, in 2014 and 2015

Table 2 NDVI (Normalizes Difference Vegetation Index) values for lettuce in 2014 and 2015

LETTUCE	2014				2015					
Treatment	**23/06/2014**		**02/07/2014**		**29/05/2015**		**16/06/2015**		**22/06/2015**	
0N	509,9	ns	735,1	ab	419,5	b	882,1	b	774,0	b
CMPA	508,3		691,1	b	597,3	a	924,5	a	835,8	ab
CMPB	514,8		741,6	ab	585,2	a	920,4	a	867,0	a
SSMPA	533,5		763,9	a	623,8	a	919,6	a	860,0	ab
SSMPB	509,0		728,2	ab	591,1	a	916,0	a	815,6	ab
TRAD	530,4		745,2	ab	582,7	a	928,8	a	868,4	a

In the Grandi farm a significant increase was found in yield of fresh lettuce for soil treatments with organic matter addition (Fig. 9) in respect to traditional plots, that achieved the +24 to + 30% after the 2nd year of SOM management, with the best performance found for the larger doses of fresh and composted digestate. The crops from organic amended soils showed strictly comparable concentration of nutrients (Figs 10, 11). with higher N content, although the variability makes the increase significant only for the SSB and SSA samples. The positive response on organic matter addition on lettuce development, was indicated also by the data of photosynthetic status (Table 2), which revealed a similar or even larger values of vegetation index in respect to TRAD treatment.

A residual effect of soil treatments on the second horticultural crop, for the first year of soil management, was revealed by an overall maintenance of yields in the fresh weight of brassicacecae in all amended plots, while in the second year a decrease was observed for both the 0N and the lower dose of fresh digestate (Fig. 12). However, all the crops in each cycle showed a comparable N and P uptakes in respect to traditional fertilized samples (Figs. 13, 14).

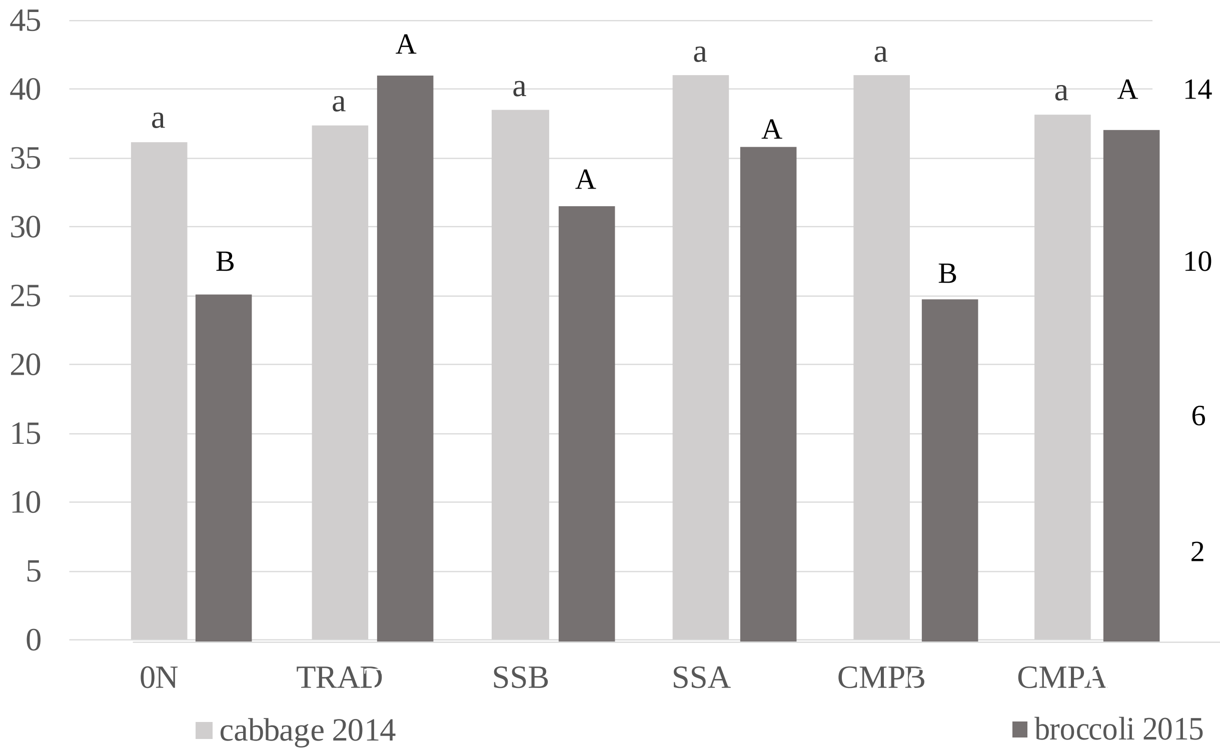


Figure 12. Fresh yields (ton ha $^{-1}$) of 2nd horticultural crops produced at Grandi experimental trial in 2014 and 2015 (different letters indicate significative differences for p=0.05 between treatments in the same crop cycle)

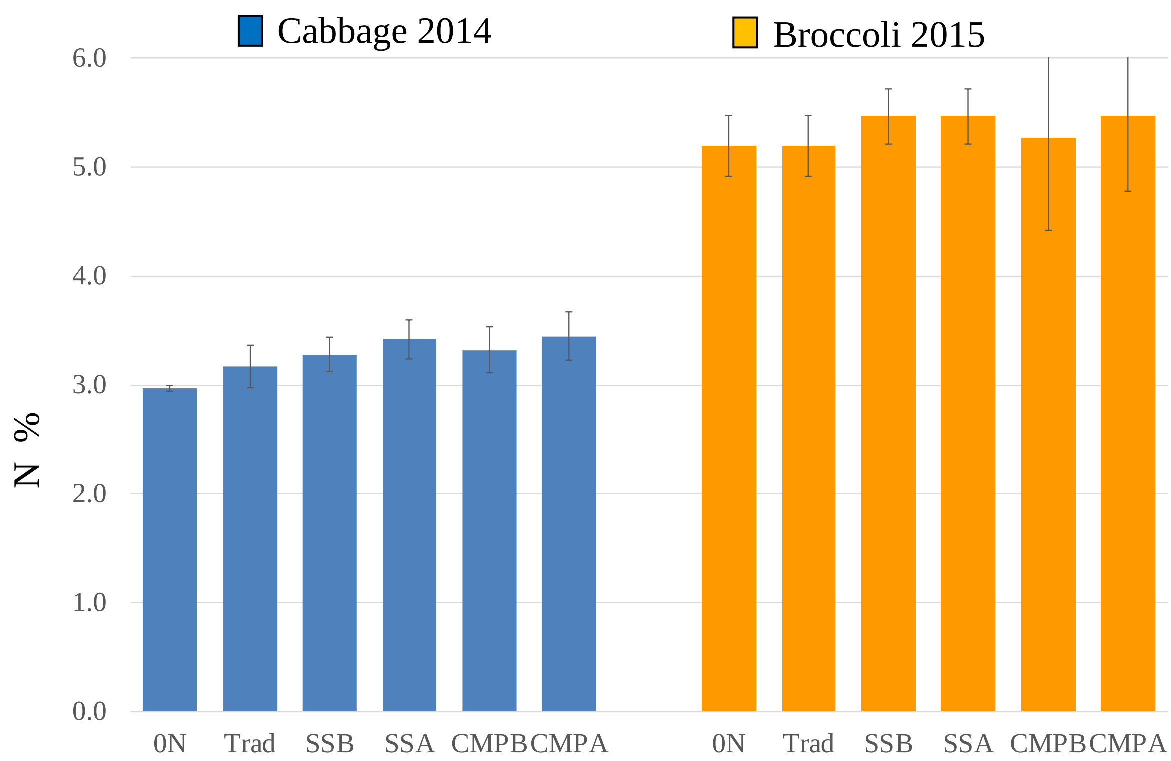


Figure 13. Nitrogen content in 2nd crops from Grandi experimental trial, in 2014 and 2015

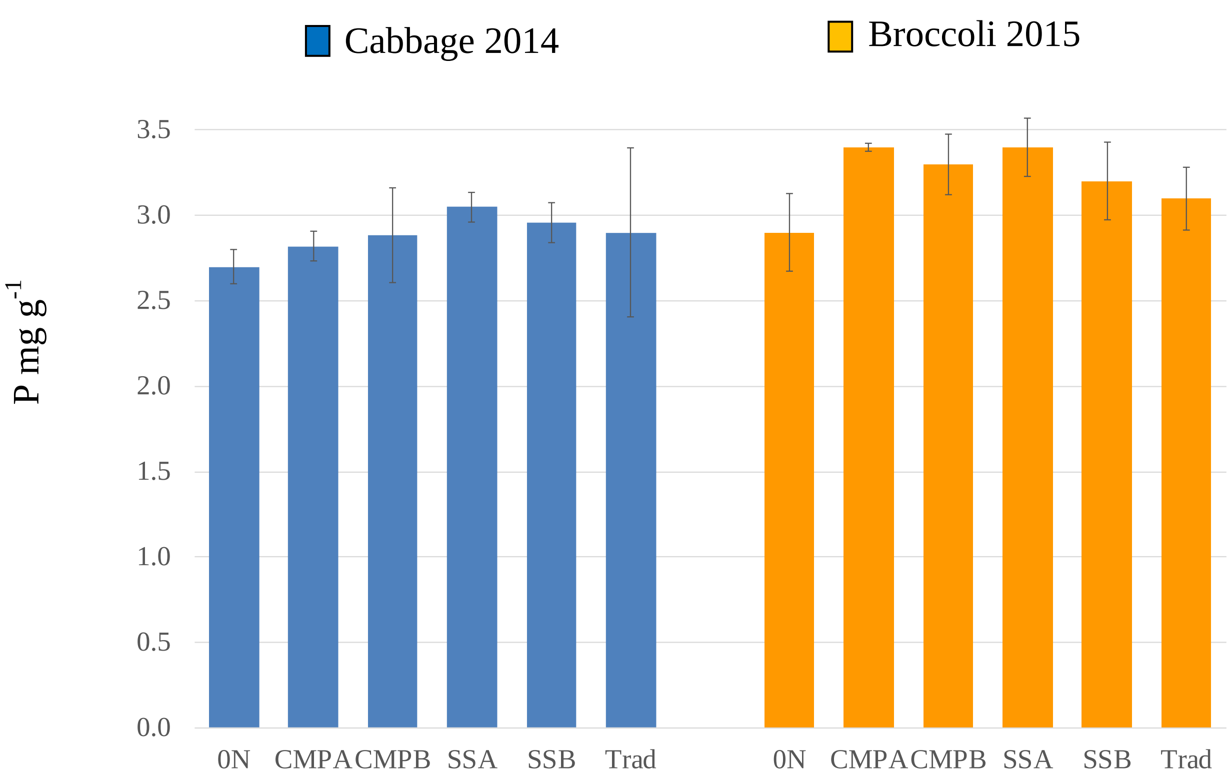


Figure 14. Phosphorus content in 2nd crops from Grandi experimental trial, in 2014 and 2015

The prompt response to SOM managements observed for the horticultural crops, in respect to maize at Tetto Frati, a part for the different plant physiological requirements, may be also related to the different effect provided by the organic management to soil properties. The larger retention of water associated with soil organic matter have a noticeable and well-known effect on the fresh vegetable. Moreover, it is possible to conceive that the use of organic materials underwent to a more fast mineralization and release of nutrients related with a more suitable and timed capability of microbial biomass in the utilization of added organic materials in the soils of Grandi farm, following the application of different organic residues as usual agronomic practices.

Steady larger yields of lettuce were found in SOM managed plots in the second period of project activities, combined with no significative differences on nutrient uptakes between treatments, thereby confirming the positive effects of added organic materials on the crop productivity (Figs. 15, 16, 17). The data were confirmed by the analyses of NDV index (Fig. 18) that indicated an extended maintenance of effective photosynthetic status for the plants in the organic treated soil samples, comparable (2016) or higher (2017) to that of traditional treatment.

Different responses were observed for the second horticultural crop cycles in 2016 and 2017 (Figs. 19, 20, 21). The Brassicaceae showed a larger yield in the traditional managed soils, although a comparable of crop productivity and nutrient uptake were still provided by the addition of organic materials. Conversely the second lettuce cycle in 2017, despite the overall yield decrease, revealed a steady positive effect for soil samples added with the fresh and composted organic materials.

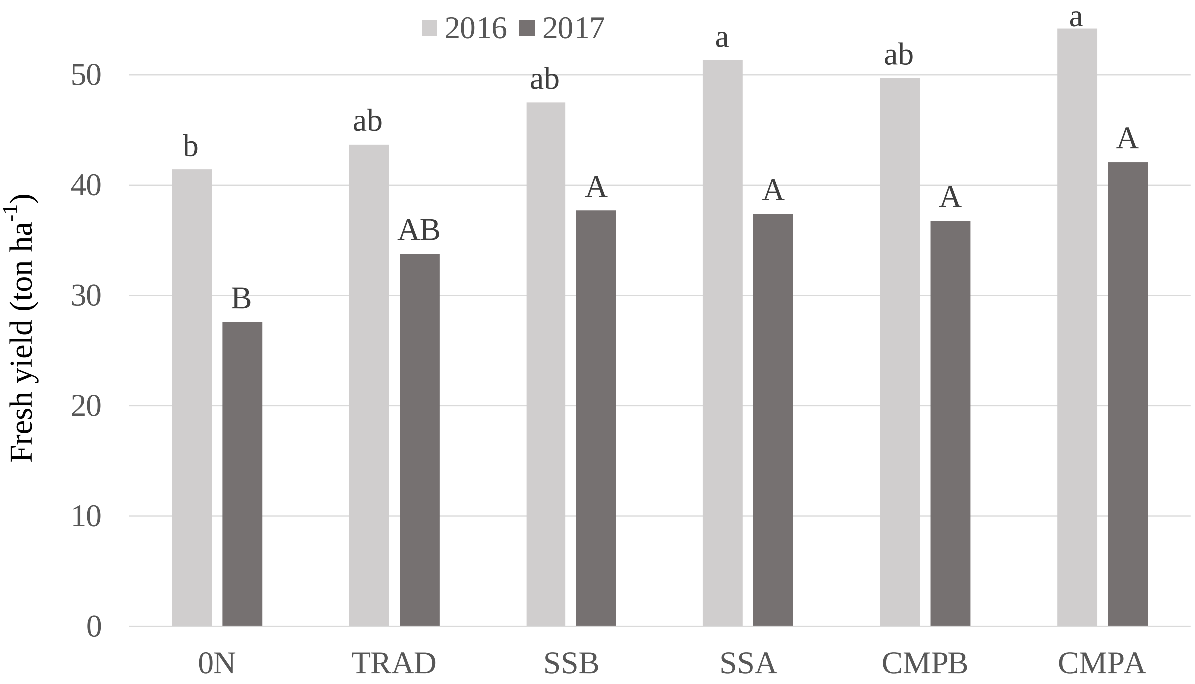


Figure 15. Fresh lettuce produced at the "Grandi" experimental trial in 2016 and 2017 (different letters indicate significative differences for p=0.05 between treatments in the same crop cycle)

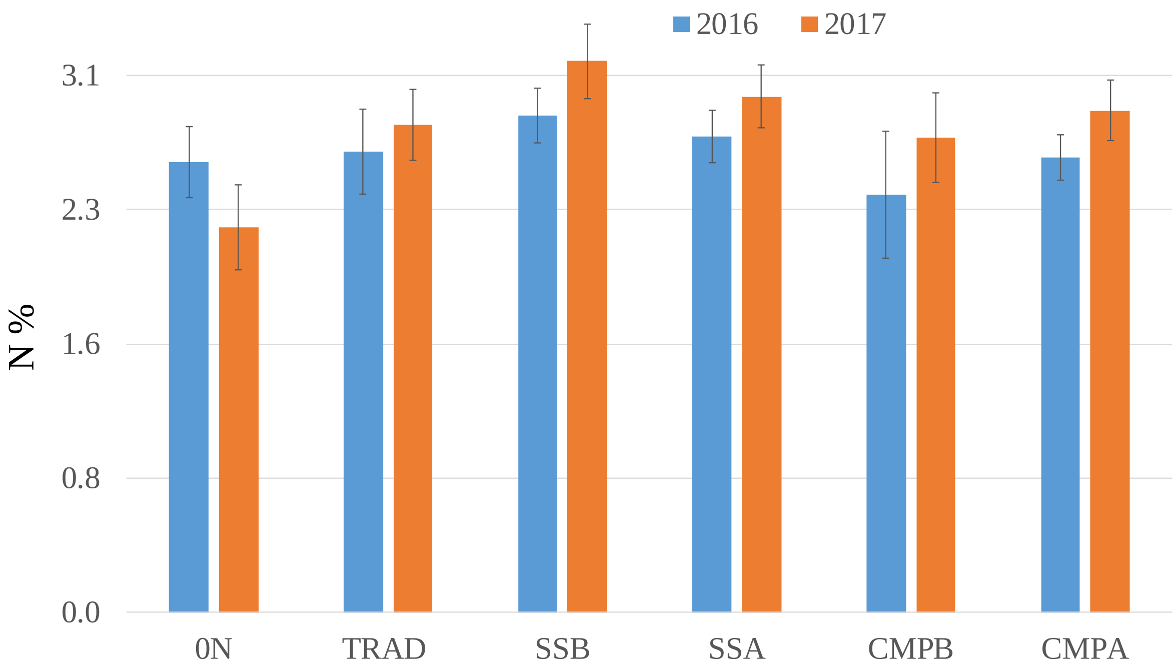


Figure 16. Nitrogen content in lettuce from Grandi experimental trial, in 2016 and 2017

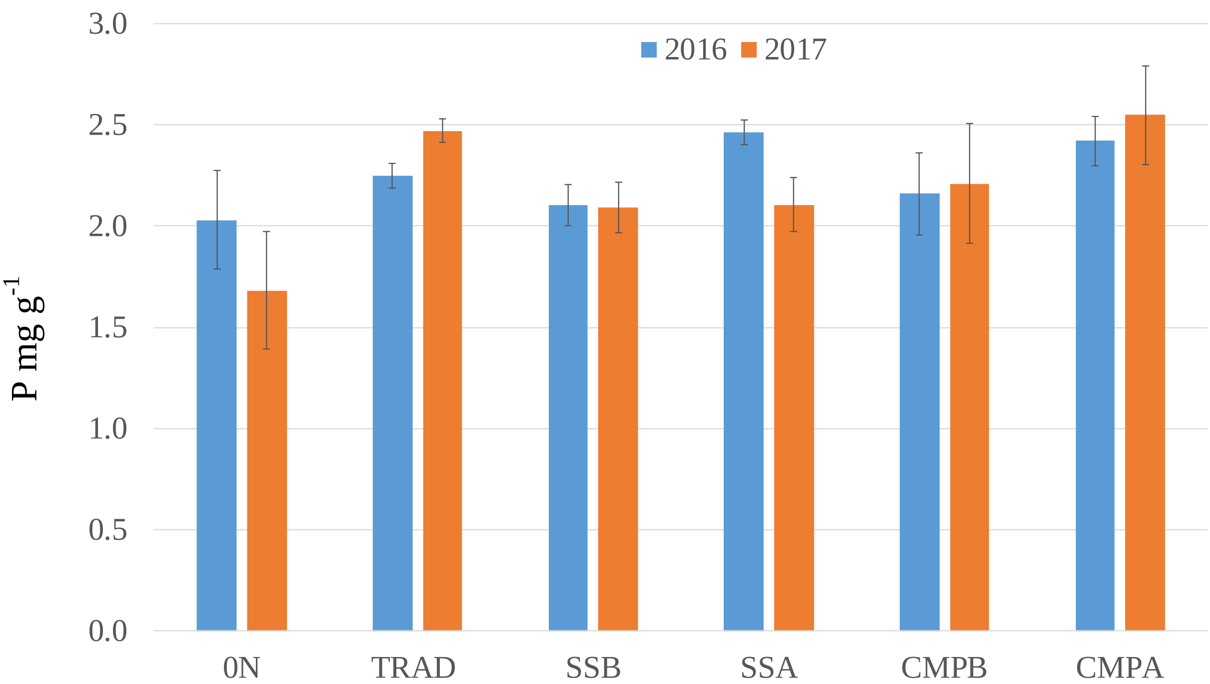


Figure 17. Phosphorus content in lettuce from Grandi experimental trial, in 2016 and 2017

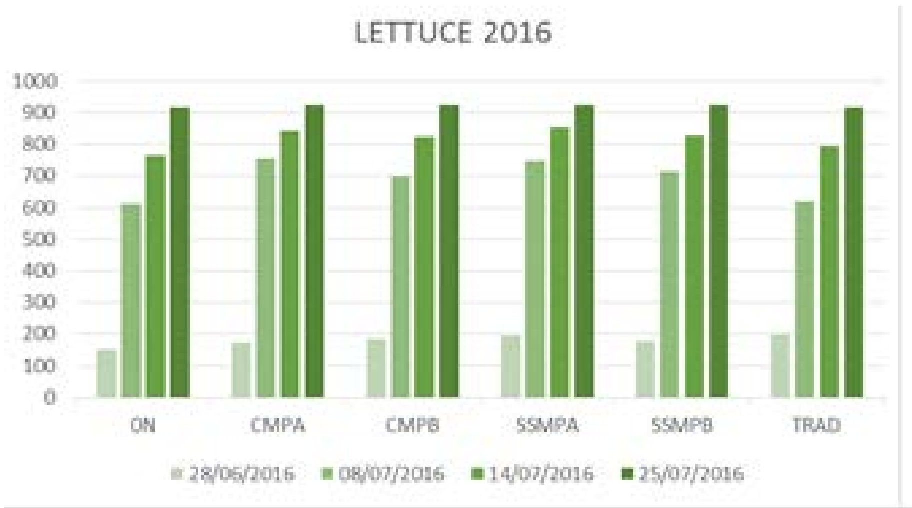
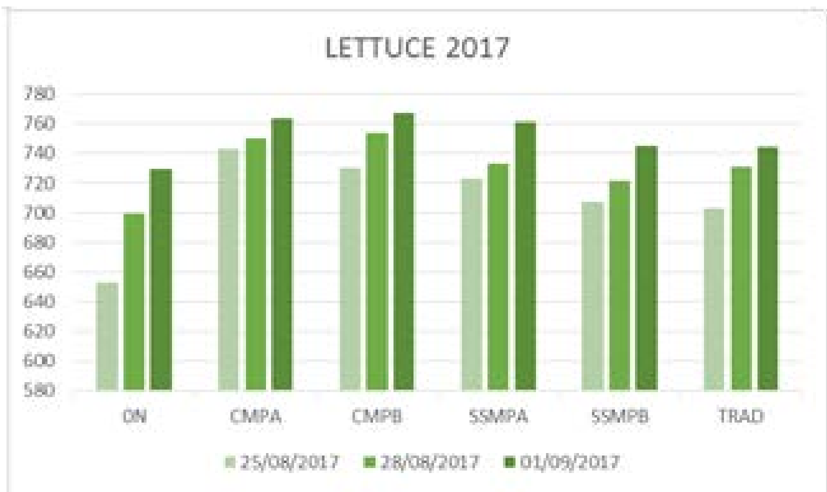


Figure 18 NDVI (Normalizes Difference Vegetation Index) values for lettuce in 2016 and 2017



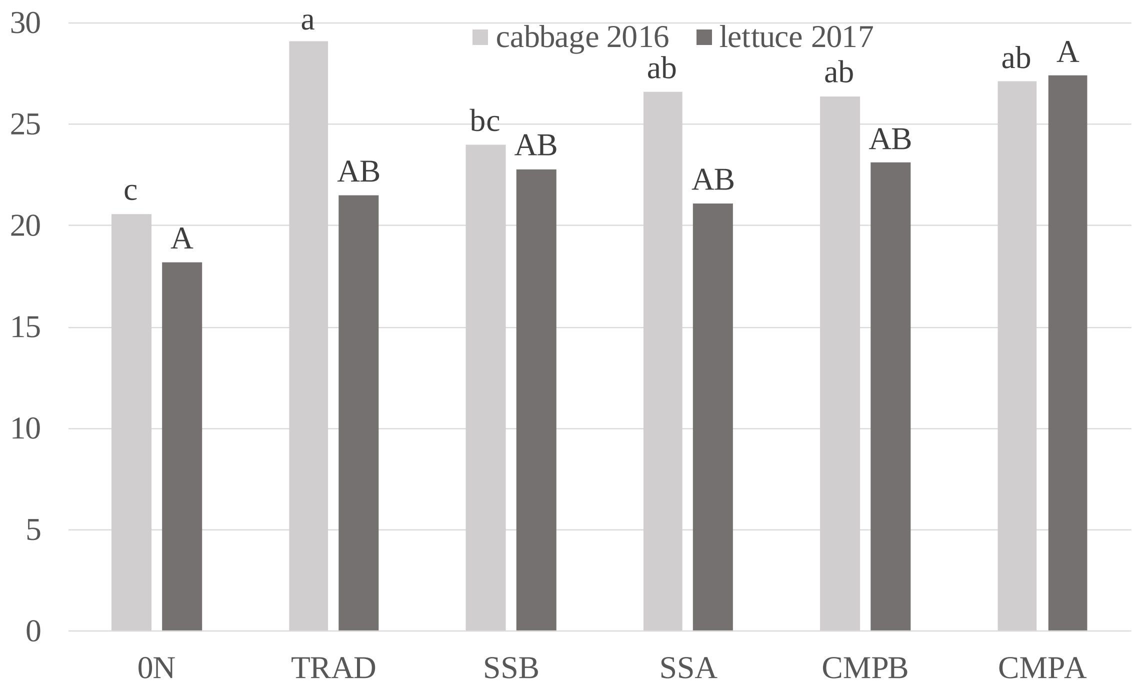


Figure 19. Fresh yields (ton ha $^{-1}$) of 2nd horticultural crops produced at Grandi experimental trial in 2016 and 2017 (different letters indicate significative differences for p=0.05 between treatments in the same crop cycle)

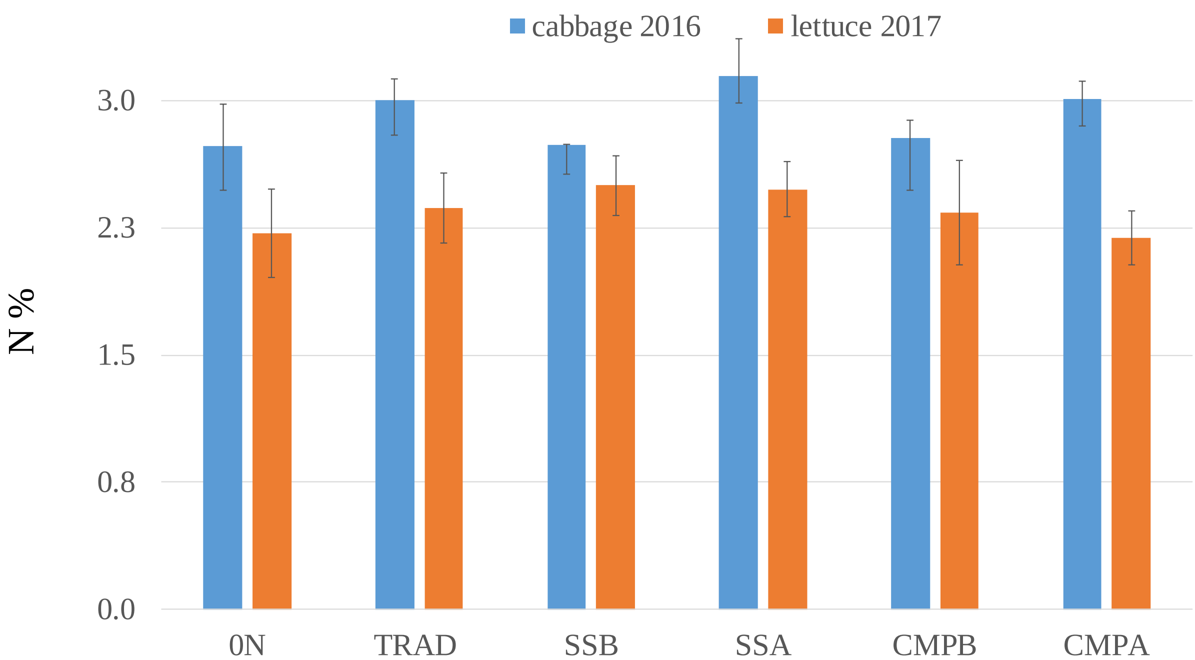


Figure 20 Nitrogen content in 2nd crops from Grandi experimental trial, in 2016 and 2017

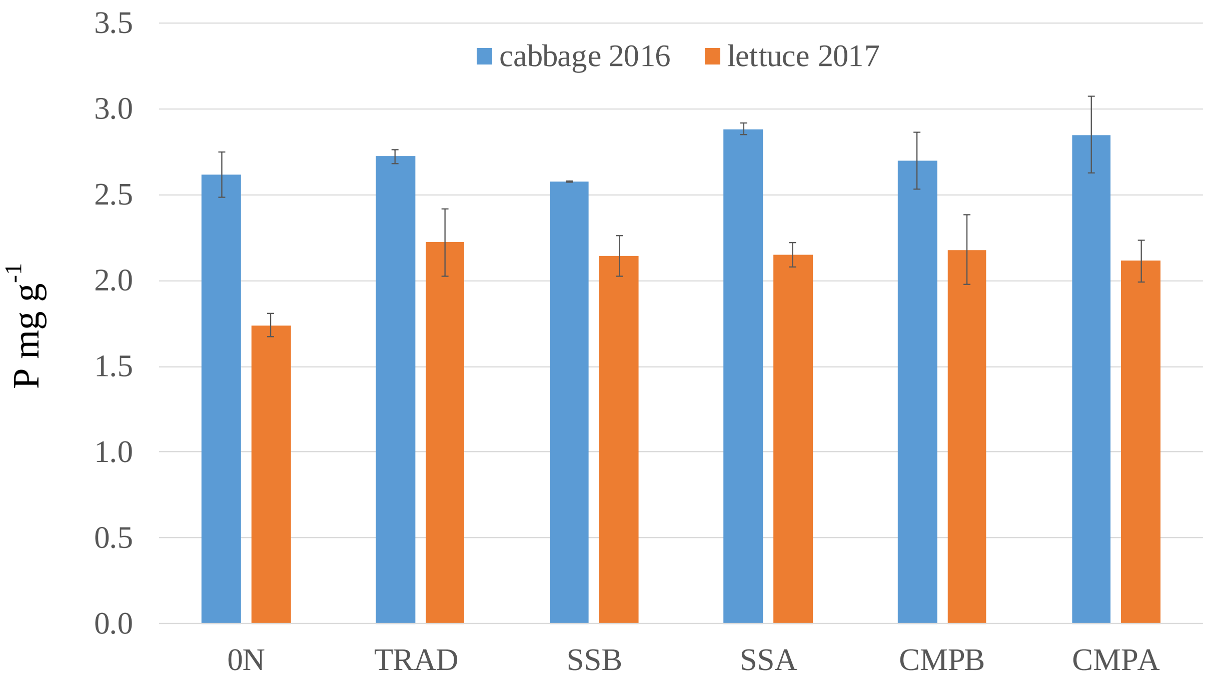


Figure 21. Phosphorus content in 2nd crops from Grandi experimental trial, in 2016 and 2017

2.3 Castel Volturno

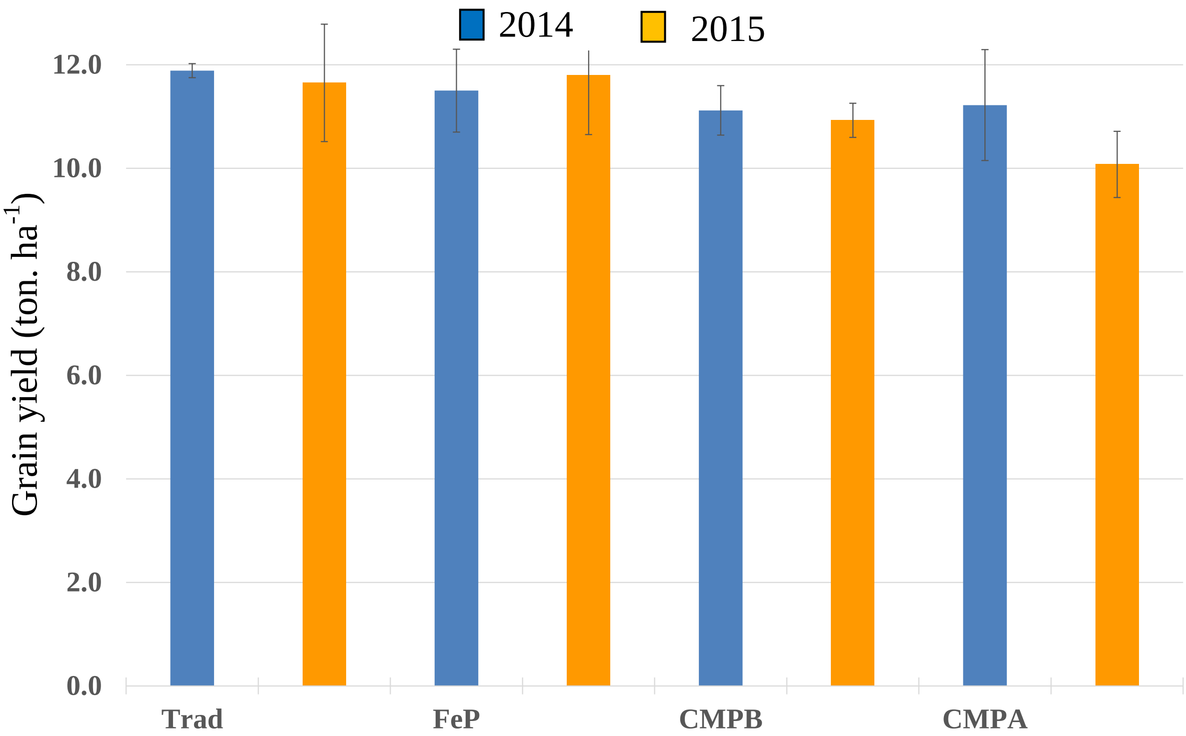


Figure 22. Grain yield of maize cultivated at CastelVolturno experimental trial in 2014 and 2015

In the first two years of project activities, the data of CastelVolturno showed a slight decrease, in the yield of maize grain, expressed as dry matter, for the field plots amended with the on-farm manure compost that ranged from -5% to -13 % for CMPA treatment in 2014 and 2195, respectively (Fig. 22). Differently from the project site of Tetto Frati the compost treatments had also a slight lower N content (Fig. 23), while no differences were found in phosphorus concentration (Fig. 24).

As outlined for the project site of Tetto Frati, the result of maize productivity at Castel Volturno suggested that the addition of organic materials promoted an initial lower supply of nitrogen uptakes in maize plants, irrespective to soil type and organic matter composition. This effect may be related to the required adaptation of soil microbial biomass for the utilization of additional organic materials as C sources with a delaying effect in the availability of organic nitrogen and possible immobilization of overall nitrogen forms. Moreover, in the heavy textured soil of Castel Volturno a slower mineralization of humified organic materials, may have further limited the N availability for maize plant, even compared with the loamy sandy soil of Tetto Frati Conversely, no limiting effects were produced by the applied SOM managements on the amount of available phosphates.

Despite the decrease in N uptake in compost amended plots, no differences were found among treatments in the photosynthetic status of maize plants, as measured by SPAD methodology (Table 3). The graph in Figure 25 show the reliability of the SPAD values with the actual amount of leaves chlorophyll contents.

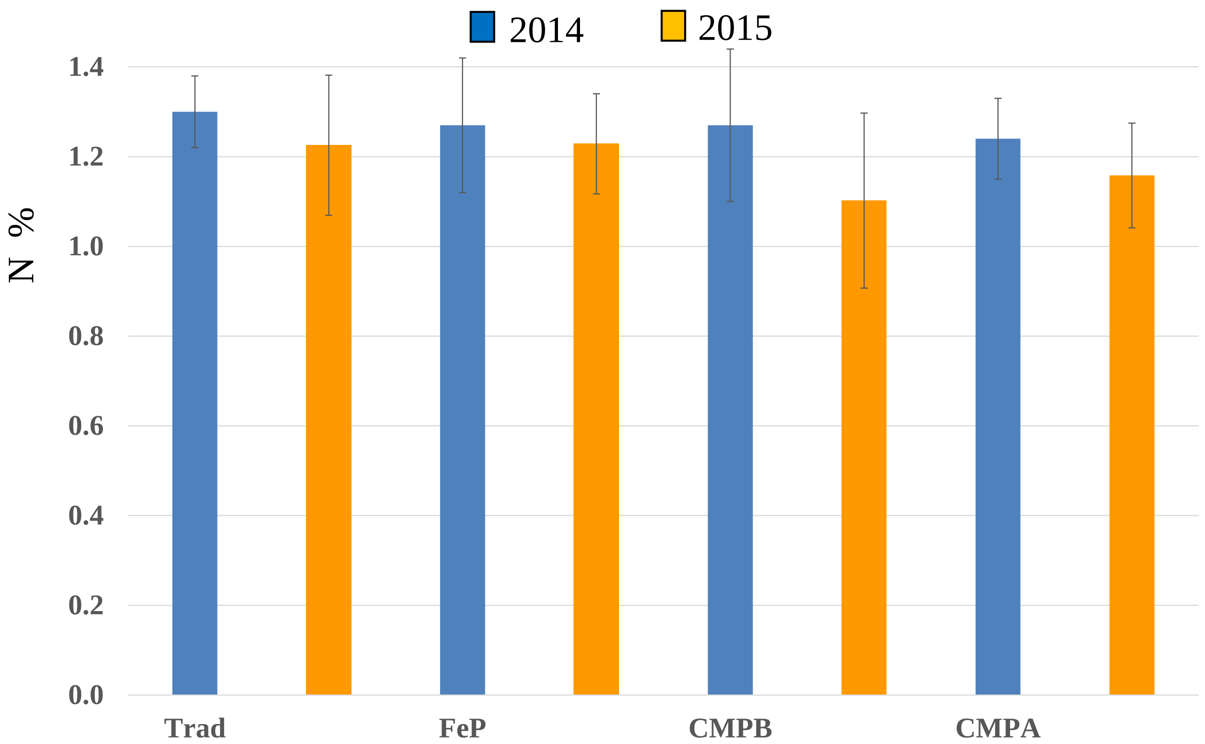


Figure 23 Nitrogen content in maize grain from Castel Volturno experimental trial, in 2014 and 2015

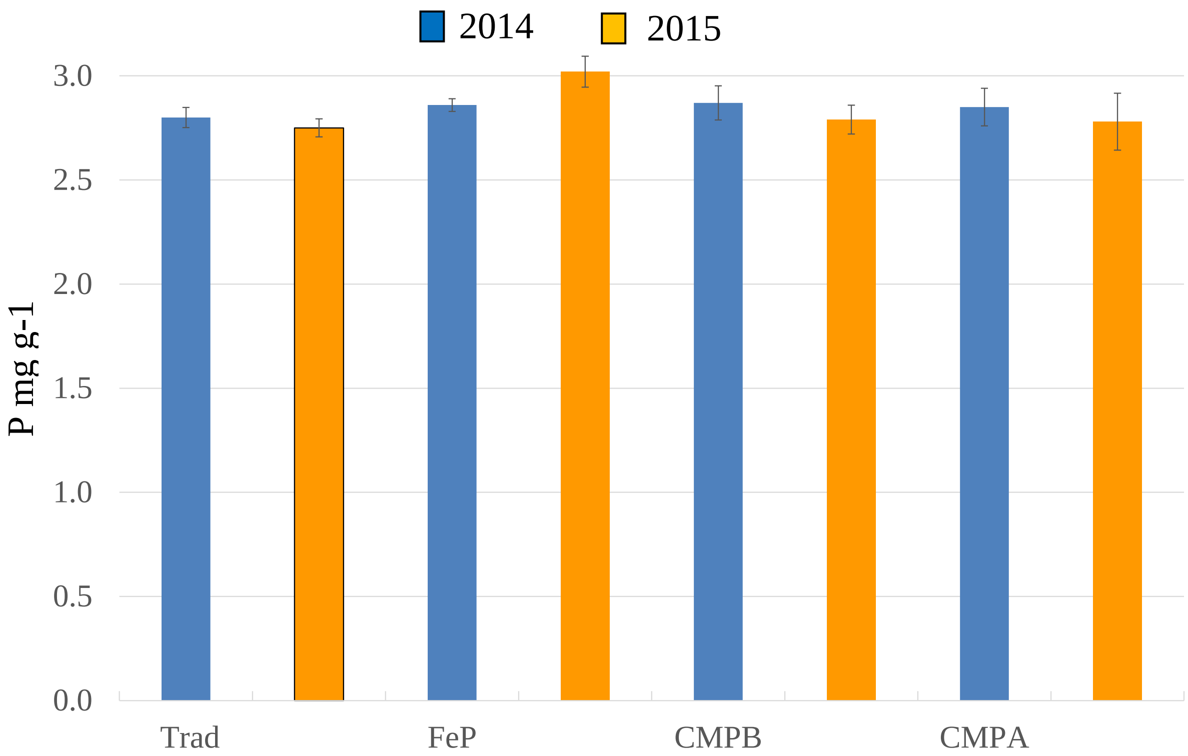
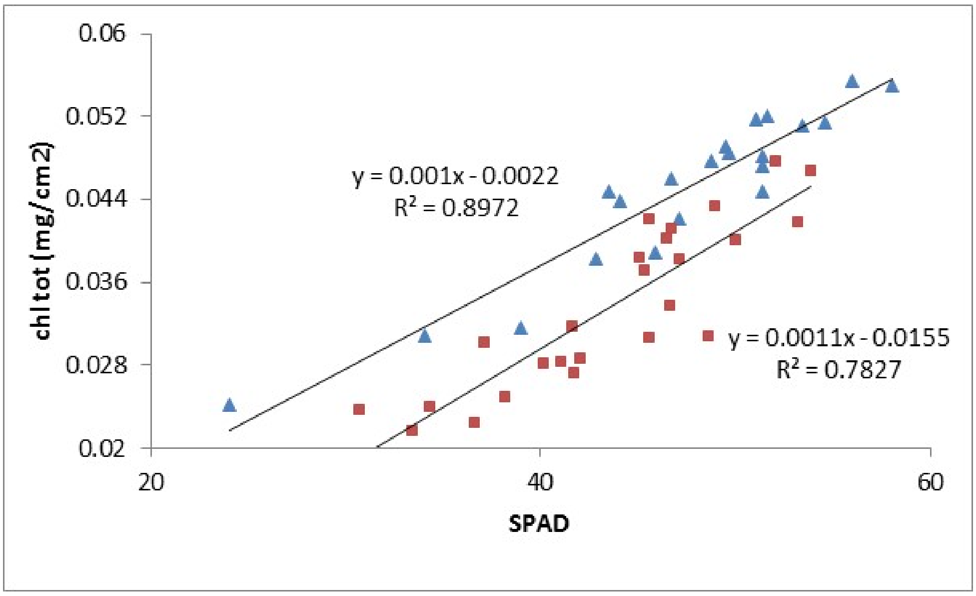


Figure 24. Phosphorus content in maize grain from CasteVolturno experimental trial in 2014 and 2015

Table 3. Photosynthetic status measured as SPAD values for maize (n= 80)

	23/07/2014	1/09/2014	16/7/2015	10/09/2015
TRAD	51.8 (2.96)	51.9 (3.47)	51.29 (2.61)	51.23 (3.41)
PORF	50.6 (4.15)	49.8 (3.77)	53.63 (3.07)	51.55 (2.65)
CMPB	52.9 (3.16)	49.9 (3.00)	52.85 (1.65)	50.35 (3.80)
CMPA	53.1 (3.93)	50.9 (2.26)	52.07 (4.18)	51.86 (3.47)

(standard deviation in parenthesis)



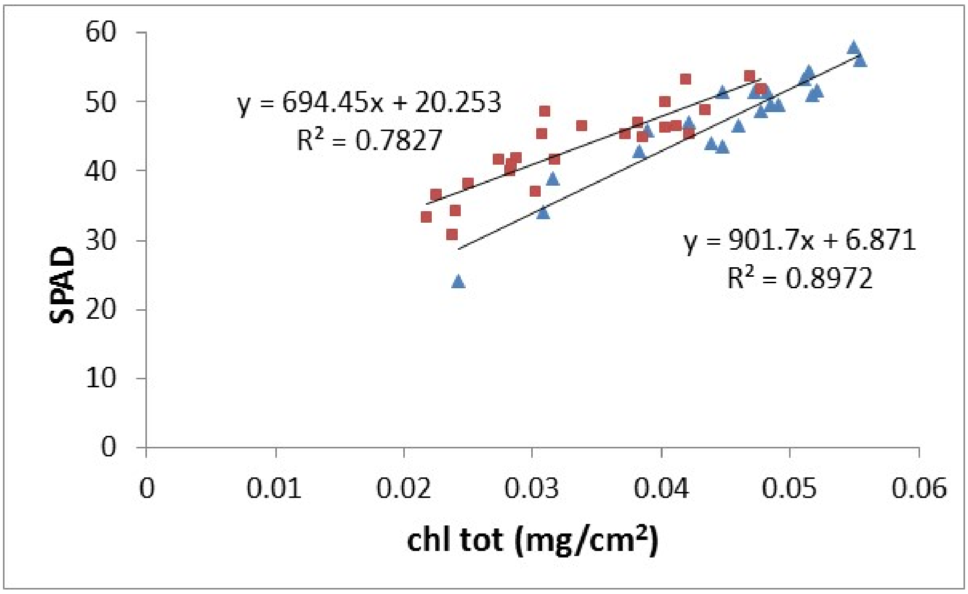


Figure 25 Direct and inverse correlation between maize leaves chlorophyll content and SPAD index

■ july 2014 ▲september 2014

Notwithstanding with the slower fertilizer potential of humified compost, in the second project period, the field plots added with on farm manure compost showed an overall maintenance in the yield of maize grain, comparable in respect to traditional managed plots, with a global amount that varied from -6% to -3 % for CMPB and CMPA samples (Fig. 26). The maize yields indicated a progressive approach, following the annual SOM managements, to new soil equilibrium that should allow a more efficient utilization of the nutrients supplied with organic amendments, as revealed also by the similar level of N and P concentrations found for each soil treatments (Figs. 27, 28). No differences were found among treatments in the photosynthetic status of maize plants, as measured by SPAD methodology (Table 4).

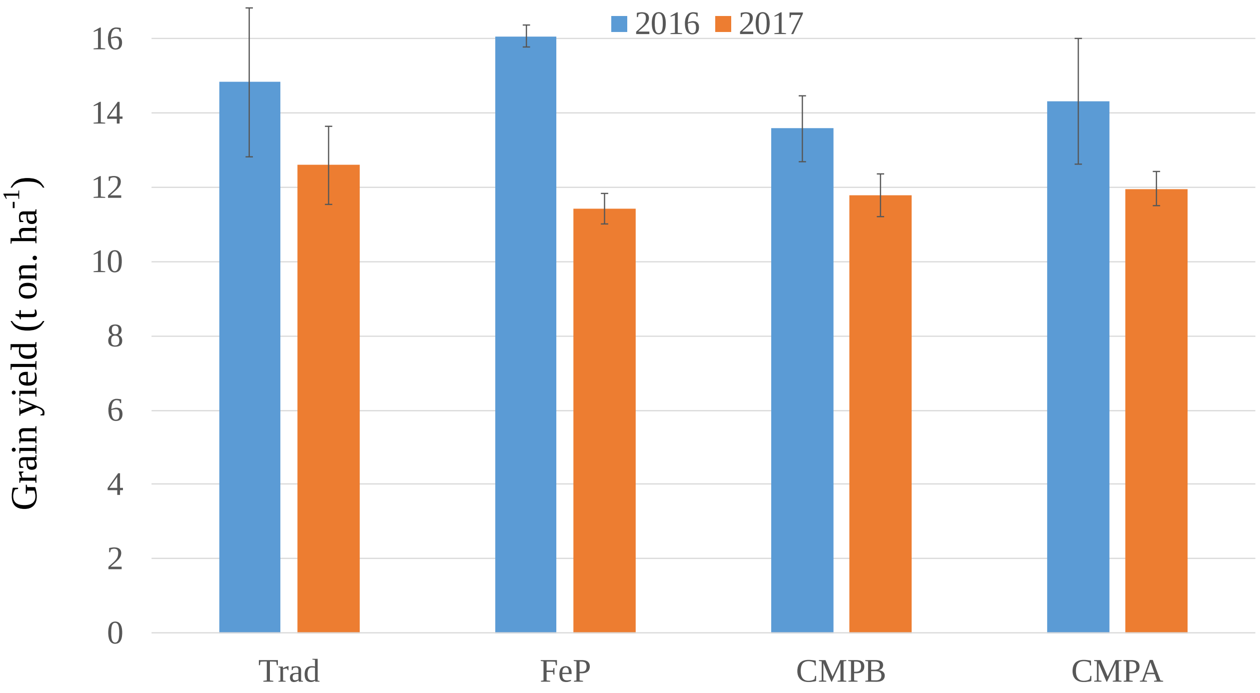


Figure 26. Grain yield of maize cultivated at CastelVolturno experimental trial in 2016 and 2017

Table 4 Photosynthetic status measured as SPAD values for maize (n= 90)

	18/07/2016		15/09/2016		28/06/2017		123/09/2017	
TRAD	47.8	(3.4)	54.7	(4.3)	51.2	(1.8)	56.1	(3.2)
PORF	52.1	(4.1)	58.6	(2.2)	50.6	(2.3)	57.6	(4.1)
CMPB	48.6	(4.1)	59.2	(2.8)	49.9	(3.7)	58.4	(1.8)
CMPA	51.0	(3.3)	57.6	(2.6)	52.3	(2.6)	55.8	(3.4)

(standard deviation in parenthesis)

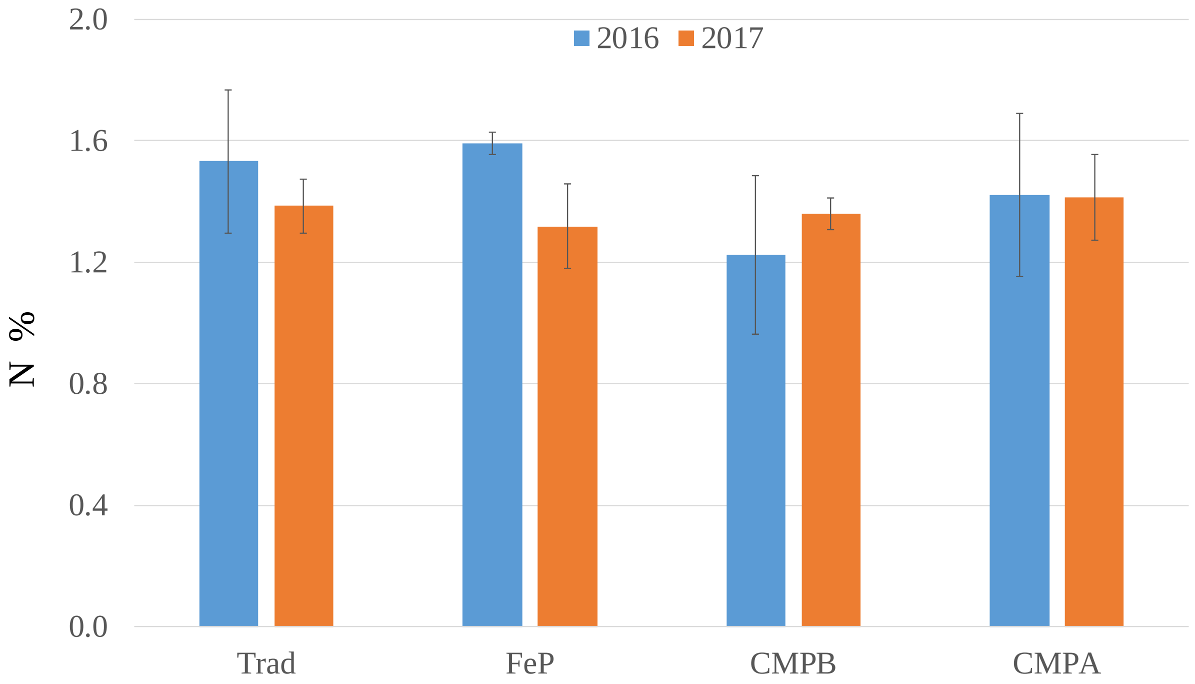


Figure 27 Nitrogen content in maize grain from Castel Volturno experimental trial, in 2016 and 2017

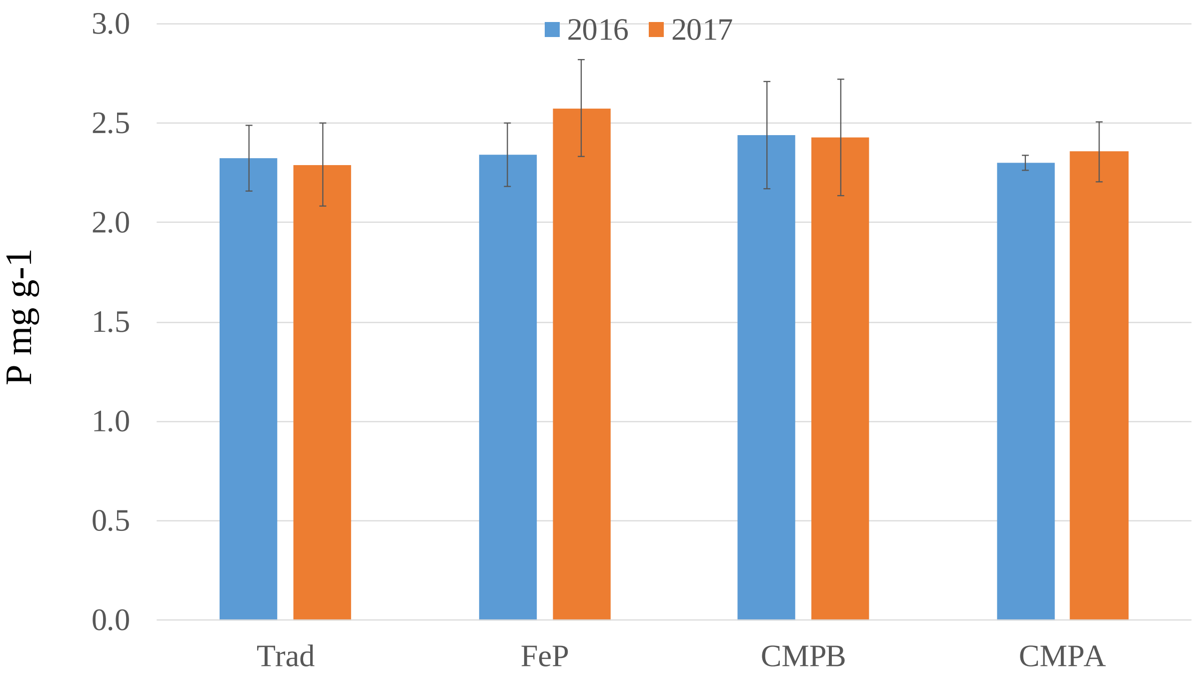


Figure 28. Phosphorus content in maize grain from CasteVolturno experimental trial in 2016 and 2017

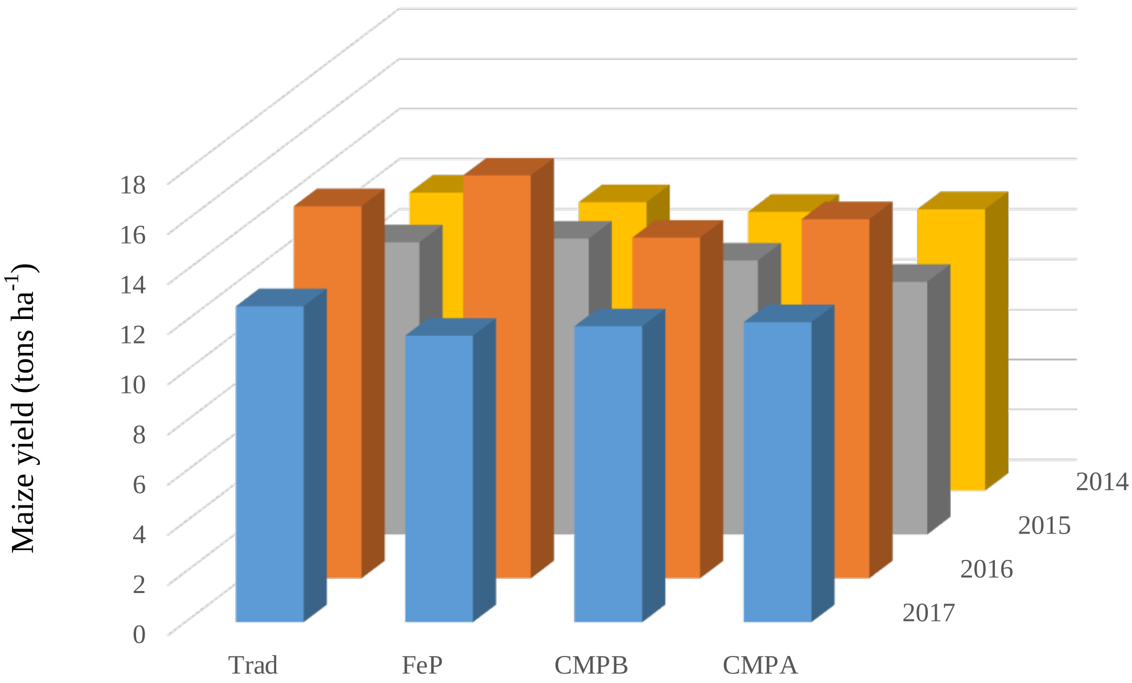


Figure29. Resume of maize yield at Castel Volturno project site

2.4 Prima Luce

Crop: Endive scarole

During the cycle, ten SPAD measures (the first on 22 October 2014 and the last just before harvesting) were made on the leaves by Konica Minolta instrument. The average SPAD values did not show significant differences in the first part of the escarole cycle, but in the period from December 2014 untill January 2015, SPAD values drastically decreased. Afterwards, only the trial A showed slightly lower SPAD values when compared to the other trials (Fig. 30).

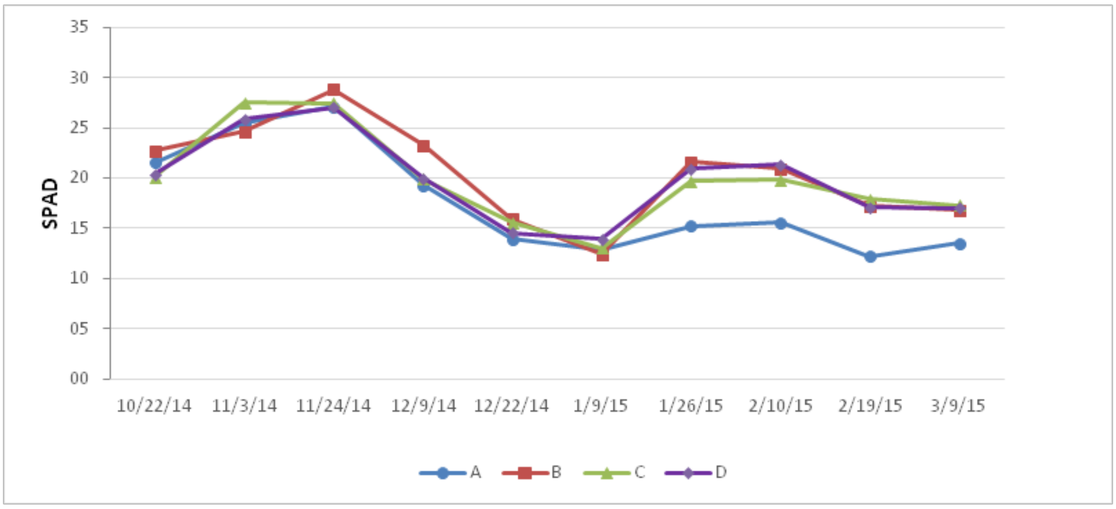
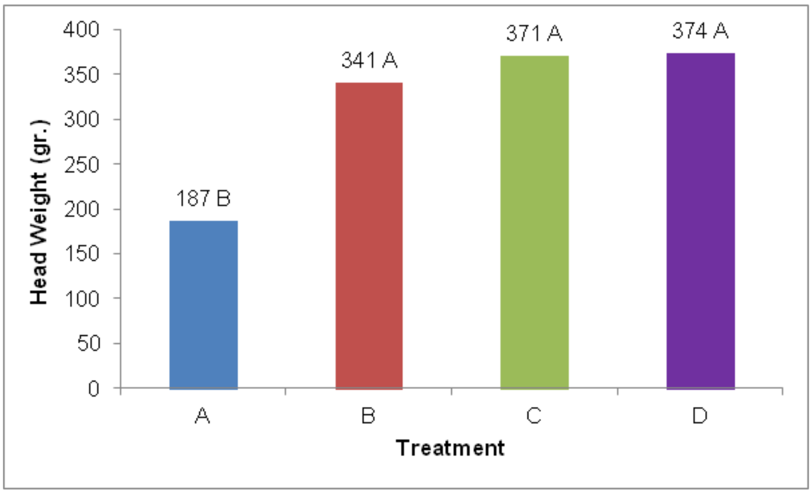


Figure 30. SPAD values monitored during the escarole cycle

A = No fertilized/No amended (control); B = organic and mineral fertilization; C = On-farm compost (10 Ton ha^{-1}) of dry matter + mineral fertilization; D = On-farm compost (20 Ton ha^{-1}) of dry matter + mineral

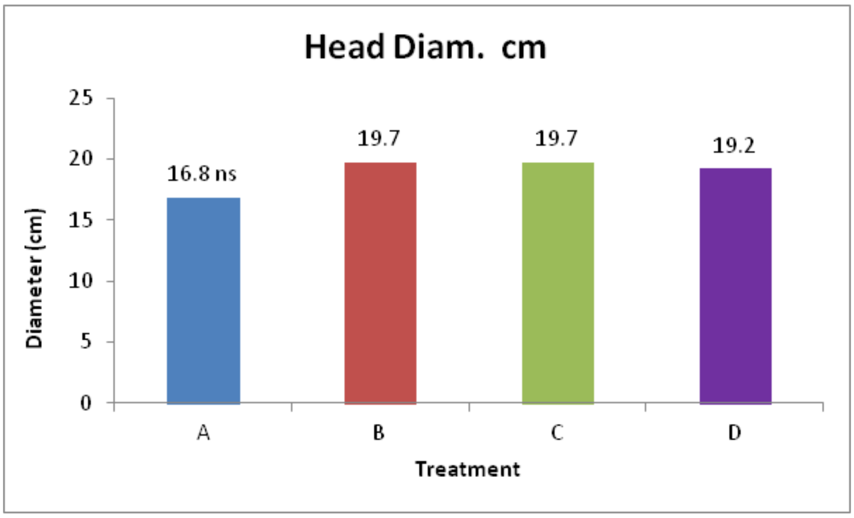
The harvest took place on 18th March 2015 and on representative soil areas some morphological relieves were carried out: weight and diameter of the cutted escarole head, dry matter content and aerial production. The average weight of the escarole head varied from a minimum of 187 gr in the control treatment (A) to a maximum of 374 gr in the most amended treatment (D). The three amended/fertilized treatments (B, C and D) did not show significant differences and the treatment A was statistically lower compared to the others (Fig. 31).



The letters indicate the different statistical values with P<0,05 (HSD Tukey test)

Figure 31. Average weight of the cutted escarole head at the end of the cycle

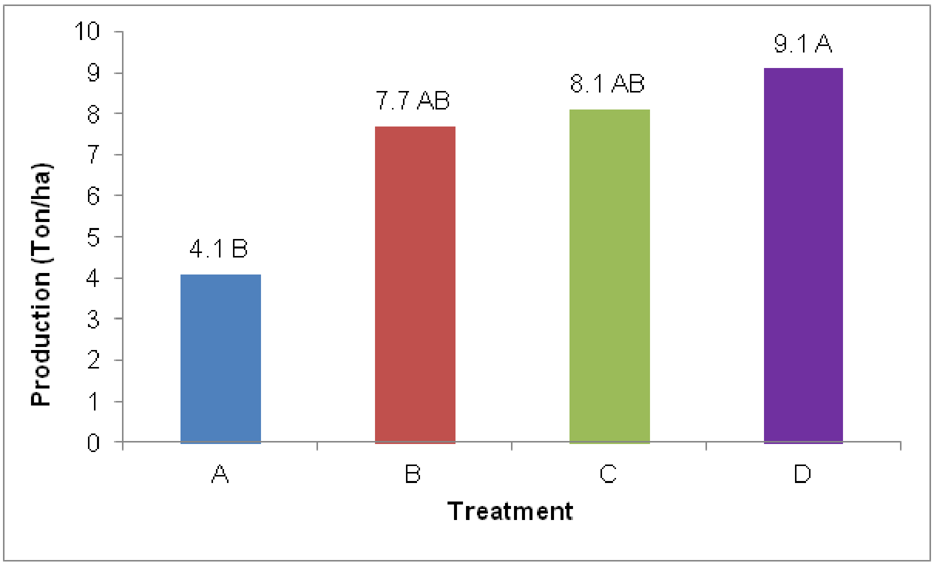
The average diameter of the escarole head did not show any significant difference between the trials but was slightly lower in the non-fertilized control (A) (Fig. 32).



The letters indicate the different statistical values with P<0,05 (HSD Tukey test)

Figure 32. Average diameter of the cutted escarole head at the end of the cycle

Aerial commercial production was significantly higher in the trial D (9,1 t ha^{-1}) which was fertilized with 20 t ha^{-1} prior to planting and with nitrogen during the season, while the non-fertilized trial gave the lower commercial production (Fig. 33).



The letters indicate the different statistical values with P<0,05 (HSD Tukey test)

Figure 33. Escarole commercial production observed between the four treatments.

Crop Pumpkin

During cultivation cycle, SPAD was measured. In the first part of the cycle the trial A showed lower values while, in the second part of the pumpkin cycle, there were no difference between the treatments (Fig. 34).

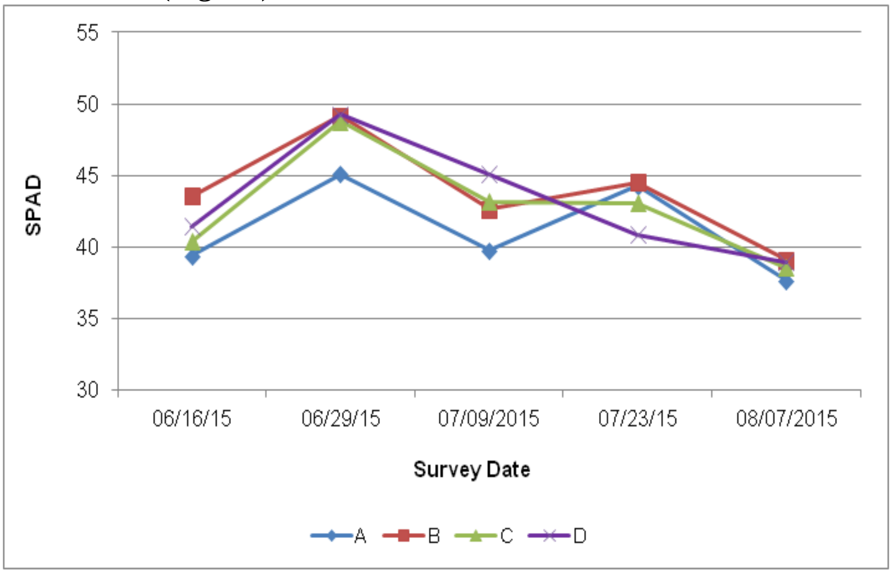


Figure 34. SPAD data collected on pumpkin plants during the cultivation cycle

The harvest took place on 25th August 2015 and the commercial fruit per plant and the related weights were measured. The average number of the fruits for single plant was 4.6, with the lowest value (3.5) for the treatment A and the highest value (5.9) for the treatment D (Fig. 35).

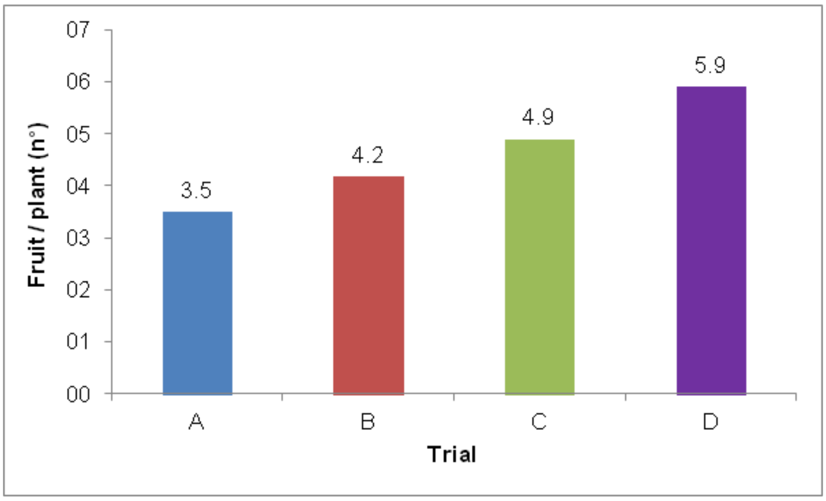


Figure 35. Number of commercial fruits verified at the end of the pumpkin cultivation cycle (ANOVA not relevant)

On the chosen representative soil areas, average weight of commercial fruits was the lowest (772 g) for treatment A, the highest (884 g) for treatment B (Fig. 36).

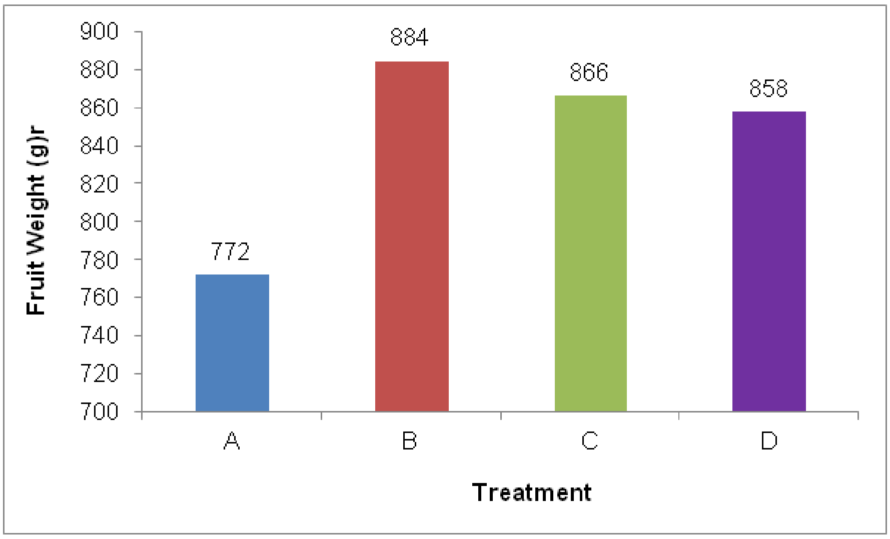


Figure 36. Average weight of the commercial fruit verified at the end of the pumpkin cultivation cycle (ANOVA not relevant)

Total production was the lowest (5,5 t ha-1) for treatment A and the highest (10.3) for treatment D (Fig. 37).

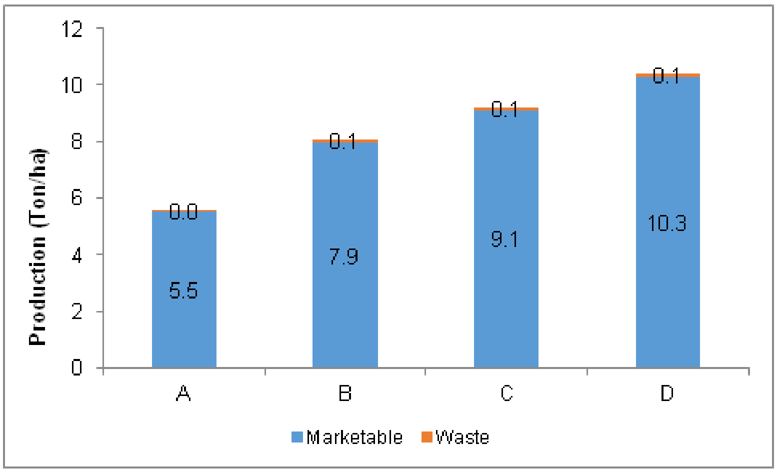


Figure 37. Pumpkin production (ANOVA not relevant)

Chemical quality of endive-escarole and pumpkin

During 2016 chemical quality of endive-escarole and pumpkin harvested on 18th March 2015 and on 25th August 2015 respectively, were analysed on cryopreserved samples. Endivia-escarole was analyzed for water content, nitrate contents and juicines; pumpkin was analyzed for water content, pH, °Brix, reducing sugars and acidity. Results are reported in **Tables 5** and **6**

Table 5. Chemical quality of endive-escarole

Treatment	Water (%)	Nitrate (ppm)	Juicines (mg H_2O/cm^2)
A	88	110	109
B	90	450	115
C	89	190	110
D	90	260	114

Table 6. Chemical quality of pumpkin

Treatment	Water (%)	pH	°Brix	Sugars (%)	Acidity (%)
A	85.4	5.90	11.02	0.81	0.18
B	87.0	5.79	8.62	0.75	0.16
C	83.9	5.86	10.06	0.96	0.18
D	85.5	5.75	11.00	1.03	0.23

Agronomic trials, SPAD measure and chemical quality of baby-broccoli

Baby-Broccoli plants (cv. Marathon), transplanted on 28th October 2015 after pumpkin cultivation, were harvested on 1st April 2016. Plants were distributed in single rows with 0.9 m between the rows and 0.5 m between the plants on the rows. The density was 22,000 plants ha^{-1}. A randomized blocks design with three repetitions was used. Experimental treatments consisted of:

A) Non-fertilized/Non-amended (control);
B) Organic and mineral fertilization (32,4 kg/ha N, 54 kg/ha P_2O_5 and 75,6 kg/ha K_2O) distributed before transplanting; during crop cycle, 53 kg/ha N was added;
C) On farm compost 10 t ha^{-1} of dry matter before transplanting; during crop cycle, 53 kg/ha N was added;
D) On farm compost 20 t ha^{-1} of dry matter before transplanting plus mineral fertilization as in C.

SPAD measured during crop cycle, showed the lowest values for treatments A and B and the highest values for treatments C and D **(Figure 38)**.

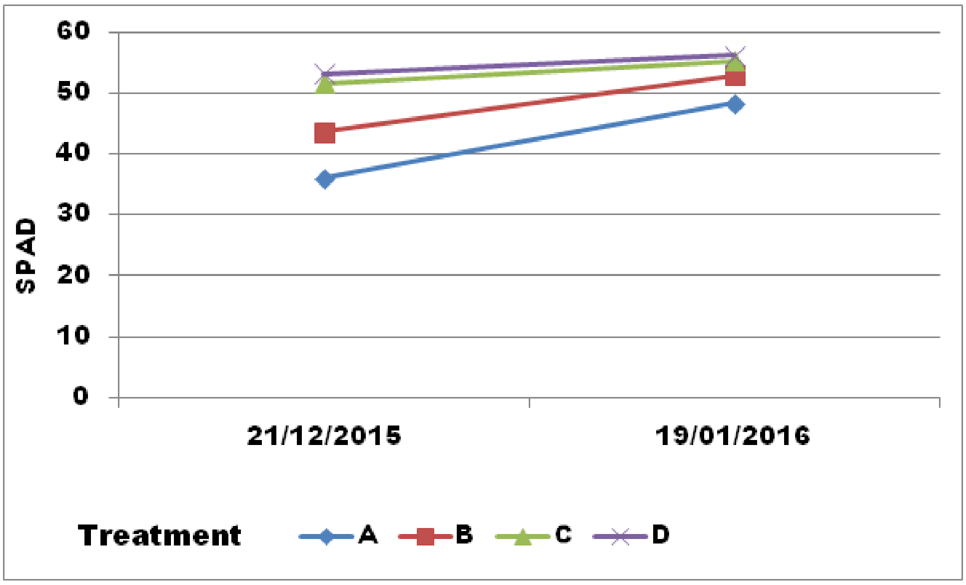


Figure 38. SPAD values during crop cycle of baby-broccoli.

Data about production and biometric relieves on baby-broccoli are reported in **Figures 39** and **40**. The highest production were registered for treatment D and C, whereas the lowest were registered for treatment A and B. The same trends were observed for length and diameter of broccoli heads and are in agreement with the SPAD values registered during crop cycle. These results show the ability of vegetable compost to improve broccoli production.
Chemical quality (water content, pH, °Brix, and acidity) of baby-broccoli are reported in **Table 4.** No particular differences were registered about them, except for the lowest values of °Brix for treatment D.

Table 7. Chemical quality of baby-broccoli harvested

Treatment	Water (%)	pH	°Brix	Acidity (%)
A	83.5	5.90	8.8	0.22
B	82.0	5.93	10.1	0.25
C	82.5	5.96	8.7	0.22
D	83.0	6.05	6.6	0.24

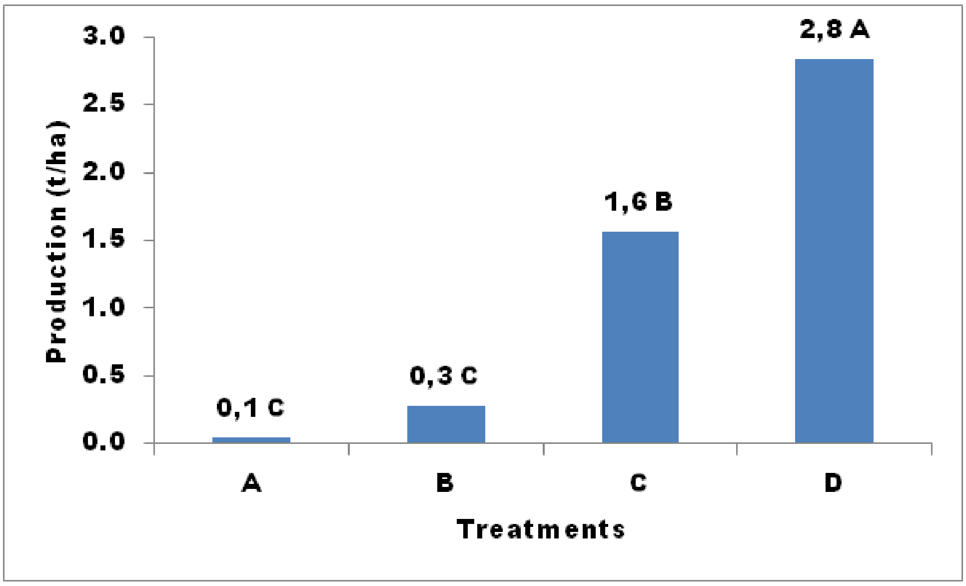


Figure 39 Production of baby-broccoli

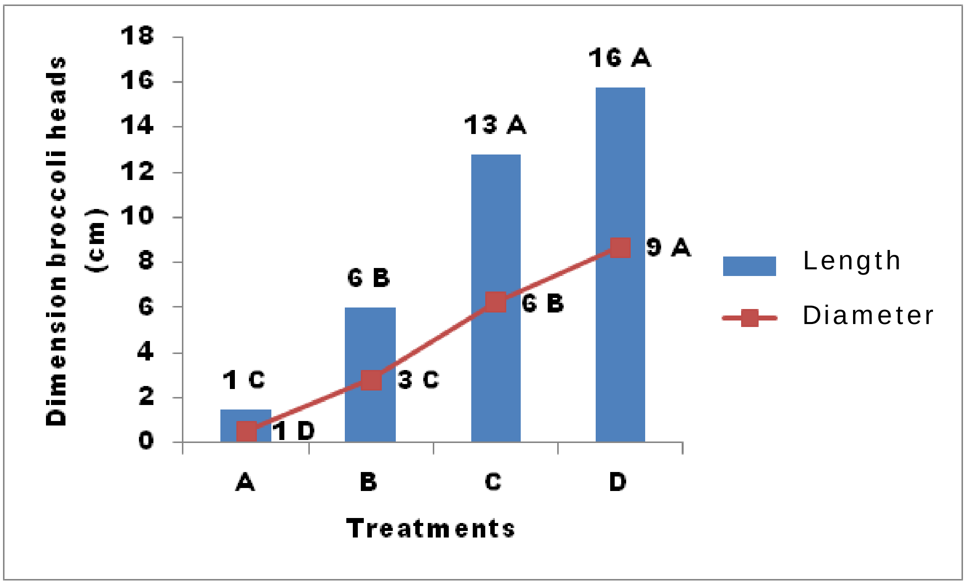


Figure 40. Dimension of baby-broccoli heads at harvesting

Agronomic trials, SPAD measure and chemical quality of bean

Bean (Borlotto type) was sown on 5th June 2016 after baby-broccoli. A randomized blocks design with three repetitions was used. Experimental treatments consisted of:

A) Non-fertilized/Non-amended (control);
B) Pelleted organic fertilizer title 3-5-7 (30 kg/ha N, 50 kg/ha P_2O_5 and 70 kg/ha K_2O) distributed before sowing. After sowing, inoculation of rhizobia into the soil was performed;
C) On farm compost 10 t ha^{-1} of dry matter before sowing. After sowing, inoculation of rhizobia into the soil was performed;
D) On farm compost 20 t ha^{-1} of dry matter before sowing. After sowing, inoculation of rhizobia into the soil was performed.

Seeds were planted at a distance of about 5 cm on the rows and 75 cm among the rows.

SPAD was measured during crop cycle. Harvesting was made at waxy maturation on 13th August 2016. SPAD measured during crop cycle, showed similar trends for all treatments, probably due to activity on nitrogen-fixing rhizobia **(Figure 41)**.

Data about production and protein content in bean seeds are reported in **Table 8**. No difference in production was registered among treatments B, C and D, whereas for treatment A was registered the highest production. About protein content, the highest content was registered for treatment D.

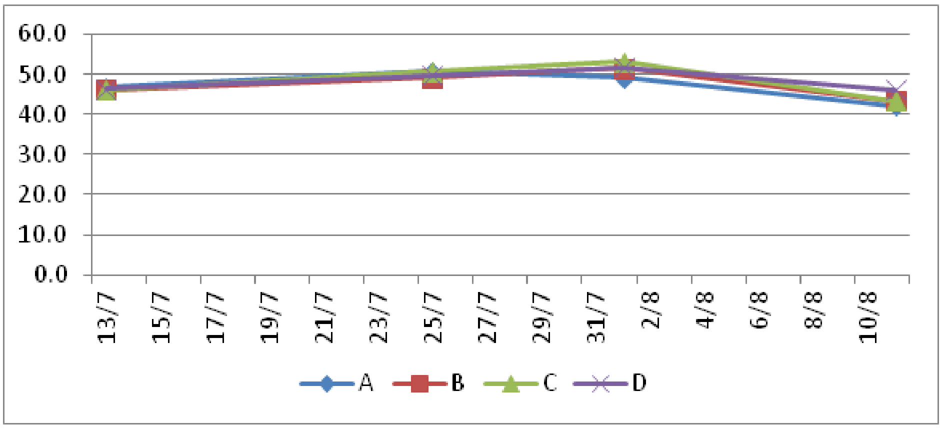


Figure 41. SPAD values during crop cycle of bean

Table 8**.** Production of bean at waxy maturation and protein content in the seeds.

Treatment	**Yield (t/ha)**	**% protein (seeds 13% umidity)**
A	2.37 a	21.26
B	1.81 b	19.04
C	1.75 b	21.09
D	1.64 b	22.86

Monitoring of nitrate content, pH and electrical conductivity in the soil

Monitoring of nitrate content, pH and electrical conductivity in the soil is reported about Winter season 2015-2016 during cabbage-broccoli cultivation and in Summer season 2016 during bean cultivation. Soil treatments were: no-fertilized control soil (A); mineral fertilized soil (B); soil treated with 10 t ha^{-1} of compost (C); soil treated with 20 t ha^{-1} of compost (D).

During the first and the second crop cycle measures of moisture, pH, electrical conductivity (EC) and nitrates, were carried out for seven times in each cycle. The moisture level achieved in the soil was monitored gravimetrically at 378.15 K and expressed as % of the soil weight; pH and EC were measured with a portable meters (HI 9811-5 Hanna Instruments). The conductivity measured in µS cm^{-1} was transformed in dS m^{-1} and expressed for gram of dry soil (Barbieri and De Pascale, web sites; Sequi, 2007; Ajres and Westcott, 1993). Nitrates were monitored with an UV-Vis spectrophotometer (DU 64 Beckman Coulter s.r.l.) in the wavelength of the ultraviolet wave (UV) and were expressed as ppm (Nemade *et al*., 2014; Edwards *et al*., 2001; Merafina, 2003; Pon 2007-2013, web site). A dilution of 1: 5 (soil: water) was used for pH and EC determinations.

Due to the rain, the humidity of soil during winter shows two peaks, 30.64% and 30.19%, at the III and the V relieve respectively. The average of humidity remained higher (28.06%) than the first year (26%). During Summer, the average of humidity was 22.07%, higher than the first year (21.98%).

During Winter, pH values were lower when nitrate concentrations were higher, probably due to fertilization; the averages values of pH were very similar among the plots (8.01, **Figure 42**). In the Summer, pH had two peaks (II-IV times) and pH mean was 8.10 **(Figure 43)**. The pH values resulted to be low when the nitrate concentrations were highest in both seasons. Both seasons during 2016 had lower values than the previous year.

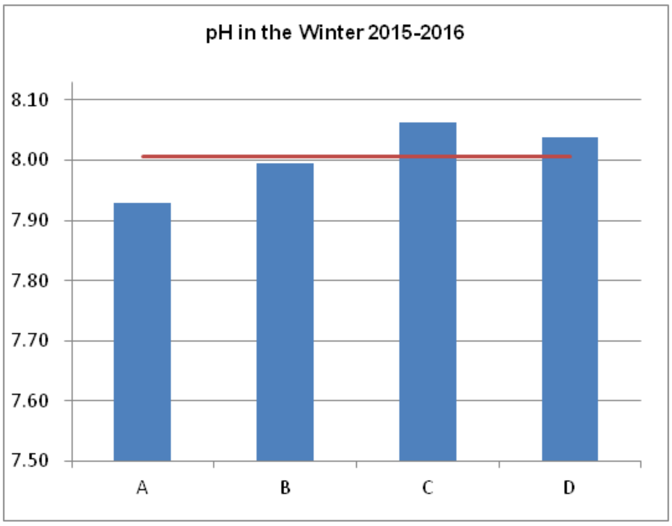


Figure 42. pH values registered during Winter 2015-2016

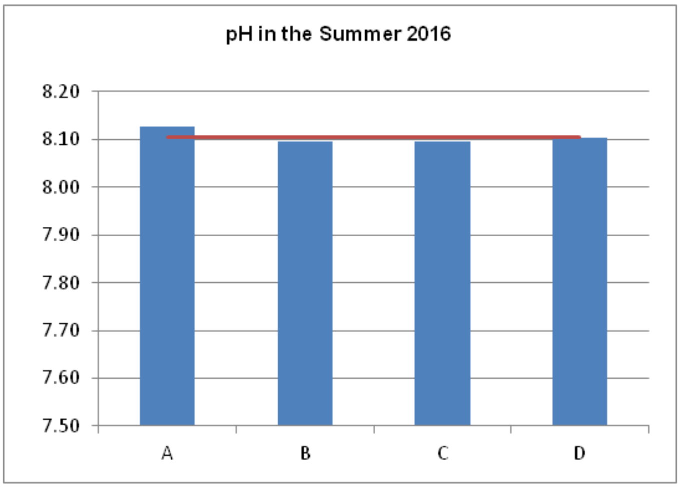


Figure 43. pH values registered during Summer 2016

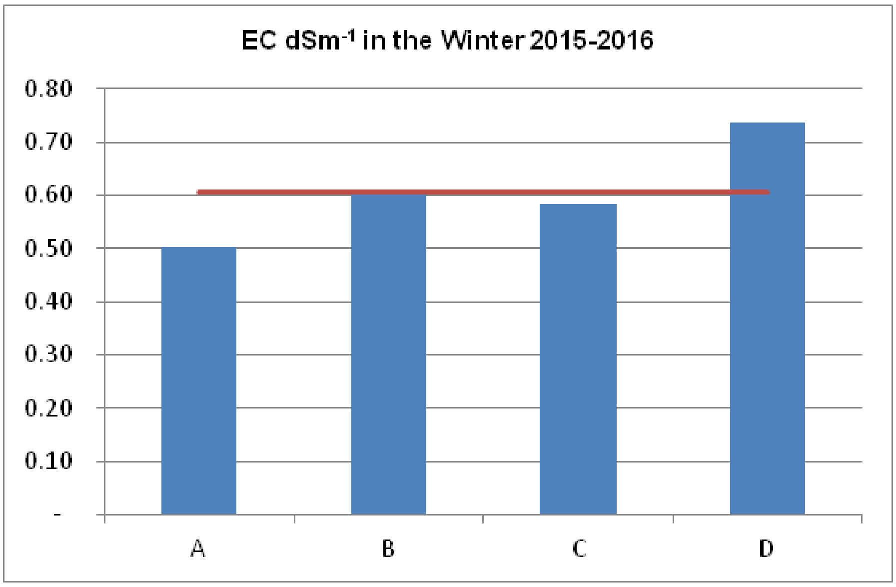


Figure 44. EC values registered during Winter 2015-2016.

Winter conductivity recorded was, on average, 0.61 dS m^{-1} for a dilution of 1:5 (soil: water). It shows a peak corresponding to a high value of nitrates **(Figure 44)**.

The Summer conductivity had the same trend of nitrates concentration. In the Summer, conductivity showed two peaks (I and VI times) with an average of 0.77 dS m^{-1}. It falls within class ‘’0-2 dSm^{-1}’, corresponding to a range of values with satured soil extract **(Figure 45)**.

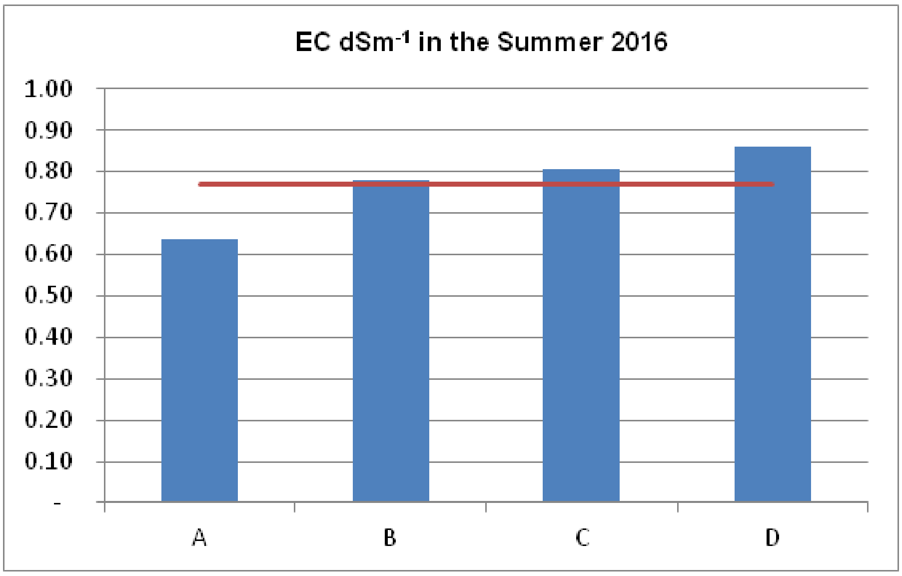


Figure 45. EC values registered during Summer 2016

Nitrates concentration determined by spectrophotometer using ultraviolet radiation showed, during Winter, a peak that reached the highest average value of 67.10 ppm and 95.18 ppm at the first and sixth sampling time, due to fertilization events. On average, nitrate was 41.62 ppm **(Figure 46)** and increase from the treatment A to the treatment D (A=20.62; B=39.23; C=51.06; D=55.57 ppm). The treatments with the compost had the highest values of nitrates and the statistical analysis is significant when it is compared to the control. During Summer season, the averages for each treatment were the following: A=23.06; B=27.56; C=58.74 and D=50.61 ppm. These averages are without the first sampling in B treatment, because it heavy influence nitrate concentration **(Figure 47)**. There were two peaks in all treatments, one in June and a larger one in October; their means were 38.02 and 40.04 ppm, respectively. This phenomenon was present in all treatments during Summer because, in this season, nitrates are produced by a remarkable degradation of organic matter. The Summer mean value of nitrates was 40.13 ppm (50.17 ppm with the first sampling in B treatment, **Figure 47**). The highest nitrates values of 99.78 and 78.21 ppm, registered in the treatments C and D respectively, were due to the mineralization of the compost amended into the soil.

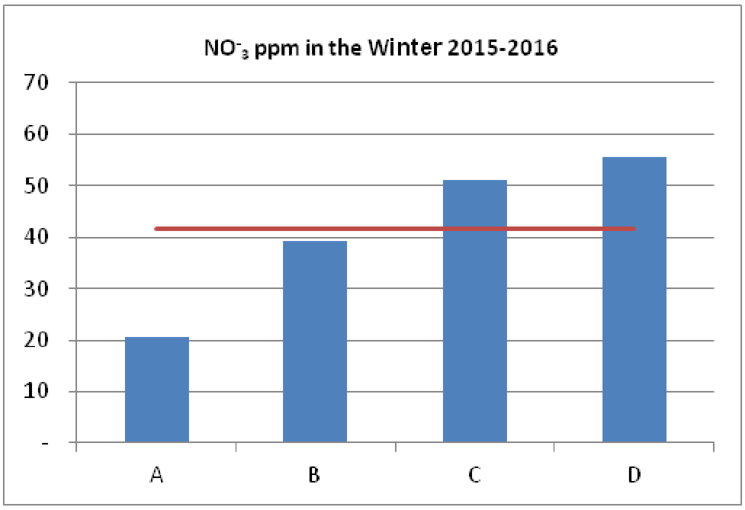


Figure 46. Nitrate content registered during Winter 2015-2016.

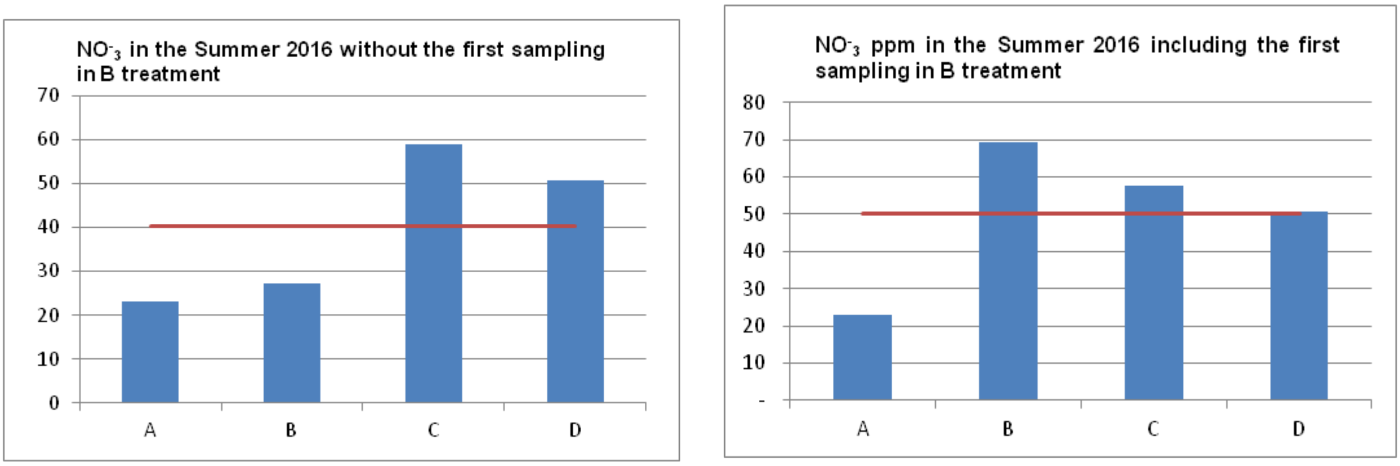


Figure 47. Nitrate content registered during Summer 2016 with and without the first sampling of B treatment

2.5 Mellone

Materials and methods

The quantitative and qualitative assessment of yields was performed by means of the classical parameters used for fruit marketing. However other unusual analysis (total phenols) were performed in order to better describe fruit quality. The quality assessment performed during the first year of activities was really useful not only for the results obtained but also for the development and calibration of the analytical methodologies.

- Fruit sampling

Five compost amendment treatments were defined in the kiwi orchard (indicated in tables as K:

- Control (0),
- Amendment with light compost, indicated with A, using a dose of 10 t ha^{-1} (KA1)
- Amendment with light compost, indicated with A, using a dose of 20 t ha^{-1} (KA2)
- Amendment with heavy compost, indicated with B, using a dose of 10 t ha^{-1} (KB1)
- Amendment with heavy compost, indicated with B, using a dose of 20 t ha^{-1} (KB2)

For each amendment treatment 6 sampling points were identified according to the ESAP methodology (for a detailed description please see the paragraph titled "Extraction of soil sampling points: ESAP methodology (C.2)" within the Life Carbonfarm n°1 Report). These points, with known geographic coordinates, marked as green points within the map, were called with a specific abbreviation (i.e. K0_4, where K0 indicates the control and 4 the 4th sampling point) (Fig. 48) and represented the points of major soil variability within the corresponding orchard.

The yield of three trees per each sampling point (for a total of 90 plants) was measured at maturation harvest (Fig. 49).

A sample of 50 fruits per plant (for a total of 1500 fruit) was then taken to calculate the mean fruit weight and size.

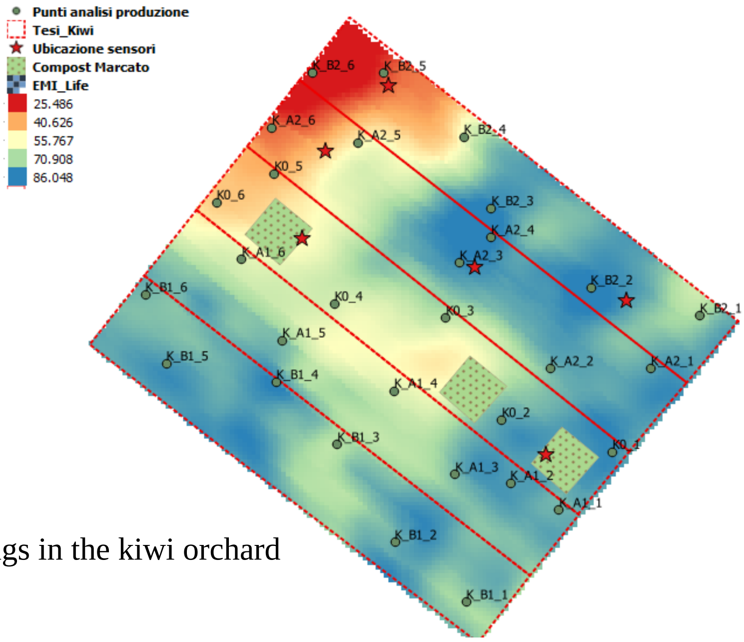


Figure 48 – EMI map used for samplings in the kiwi orchard

Figura 49 - Kiwifruit harvest

Otherwise, in the peach orchard five trees were randomly chosen for each treatment (which were the same of those performed in the kiwi orchard: amendment with light and heavy compost at two different doses - P0: control; PA1; PA2; PB1; PB2). On these plants two different maturation harvests were performed.

- Size

The assessment for the kiwifruit size was referred to the number of fruit per 3kg of product according to the commercial calibration scale reported in Table 9. The measurements were carried out with a digital balance.

Table 9 - Commercial calibration scale for kiwifruit

Size	Weight (g)
18	150 +
20	135/150
23	125/135
25	115/125
27	105/1015
30	95/105
33	85/95
36	80/85
39	75/80
42	70/75
46	65/70
49	60/65
50	50/60
Flat kiwifruit	-
Buttefly kiwifruit	-

The size of the flat peaches was determined by measuring the equatorial diameter with a Vernier caliper (Fig.50). Peaches were classified according to the calibration scale reported in Table 10.

Figure 50 – Vernier caliper

Table 10 - Commercial calibration scale for kiwifruit

Class	Diameter
AAA	80+
AA	76-80
AA	72-76
A	70-72
A	68-70
B	64-68
B	61-64
C	56-41

- Dry matter content

Dry matter content was measured by weight loss method after fruit drying in an air-forced oven at 60°C till they reached constant dry weight.

Figure 51 – Fresh weight of flat peaches measured with a digital balance

- Firmness

Thirty kiwifruit (5 per sampling point)and ten peaches per treatment (sampled in each harvest) were used for fruit firmness (N) determination. These determinations were performed on 2 (kiwifruit) and 4 (peach) opposite sides of the fruit equatorial surfaces by means of a steel cutter for peel removing (about 2 mm thickness) and a digital penetrometer (PCE-PTR200) equipped with an 8 mm tip (Fig. 52).

Figure 52 - Digital penetrometer

- Colour

Colour was measured on ten peaches per treatment by means of a digital colorimeter (Konica Minolta Chroma Meter CR 400). Particularly, 4 readings per each face of one fruit were performed. The color was expressed as CIE 1976 L*a*b*, chroma (C*) and hue (h*), with L* representing the lightness of the color, a* the redness to greeness of colour, b* the yellowness to blueness of colour. C* represents the intensity or purity of color, while h* represents hue of colour.

- Solid soluble content

For both, peaches and kiwifruit, total soluble solids were measured by means of a portable refractometer (Fig. 53) making the average of two readings per fruit. Kiwifruit juice was extracted from the two fruit parts cut about 15 mm from the base and the top. Thirty kiwifruit (5 for each sampling point) were used. Peach juice was extracted from the two fruit parts opposite to the equatorial axis. Ten peaches per harvest were used for solid soluble content determination.

Figure 53 - Portable refractometer

- Titratable acidity

Titratable acidity was measured on fruit juices by titration with 0.1 N NaOH and phenolphthalen (as indicator). Titratable acidity was referred to the equivalent content of malic and citric acids for peaches and kiwifruit, respectively. Analysis were performed on 12 samples per treatment, each composed by 2.5 kiwifruit, and 4 samples per treatment, each composed by 3 peaches.

Figure 54 – On the left: kiwifruit samples. On the right: titration for acidity determination

- Total Nitrogen (Kjeldhal method)

Total nitrogen was measured by Kjeldhal method. Six N determinations per treatment (one for each sampling point) were performed on kiwifruit. Four N determinations (2 per harvest) per treatment were carried out on composite peach samples.

- Total polyphenols

Total polyphenols, expressed as mg 100 g^{-1}, was determined by the Folin Ciocalteu colorimetric method, using gallic acid as a standard. Analysis were performed on water extracts of peeled fruits (4 subsamples per treatment, each coming from a composite sample made of 3 peaches; 6 kiwifruit per treatment with two juice extraction per fruit). The absorbance was measured at 743 nm by a spectrophotometry.

Figure 55 – Total polyphenols: sample preparation and spectrophotometric analysis

-Antioxidant activity

Antioxidant activity was measured only on kiwifruit juice (the same samples used for total polyphenols analysis). It was measured as the absorbance reduction of the ABTS radical cation at a 734 nm (Re et al., 1999). The measurement was calculated as the decrease percentage of the absorbance, defined as "inhibition percentage". The clibration curve was obtained by Trolox and data were expressed as mmol Trolox equivalent 100 g^{-1} on fresh weight basis.

Figure 56 – Spectrophotometer

- Statistical analysis

Data were analyzed by one-way ANOVA (Statistica 10.0 software). Means were separated according to Duncan's test ($p \leq 0.05$).

2.5.1 Kiwi

Fruit quality assessment at maturity harvest

Generally, no significant differences were found among the 5 treatments for the measured quantitative and qualitative variables, thereby supporting the maintenance of fruit productivity with the applied SOM management based on the on-farm green composts Some differences in the first year of project activities (i.e. mean fruit weight per K0 and KA1 treatments) (Tab. 11) seem to be due to fruit thinning rather than compost distribution. In fact, decrease in crop loads corresponded to an increase of the mean fruit weight. However, an estimated effect of the compost application on plant physiology was suggested by the steady increase in the mean percentage of quality parameters such as dry matter content, solid soluble, total dry matter and total N, although without any clear correspondence with compost type and annual dose (Tabs. 11, 12)

Table 11 - Kiwi: mean values and variance analysis of some parameters of fruit sampled at maturity harvest. K0: control; KA1: light compost using a dose of 10 t ha^{-1}; KA2: light compost using a dose of 20 t ha^{-1}; KB1: heavy compost using a dose of 10 t ha^{-1}; KB2: heavy compost using a dose of 20 t ha^{-1}

Treatment	Fresh fruit weight (g fruit^{-1})		Dry matter content (% fresh weight)		Firmness (kg cm^{-2})		Solid soluble content (°Brix)		Titratable acidity (% citric acid)	
	2015									
K0	134.9	a	17.2	b	6.2	a	6.7	ab	2.2	a
KA1	126.3	b	17.6	b	6.1	a	6.4	b	2.2	a
KA2	130.28	ab	18.4	a	5.9	b	7.1	a	1.9	b
KB1	129.46	ab	18.4	a	6.6	a	6.7	ab	1.8	b
KB2	130.15	ab	18.1	ab	6.4	a	6.9	ab	2.1	ab
	2016									
K0	128.9	a	16.1	ab	6.3	a	7.0	a	1.9	ab
KA1	124.2	ab	17.9	a	6.0	a	6.9	a	1.8	ab
KA2	128.8	a	18.8	a	5.8	a	7.0	a	2.1	a
KB1	127.5	a	17.7	a	6.5	a	7.1	a	2.2	a
KB2	122.1	ab	18.2	A	6.2	a	6.8	a	2.0	a
	2017									
K0	126.2	ab	17.5	ab	6.7	a	6.7	a	2.0	ab
KA1	116.2	b	21.7	a	5.9	b	6.8	a	2.1	a
KA2	131.4	a	19.6	a	6.3	a	7.2	a	2.2	a
KB1	134.1	a	20.2	a	6.8	a	7.2	a	2.2	a
KB2	128.0	ab	21.0	a	6.3	a	7.2	a	2.2	a

Table 12 - Kiwi: mean values and variance analysis of some fruit quality parameters of fruit sampled at maturity harvest. K0: control; KA1: light compost using a dose of 10 t ha^{-1}; KA2: light compost using a dose of 20 t ha^{-1}; KB1: heavy compost using a dose of 10 t ha^{-1}; KB2: heavy compost using a dose of 20 t ha^{-1}

Treatment	Size		Yield		Total dry matter		Mean total N		Total N	
	(class)		(t ha^{-1})		(t ha^{-1})		(%)		Kg	
	2015									
K0	AA	a	13.38	a	2.30	A	1.14	a	26.27	a
KA1	AA	a	18.03	a	3.17	A	1.15	a	36.51	a
KA2	AA	a	13.52	a	2.49	A	1.03	b	25.75	a
KB1	AA	a	15.36	a	2.83	A	1.06	ab	29.89	a
KB2	AA	a	16.43	a	2.97	A	1.01	b	29.96	a
	2016									
K0	AA	a	15.6	a	2.6	a	1.12	a	29.1	ab
KA1	AA	a	16.2	a	2.7	a	1.22	a	33.0	a
KA2	AA	a	19.4	a	3.2	a	1.16	a	37.0	a
KB1	AA	a	15.7	a	2.7	a	1.14	a	30.5	ab
KB2	AA	a	17.7	a	3.0	a	1.24	a	37.1	a
	2017									
K0	AA	a	15.3	ab	2.2	ab	1.15	ab	25.0	ab
KA1	AA	a	19.0	a	3.4	a	1.12	ab	38.5	a
KA2	AA	a	19.5	a	2.2	ab	1.23	ab	21.8	ab
KB1	AA	a	15.2	ab	2.9	a	1.18	ab	29.6	a
KB2	AA	a	15.8	ab	3.0	a	1.38	ab	34.8	aa

Fruit quality assessment at post-harvest

Table 13 shows the fresh weight data, firmness, total soluble solids, total polyphenols content and antioxidant activity in kiwifruit sampled 60 days from harvest. Differences in polyphenols data are highlighted since the first year of compost distribution. Particularly, the content in total polyphenols is high especially in treatments whit larger quantities of both *summer* and *winter* compost types (A2 and B2). A positive response to compost addition was shown by the measurement of antioxidant activity. Table 14 once again takes into account shelf life fruit parameters (firmness and total soluble solids) at 100 days from harvest. While at 60 days there are still no large discrepancies in firmness, at 100 days steady significant differences were recorded by the treatments with compost addition with the best performance shown for B2 samples

Table 13 - Kiwi: mean values and variance analysis of some fruit quality parameters of fruit sampled after 60 days of refrigerated storage. K0: control; KA1: light compost 10 t ha^{-1}; KA2: light compost 20 t ha^{-1}; KB1: heavy compost 10 t ha^{-1}; KB2: heavy compost 20 t ha^{-1}

Treatment	Fruit fresh weight (g $fruit^{-1}$)		Firmness ($Kg\ cm^{-2}$)		Solid soluble content (°Brix)		Total polyphenols (mg 100 g^{-1})[a]		Antioxidant activity (mmol 100 g^{-1})[b]	
	2015									
K0	138.47	a	3.83	a	13.60	a	53.2	bc	0.17	a
KA1	121.38	b	3.55	a	13.84	ab	55.8	abc	0.15	a
KA2	130.31	a	3.38	a	14.19	b	57.2	ab	0.18	a
KB1	122.61	b	3.46	a	14.21	b	52.09	c	0.21	a
KB2	131.30	a	3.34	a	14.20	b	58.2	a	0.23	a
	2016									
K0	134.2	a	3.4	a	13.3	a	50.1	b	0.19	a
KA1	128.1	b	3.3	a	12.9	ab	54.6	ab	0.17	a
KA2	134.1	a	3.3	a	13.9	a	58.1	a	0.25	a
KB1	124.5	b	3.5	a	14.1	a	53.8	ab	0.22	a
KB2	136.2	a	3.2	a	14.4	a	57.9	a	0.24	a
	2017									
K0	132.7	a	3.71	a	13.8	b	52.3	b	0.16	ab
KA1	131.6	a	3.68	a	14.3	ab	56.4	ab	0.18	ab
KA2	136.1	a	3.42	a	14.3	ab	56.8	ab	0.24	a
KB1	135.5	a	3.44	a	14.3	ab	56.6	ab	0.21	a
KB2	138.7	a	3.41	a	15.7	a	58.4	a	0.23	a

aGallic acid equivalent (on fresh weight basis) bTrolox equivalent (on fresh weight basis)

Table 14 - Kiwi: mean values and variance analysis of some fruit quality parameters of fruit sampled after 100 days of refrigerated storage. K0: control; KA1: light compost 10 t ha^{-1}; KA2: light compost 20 t ha^{-1}; KB1: heavy compost 10 t ha^{-1}; KB2: heavy compost 20 t ha^{-1}

Treatment	Fruit fresh weight		Firmness		Solid soluble content	
	(g $fruit^{-1}$)		**(Kg cm^{-2})**		**(°Brix)**	
	2015					
K0	126.05	a	2.41	b	14.30	b
KA1	122.15	a	2.78	b	14.66	ab
KA2	123.85	a	2.31	b	14.87	a
KB1	124.72	a	2.58	ab	14.53	ab
KB2	130.52	a	3.15	a	14.77	a
	2016					
K0	122.2	a	2.2	b	13.9	b
KA1	125.3	a	2.5	ab	14.3	ab
KA2	124.8	a	2.7	a	14.9	a
KB1	126.1	a	2.3	b	14.4	ab
KB2	127.4	a	2.9	a	15.1	a
	2017					
K0	115.6	ab	2.31	b	13.8	ab
KA1	120.6	ab	2.74	ab	14.8	ab
KA2	123.4	ab	2.64	ab	14.9	ab
KB1	124.0	ab	2.74	ab	16.0	ab
KB2	132.1	ab	3.13	a	15.4	ab

2.5.2 Peach

Figure 57 - Compost distribution in the peach orchard

In peach orchard the variation found in the yield for plants and for hectare smoothed the differences in the total yield between various treatments. However, a positive response was shown for compost amended plots in quality parameters (dry matter content, total soluble solids, N content, colour), thereby further indicating the nutrients performance of compost amendments in respect to conventional fertilization (Tabs 15, 16, 17)

Table 15 - Peach: mean values and variance analysis of some fruit quality parameters of fruit sampled at maturity harvest. P0: control; PA1: light compost 10 t ha^{-1}; PA2: light compost 20 t ha^{-1}; PB1: heavy compost 10 t ha^{-1}; PB2: heavy compost 20 t ha^{-1}

Treatment	Fruit fresh weight (g $fruit^{-1}$)		Yield per plant (kg $plant^{-1}$)		Yield per ha (t ha^{-1})		Dry matter content (%)		Total dry matter content (t ha^{-1})	
	2016									
PO	141.2	a	8.2	a	11.3	b	16.8	ab	1.90	b
PA1	144.7	a	9.3	a	13.2	ab	17.3	ab	2.28	ab
PA2	151.2	a	10.1	a	14.0	ab	17.7	ab	2.48	ab
PB1	149.6	a	12.2	a	16.9	a	18.1	a	3.05	a
PB2	152.2	a	10.9	a	15.3	ab	18.5	a	2.83	a
	2017									
PO	136.3	ab	10.7	ab	15.4	ab	17.3	ab	2.7	ab
PA1	135.0	ab	12.0	ab	17.5	ab	17.2	ab	3.0	ab
PA2	156.8	a	11.7	ab	16.8	ab	17.6	ab	3.0	ab
PB1	154.3	a	13.6	a	19.2	a	17.4	ab	3.3	a
PB2	148.9	ab	12.7	ab	18.4	a	18.8	a	3.4	a

Table16 - Peach: mean values and variance analysis of some fruit quality parameters of fruit sampled at maturity harvest. P0: control; PA1: light compost 10 t ha^{-1}; PA2: light compost 20 t ha^{-1}; PB1: heavy compost 10 t ha^{-1}; PB2: heavy compost 20 t ha^{-1}

Treatment	Firmness (kg cm^{-2})		Total soluble solids (°Brix)		Titrable acidity (% malic acid)		Mean total N (%)		Total N (Kg ha^{-1})	
	2016									
PO	4.9	a	15.2	a	0.32	a	0.71	a	13.5	b
PA1	4.8	a	15.4	a	0.29	a	0.73	a	16.6	b
PA2	5.1	a	14.9	a	0.31	a	0.78	a	19.3	ab
PB1	4.6	a	14.5	a	0.28	a	0.75	a	22.9	a
PB2	4.5	a	15.1	a	0.31	a	0.81	a	22.9	a
	2017									
PO	4.8	a	14.5	b	0.30	a	0.76	a	20.1	ab
PA1	5.0	a	15.4	ab	0.26	a	0.87	a	26.1	a
PA2	4.9	a	15.3	ab	0.29	a	0.74	a	21.7	ab
PB1	4.6	a	14.8	ab	0.29	a	0.76	a	25.4	a
PB2	4.4	a	16.6	a	0.32	a	0.72	a	25.0	a

Table17 - Peach: mean values and variance analysis of some fruit quality parameters of fruit sampled at maturity harvest. P0: control; PA1: light compost 10 t ha^{-1}; PA2: light compost 20 t ha^{-1}; PB1: heavy compost 10 t ha^{-1}; PB2: heavy compost 20 t ha^{-1}

Treatment	Colour						Total polyphenols	
	Lx (lightness)		C (chroma)		H (hue)		(mg $100g^{-1}$)*	
	2016							
PO	49.4	a	516.8	a	0.9	a	37.2	a
PA1	55.1	a	511.4	a	0.7	a	36.4	a
PA2	48.9	a	519.2	a	0.8	a	32.3	a
PB1	56.1	a	518.6	a	1.0	a	38.2	a
PB2	52.6	a	522.0	a	0.9	a	42.4	a
	2017							
PO	50.5	ab	510.3	ab	0.9	a	36.9	ab
PA1	56.1	a	519.3	a	0.8	a	35.3	ab
PA2	55.2	a	519.8	a	0.9	a	40.7	a
PB1	54.9	a	516.7	ab	0.9	a	39.2	a
PB2	52.3	a	521.5	a	0.8	a	43.9	a

*Gallic acid equivalent (on fresh weight basis)

2.6 Practical sustainability of compost application

No specific problems associated with compost application were detected in different project sites neither for the field distribution nor on cropping systems. The careful check of starting organic biomasses, composting processes and final products, allowed the attainment of final composts with optimized defined physical, chemical and biological properties. In particular the use of on-farm composting methodology allowed to maintain an almost uniform and steady level of physical and chemical characteristics of compost materials for all the project duration

The continuous monitoring of compost quality reduced the risk of phytotoxicity associated with the use of not completed stabilized organic materials. The final low moisture content of applied mature composts, that ranged from < 5% for the green compost of Prima Luce, to <20% for the manure based composts of Piemonte and Castel Volturno, greatly improve the easiness of compost distribution and incorporation on both light textured soils of project sites Grandi and Tetto Frati, as well as on heavy clayey soils of the project sited located in Campania (Castel Volturno, Mellone, Prima Luce) No specific problems were shown in respect to the emission of irritating volatile products, unwanted odours and weed development. As outlined by the LCA evaluation the compost distribution did not have a significant impact on the timing, energy consume and costs of field working procedures that were comparable with those of conventional managements.

Conversely a significant improvement of specific agronomic parameters were indicated by the farmers involved in the application of on-farm green composts produced by the composting prototype of Prima Luce:

- the progressive increase of soil porosity and decrease of the bulk density of ploughed soil horizons, produced a positive influence on the effectiveness of tilling practices on open fields and on the transplanting procedure in greenhouse cultivation
- larger structural stability with a decrease of superficial runoff events, increase of water infiltration and improvement of water retention capacity
- significant decrease of pathogens attack and of plant losses, and larger yields of fresh products
- larger consistence of horticultural products This parameter associated with the maintenance of the nutrient value, is an important commercial characteristic that determine the “shelf-life” of the product which extension represent an important target for the marketing of fresh vegetables to external countries.

Annex 4 – Closing Report on C.4 Action

Monitoring of greenhouse gases emissions

Project responsible: CERMANU
Field Measurements: Tetto Frati- AGROSELVIT; Castel Volturno CERMANU; Mellone: UNIBAS

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1. Methods

1.1 Field treatments

- Tetto Frati: crop system maize

7 soil treatments: Trad (chemical fertilizers); 0N no nitrogen addition; CMB-B and CMP-A: low (1000 kgOC/ha) and high (2000 kgOC/ha) doses of compost from bio-digestate; SS-B and SS-A: low (1000 kgOC/ha) and high (2000 kgOC/ha) doses of fresh solid digestate; FeP: like TRAD with the addition of biomimetic catalyst (5 kg/ha)

- Castel Volturno: crop system maize

4 soil treatments: Trad (chemical fertilizers); CMB-B and CMP-A: low (10 tn/ha) and high (20 tn/ha) doses of on-farm compost; FeP: soil addition of biomimetic catalyst (5 kg/ha)

- Mellone two systems: kiwi and peach; two composts: A “summer” and B “winter” depending on the crop residues used in composting process; three compost doses 0-control , 1- 10 tons/ha, 2 - 20 tons/ha

1.2 Field measurements

At CastelVolturno the field measurements are provided by stable system placed in a dedicated field facility. The GHG measurements are carried out with automated closed-chamber system coupled to a 1412-Photoacoustic Field Gas Monitors (Fig. 1). Two chamber are placed in soil for each treatment plus two chambers between treatments as control. Each chamber provided daily, on average, 7/8 measurements.

The analyses are currently stopped during December and January for the decreasing sensibility of analytical system derived from the low temperature and high moisture contents of soil plots. The analytical systems provide high-time resolution of gas fluxes data, being able to perform day-long analytical cycle of 20 minutes for each chamber with a maximum of 10 operating chambers.

At the project sites of Tetto Frati the analyses are performed with similar removable systems, based on sampling chambers and photoacustic gas monitor (1412-Photoacustic Field Gas Monitors); the gas-fluxes measurements are carried out periodically for 15 days for each months. The evaluations of filed emissions are performed placing two/three chambers for each treatment, plus two chambers in non cultivated plots as control, with a weekly alternation between soil treatments (1st week 8 chambers 4 treatments 2nd week 8 chambers 3 treatments).

Photoacoustic Field Gas Monitor operates collecting gas samples by means of pump from closed chamber; the gas sample is allocated in a small chamber (3 ml). Chamber is irradiated with pulsed, modulated by a chopper, narrow-band light. Gas absorbs light proportional to its concentration and converts it to heat. Temperature fluctuations determined by modulation generate pressure waves detected by sensitive microphones. Gases specific carousel, are available to select the appropriate light wavelength. The instrument is capable to measure gas concentration in few seconds.

Each chamber (Ø= 30cm, h= 10cm) is automated by means of electronic engine in order to modulate opening/closing cycle for the accumulation of soil air fluxes. Each cycle was run by a multiple channel sampler provided of 10 channels. Each chamber was equipped with a vent valve to avoid pressure variations inside the chamber Inlet and outlet tubes allow air circulation from chamber to detection instrument. Soil gases fluxes have been calculated for each chamber, considering a cycle of 10 measurements with open chamber and 10 measurements with closed chamber, covering a total time of about 20 minutes.

For Mellone site, an innovative specific instrumentation for the field GHG evaluation was developed by Beneficiary Unibas. The equipment is made by automatic independent system with sampling chamber, analyser, solar battery, electric motor and remote sensing control that allows both a wi-fi transmission of acquired data and the check of working procedure. The CO2 Analyzer module uses thePP-Systems SBA-5 probe for precise measurement of the carbon dioxide content. The measurement is based on the specific gas absorption within the infrared spectrum. This technique allows a precise qualitative identification and reliable quantitative evaluation, being able to measure concentrations of a few ppm (parts per million) of gas. The assembled module is equipped with an additional sampling pump, air filters and perform a precise calibration with few ppm (parts per million) of gas. The analyzer can be used both indoors and outdoors having a degree of protection IP67. he system is modular and therefore easily configurable. The modules can be connected thanks to the already prepared cables and following the information given by the labels identify each connector. The block diagram highlights the codes of the modules and the cables and connectors used. The system thus configured is already functioning and able to connect to the wireless (WSN) network. The main advantage in respect to the traditional system is the wireless characteristic with an effective displacement of sampling chambers in large plots other thereby allowing to perform analyses with a limited spatial restriction. Each chamber needs about 30 minutes to attain the correct internal equilibrium and is able to carry out continuous analyses with an average of 2 minutes for each analysis

The amount of released C CO2 is derived by the mass proportion

CO2 (kg ha-1) x 12(mass of Carbon)/44 (mass of CO2)

<u>1.3 Laboratory measurements</u>

All organic materials used at different project sited for field treatments were tested for NH3 volatilization and GHG (N2O, CO2 and CH4) emissions under potential conditions, in climate room incubation. The tested organic materials used at different filed sites are: fresh solid digestate, compost from digestate (Agroselvit); on farm manure compost (Cermnau); on farm green compost (Unibas-Prima Luce). For GHG measurements, the incubation experiment was carried out in a controlled climate (20° C, 65% relative humidity) and set-up as a randomised complete block design, with four replicates. Each experimental unit consisted of a cylindrical glass jar (volume 5 L).). Air-dried and sieved soil (3 kg for each jar) moistened with deionized water in order to reach 75% saturation. Two types of model soils with different properties (Table 1) were used for the experiment. All jars were fertilizes at 170 kg N ha-1. GHG emissions were measured by means of a closed-chamber technique.

Table 1 Textural composition and main characteristic of the two soils used in the experiment.

Soil type	Sand	Silt	Clay	Porosity[a]	$CaCO_3$	SOC	TN	C/N	pH	CEC	P[b]	K[c]	Ca[c]	Mg[c]
	%							-	-	(cmol$_c$ g^{-1})	(cmol$_c$ kg^{-1})			
SL	17,2	71,1	11,7	49,2	0,4	1,2	0,2	8,0	6,1	12,4	23	42	1452	179
SY	89,7	5,5	4,8	45,3	15,3	0,52	0,057	9,1	8,3	5,4	14	28	980	21

Abbreviations: SL= silt-loam, SY= sandy, SOC = soil organic carbon, TN= total nitrogen, CEC= cation exchange capacity (cmolc kg-1); a Calculated on the basis of bull density and particle density; b Available (Olsen P); c Exchangeable.

2. Results

For each project sites the lower yields of nitrous oxide emissions, equivalent to less than 0.013 kg/ha/d (1500/2000 times lower than CO2 levels), and the consequent larger variability (± 75%), did not allow a reliable estimation of N2O contribution to soil GHG.

2.1 Field measurements

- *Tetto Frati*

The analyses of CO2 evolution from field treatments in the first cycle of filed analyses (Fig. 1) revealed higher emissions from soil amended with fresh solid digestate, while the lower values were found for the soil without fertilization (0N) and with biomimetic catalyst addition (FeP). The large emissions found in late spring and summer periods are associated with the contribution of root and rizho-sphere biomass associated with maize crop cycle

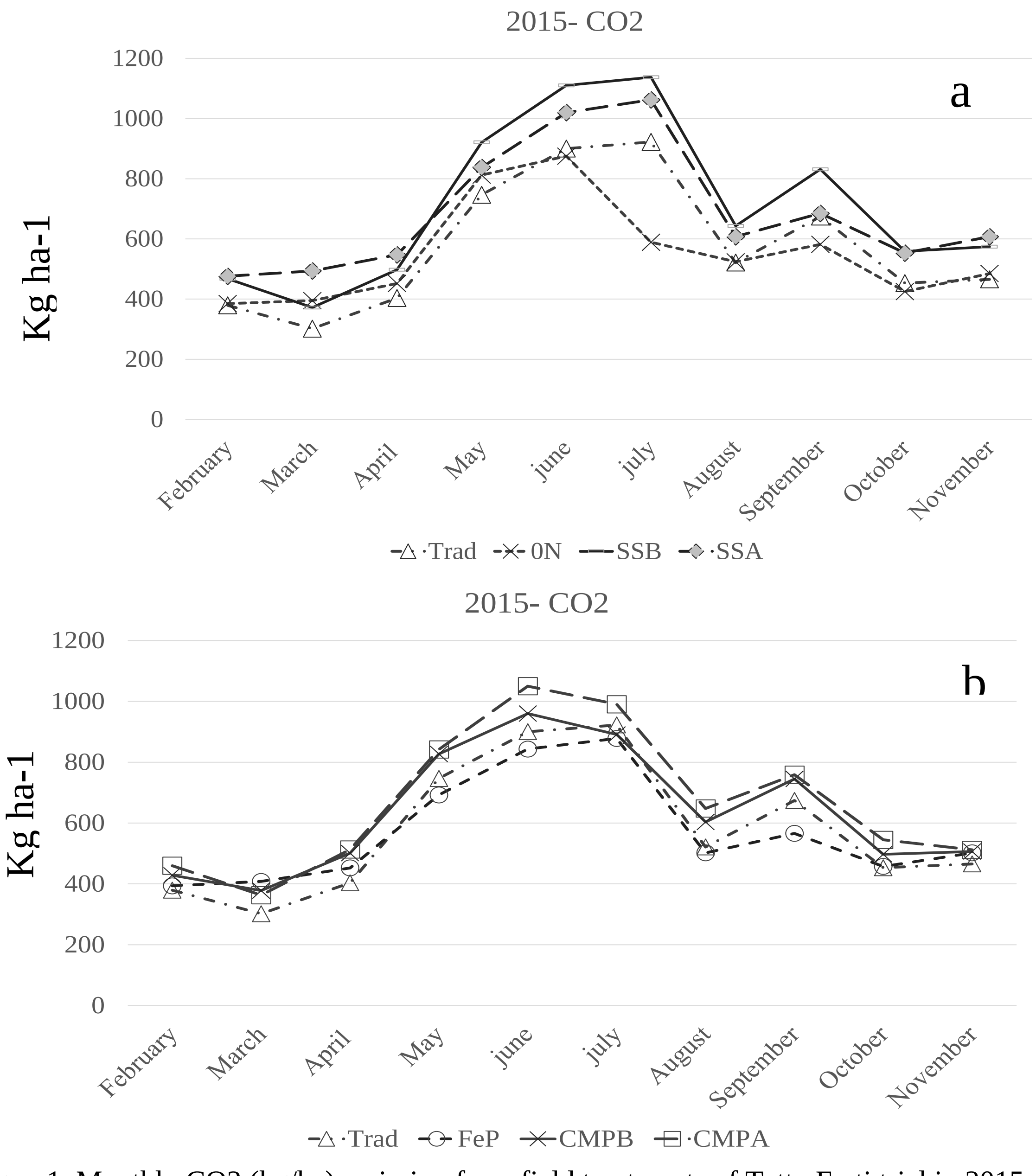


Figure1. Monthly CO2 (kg/ha) emission from field treatments of Tetto Frati trial in 2015

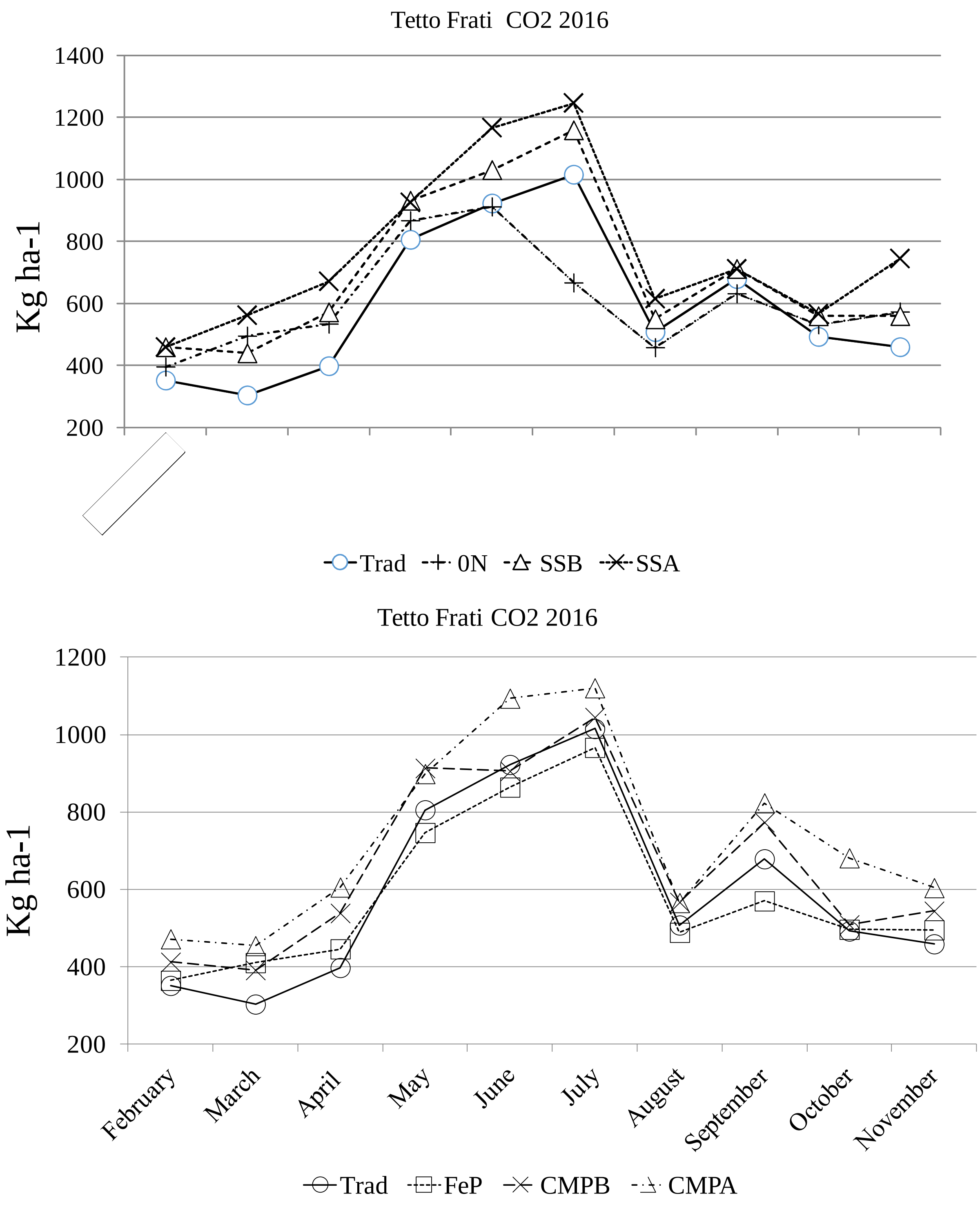
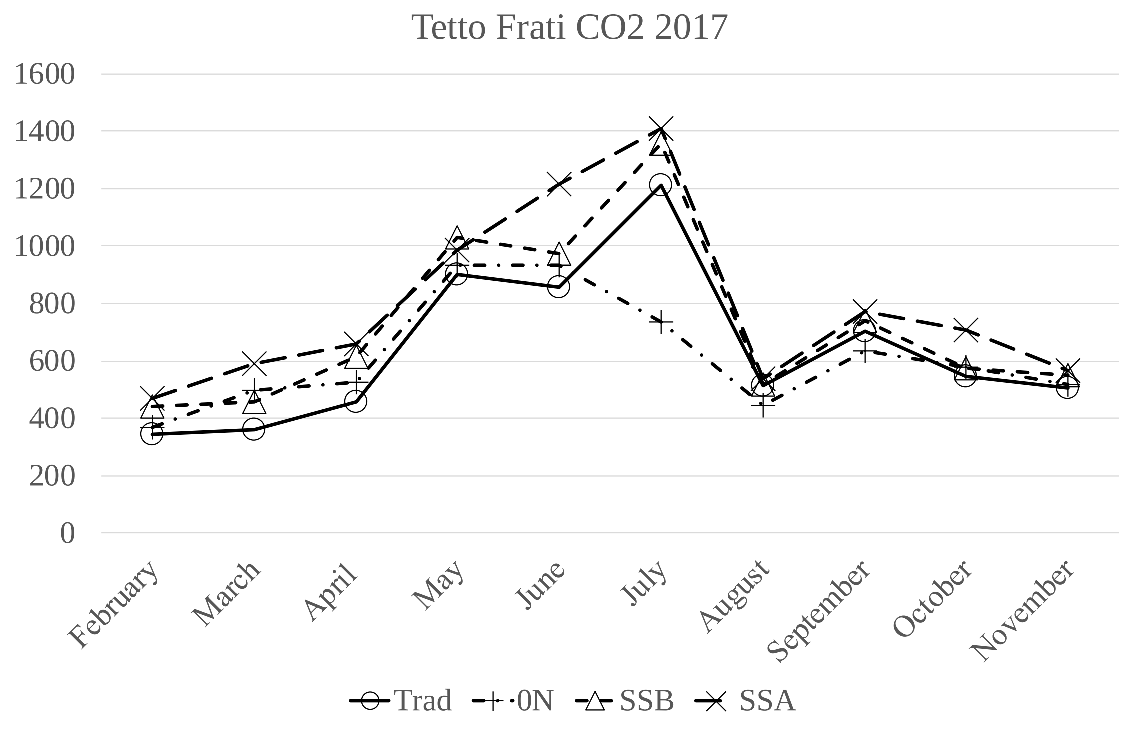


Figure 2. Monthly CO2 (kg/ha) emission from field treatments of Tetto Frati 2016:

The analyses of CO2 evolution from field treatments in 2016 and 2017 (Figs 2, 3) revealed larger emissions from soil plots added with organic materials, while the lower values were found for the soil without fertilization (0N). The soil treatments with fresh solid digestate showed a slight largest C mineralization in respect to compost amended samples. The large emissions found in late spring and summer periods are associated with the contribution of root and rhizosphere biomass associated with maize crop cycle. The average estimated annual cumulative C losses ranged from 1.7, 1.9 to 2.1 t ha^{-1} for Trad, SS and CMP treatments respectively



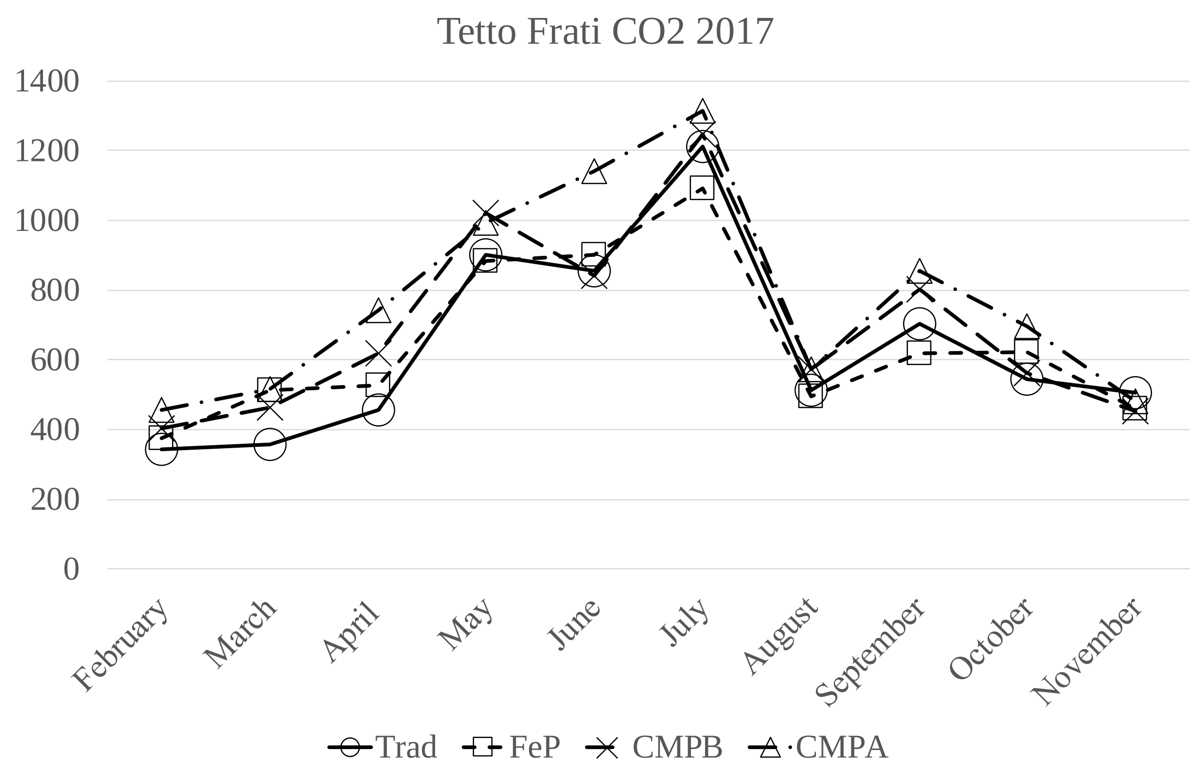
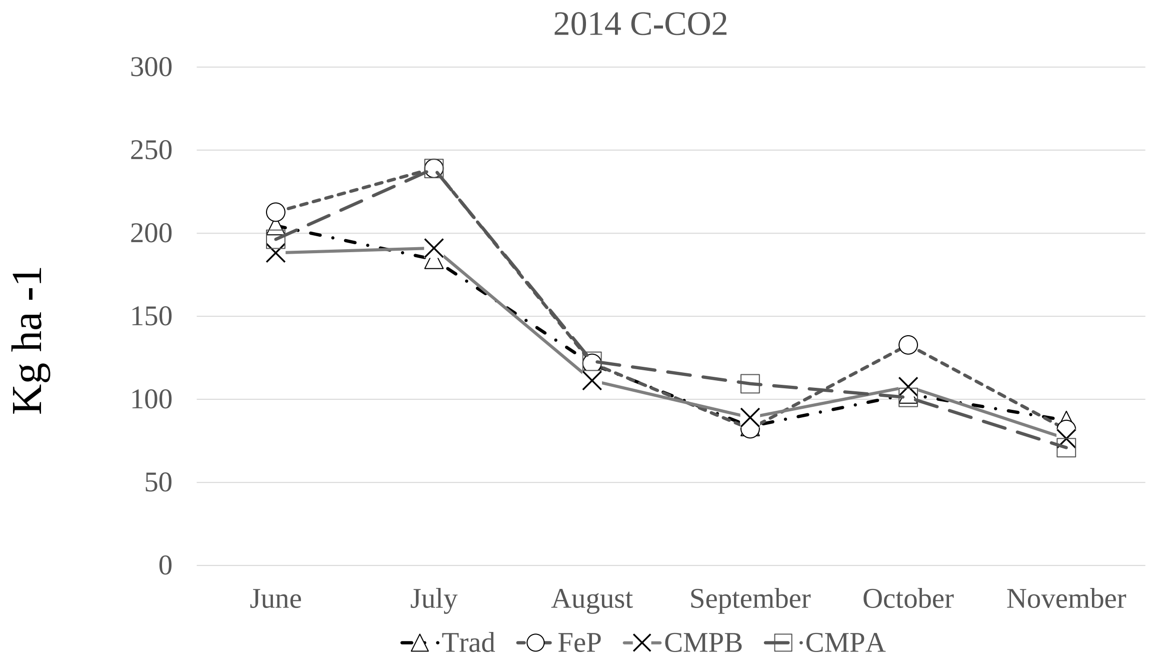


Figure 3. Monthly CO2 (kg/ha) emission from field treatments of Tetto Frati 2016

- *CastelVolturno*



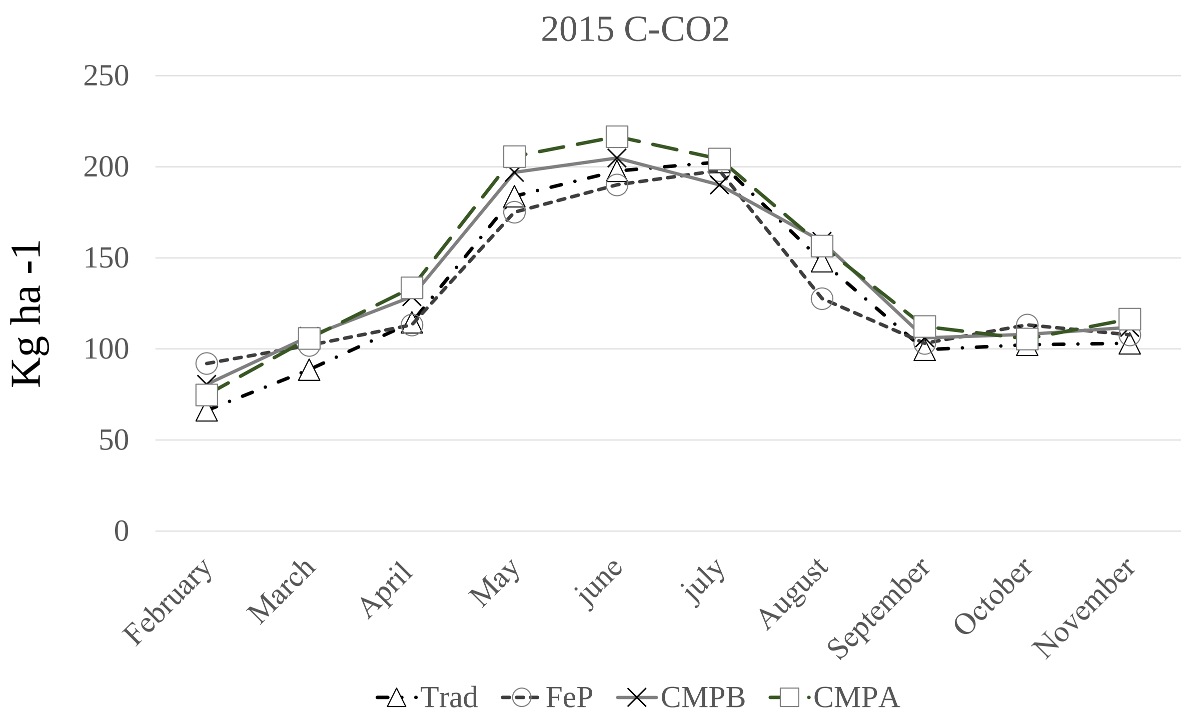
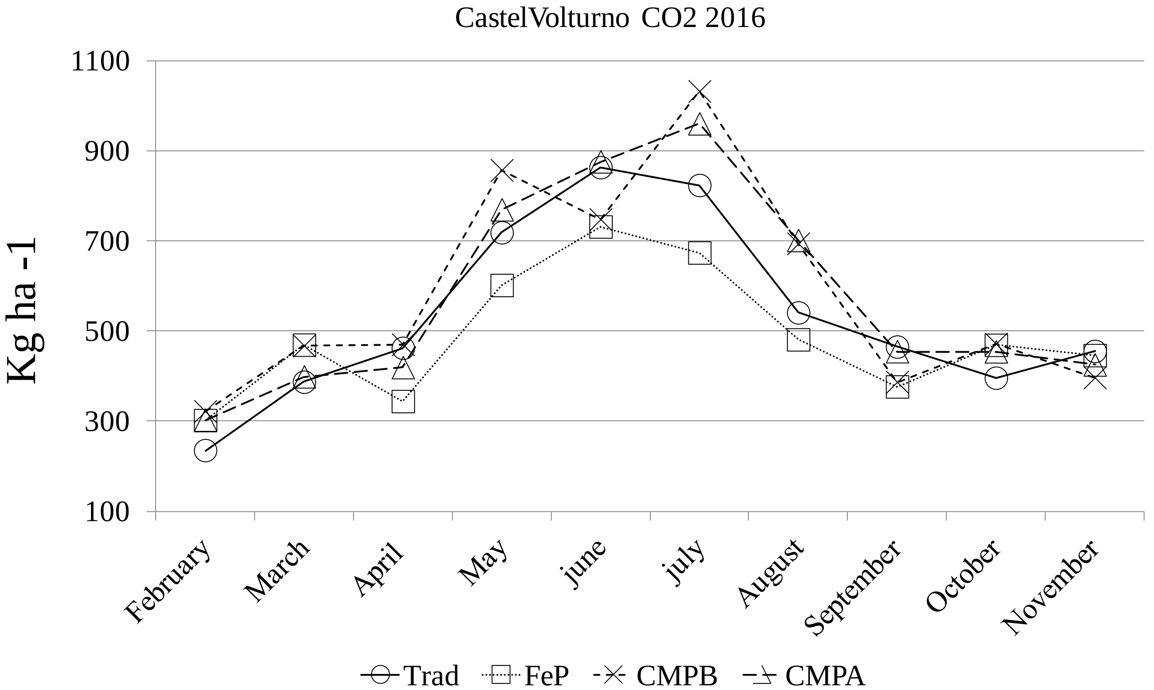


Figure 4 Monthly CO2 emission from field treatments of CastelVolturno in 2014 and 2015: TRAD (chemical fertilizers); CMB-B and CMP-A: low (10 tn/ha) and high (20 tn/ha) doses of on-farm compost; FeP: soil addition of biomimetic catalyst (5 kg/ha)

Although no significative differences were found in the monthly CO2 fluxes from various field treatments in the project site of Castel Volturno (Figs. 4, 5), global slight larger losses were found in compost amended plots (+ 150-200 kg OC ha^{-1}) after subsequent addition of organic input in respect to traditional managed soil. Conversely at the end of last crop cycle a decrease in CO2 loss was found in Fe-P treatment (- 100 kg OC ha^{-1}). Again, the larger values shown in June and July may be associated to the contribution of crop roots and to the rhizo-biomass microbial activity.



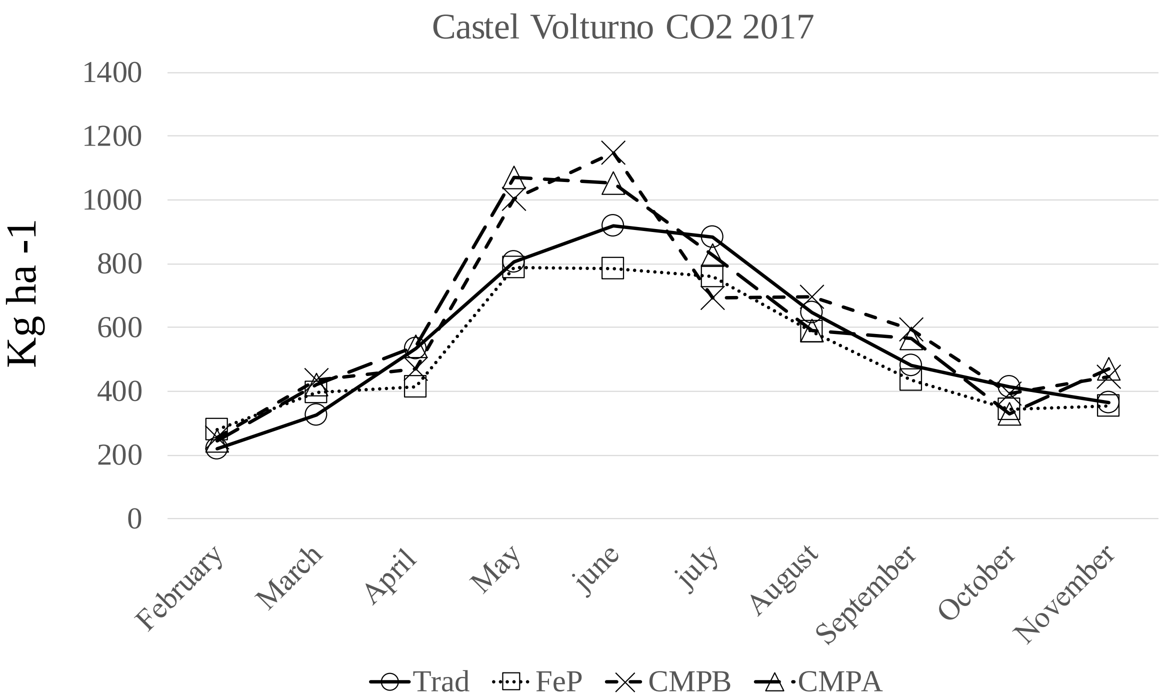


Figure 5 Monthly CO2 emission from field treatments of CastelVolturno in 2016 and 2017: TRAD (chemical fertilizers); CMB-B and CMP-A: low (10 tn/ha) and high (20 tn/ha) doses of on-farm compost; FeP: soil addition of biomimetic catalyst (5 kg/ha)

- *Mellone*

A larger CO2 emission was found in the projectt site of Mellone farm (Figs. 6, 7), for the permanent orchard system as compared to the maize fields, thereby strengthening the validity to include in the C4 action different cropping systems. The large extension of root systems and the presence of cover grass in the inter-layers between plant rows may play a role in the CO2 emission of orchard soils. For kiwi system besides the global emission also a different monthly distribution was revealed by the field measurements with an increase in the spring period followed by a sharp decrease observed in the summer period, related to the drying condition that probably influence both the grass cover and the activity of soil biomass. Minor differences in both orchard systems were found for either soil treatments with different amounts of compost addition or among compos types, thereby confirming that while the different initial biomass composition affect the final yield of dry compost, the on-farm composting systems produce a steady quality of humified mature compost. However, in the second period of field analyses for kiwi field, a slight increase of measured CO2 was found in soil treatments with the high dose of "summer" compost (A-20) that revealed a larger global emission, corresponding to about a difference of +1 and +0.5 ton OC ha^{-1} in respect to Control and B-20 soil plots respectively

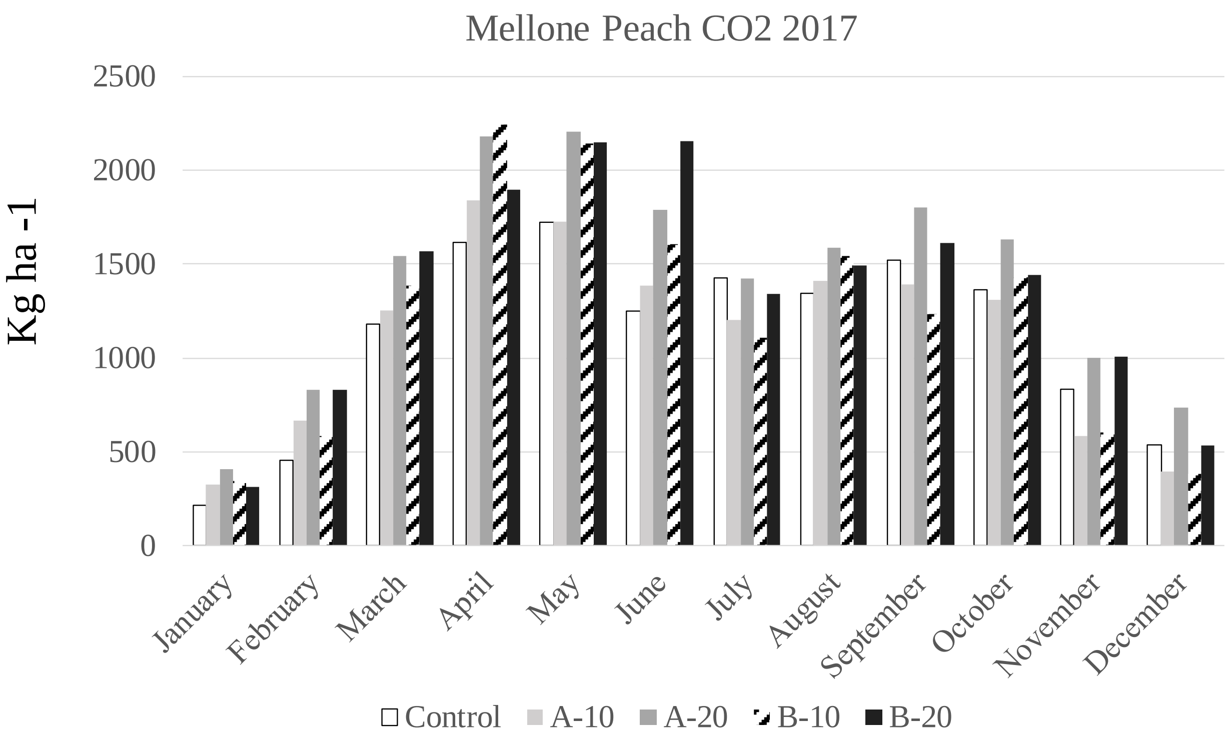
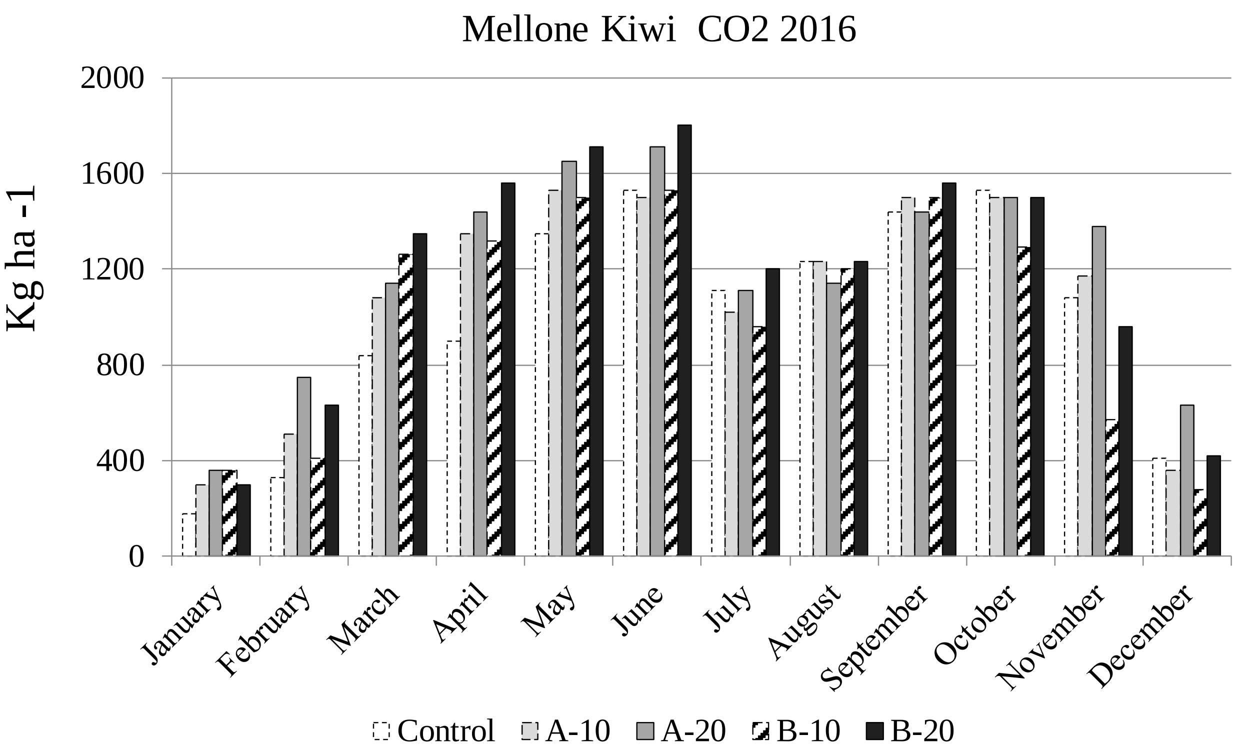


Figure 6 Monthly CO2 emission from field treatments of Mellone inpeach system (2017): Control – no compost; A-10, A20: 10 and 20 tons/ha of *summer/light* compost; B-10, B-20 10 and 20 tons/ha of *winter/heavy* compost



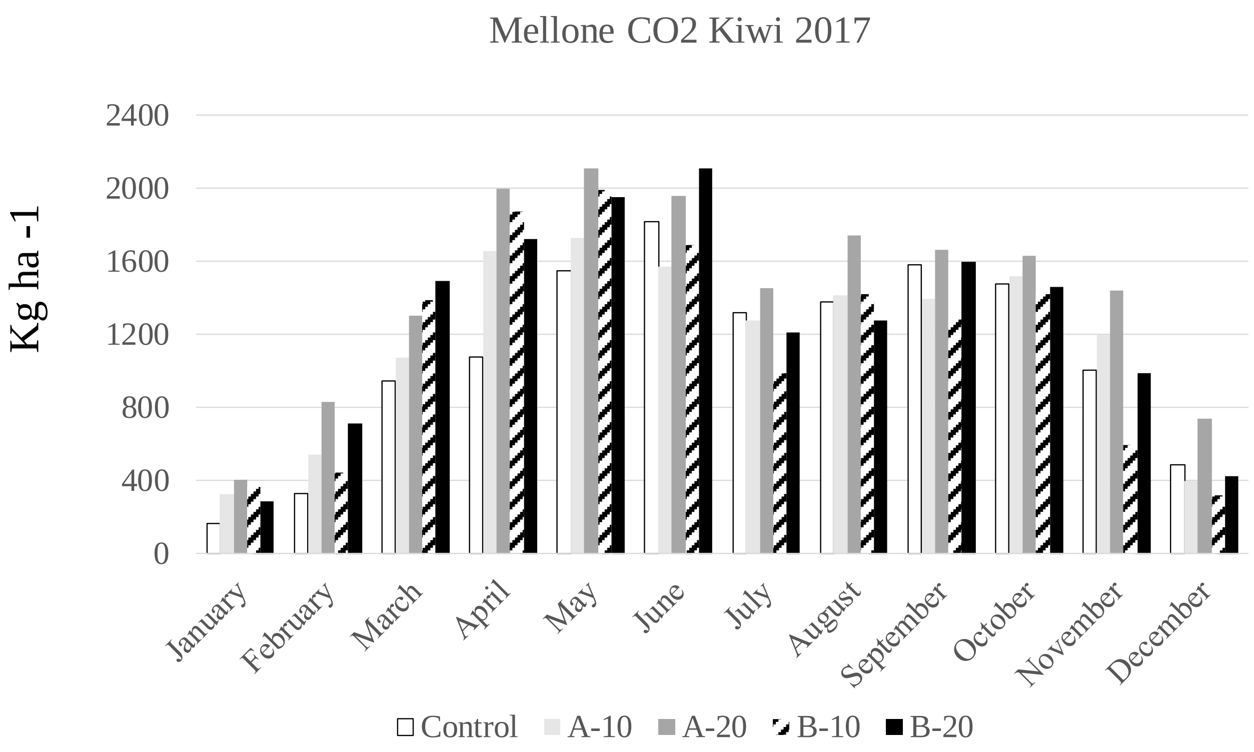


Figure 7 Monthly CO2 emission from field treatments of Mellone in kiwi system (2016, 2017): Control –no compost; A-10, A20: 10 and 20 tons/ha of *summer/light* compost; B-10, B-20 10 and 20 tons/ha of *winter/heavy* compost

2.2 Laboratory analyses

The GHG emissions from organic matrices measured during the 93-day incubation for SY and SL soils are shown in Figures 8and 9 and Figures 10 and 11, for carbon dioxide and nitrous oxide in the order. For both model soils the larger CO2 emission was found for the less stabilized fresh solid digestate, while the composted materials has CO2 fluxes comparable with the soil treatment with mineral fertilizer

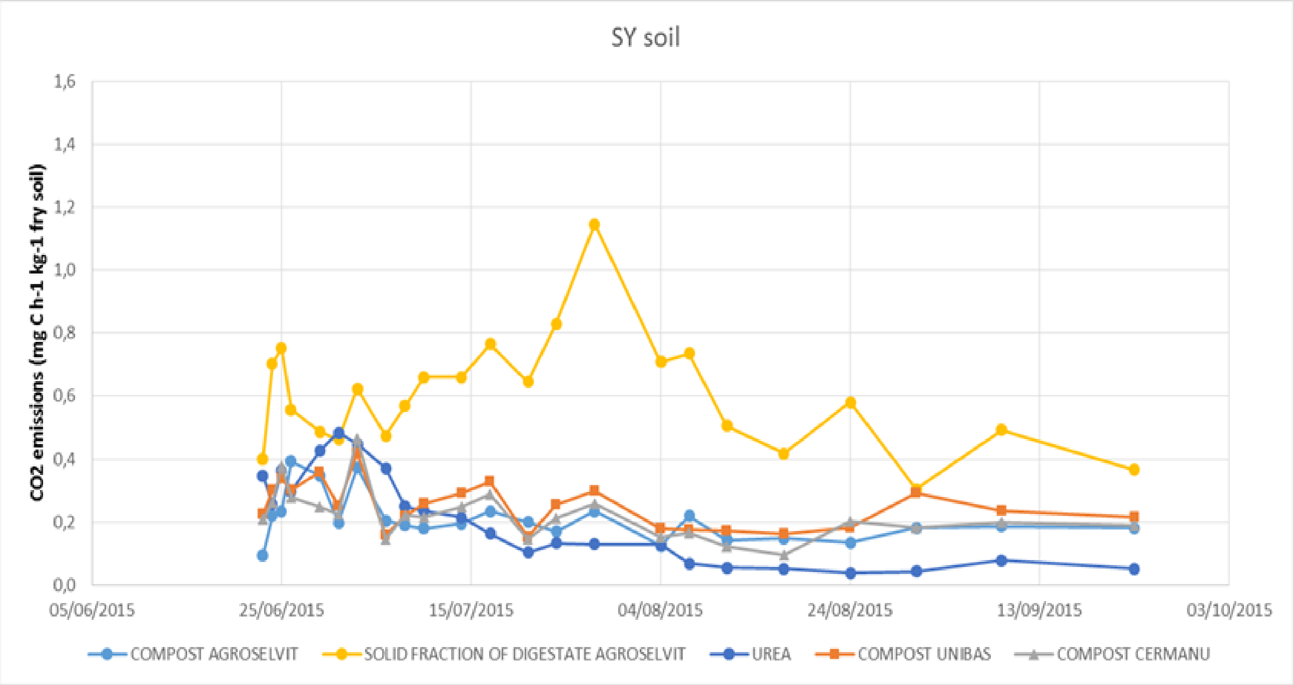


Figure 8. CO2 emissions for SY soil.

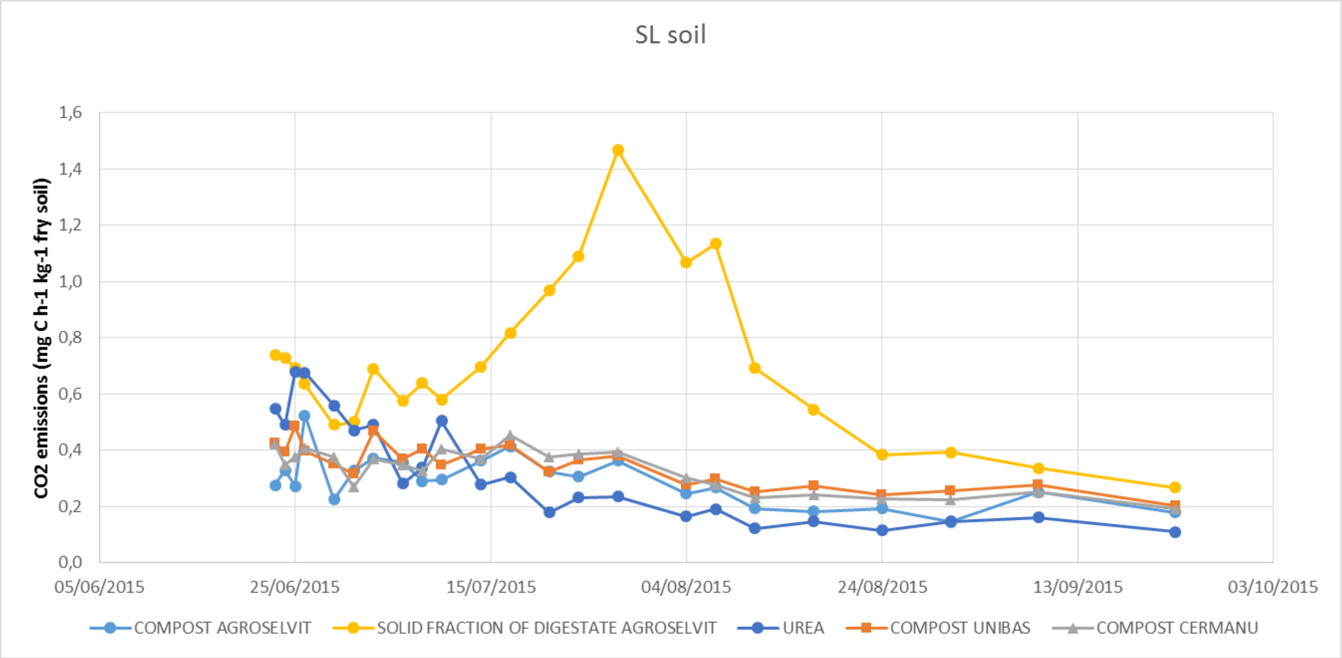


Figure 9. CO2 emissions for SL soil.

The analyses of nitrous oxide revealed an intense initial pulse emission from soil samples added with urea and minor losses from fresh solid digestate, may be associated with the release of available nitrogen, followed by a sharp lowering to the steady ground values of other organic treatments

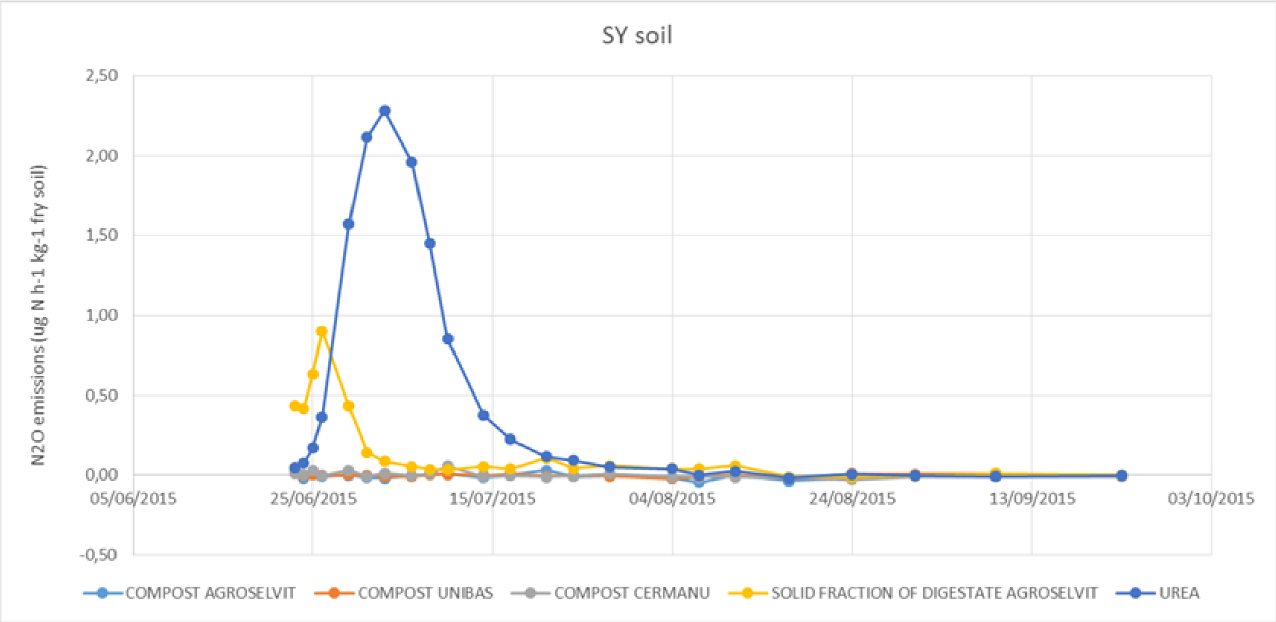


Figure 10. N2O emissions for SY soil

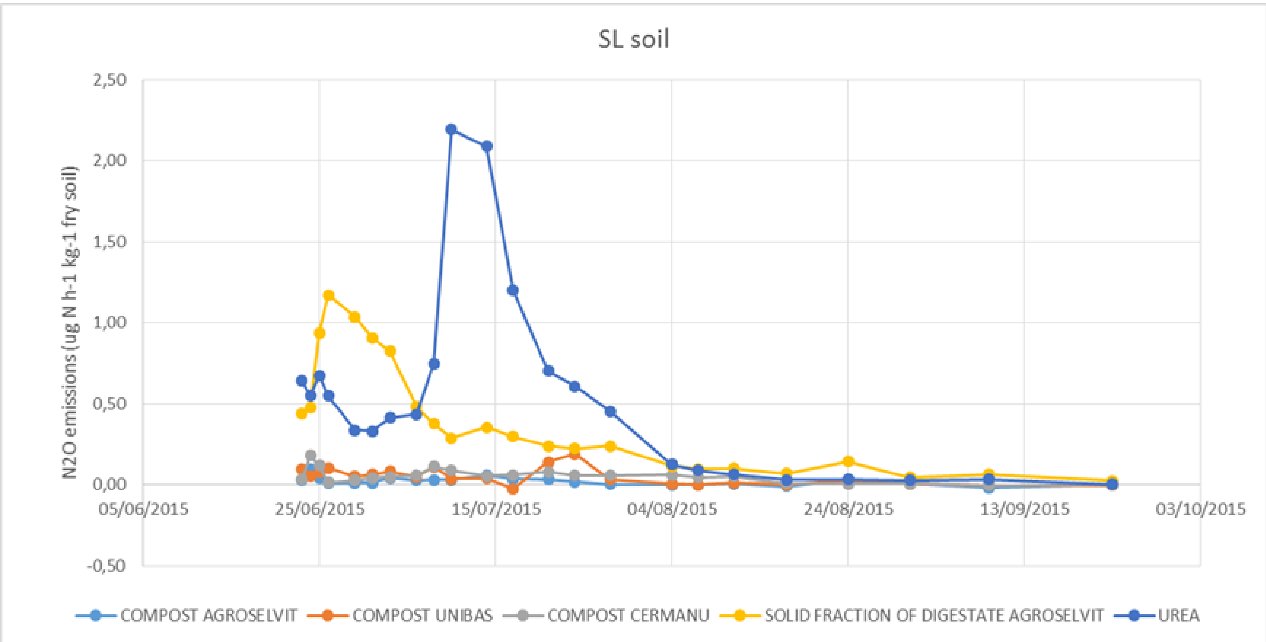


Figure 11. N_2O emissions for SL soil

Annex 5 - Closing Report on C.5 Action

Monitoring the environmental and economical sustainability of proposed strategies

Project responsible AGROSELVIT

Responsibilities in case several beneficiaries are implicated:
On farm composting facility and composting process: UNIBAS
Field SOM managements: Tetto Frati, Grandi, Castel-Volturno AGROSELVIT;

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1 LCA evaluation of on-farm composting plant

1.1 Materials and methods

Composting trial were carried out at "Prima Luce" plant. Particularly, two composting alternatives were taken into account. The alternatives differed for the organic residues used for composting: in one case, lettuce and other similar "light residues", in the other case heavy biomass such as artichokes and cauliflowers residues, tomatoes' stems or walnut husks. The typology of composted materials was very important because the compost yield depended on it. Indeed, in the first case, the production of the so-called "LIGHT COMPOST" (LC) accounted for 7.5 t/week; in the second one, the production of "HEAVY COMPOST" (HC) was around 10 t/week.

1.1.1 Environmental analysis

The environmental analysis was carried out according to LCA methodology from ISO 14040 and 14044 standards (ISO 14040:2006a; ISO 14044:2006b). LCA is a methodology for determining the environmental impacts associated with a product, process or service from cradle to grave. The SimaPro v. 8.04 software (PRé Consultants, 2012) was used to determine the environmental impacts of the examined composting system during a reference period of 20 years (corresponding to the lifespan of the plant). The impact assessment was performed following the CML 2001 methodology guidelines, developed by the Centre of Environmental Science of Leiden University (Guinée, 2001) and the following impact categories were selected: abiotic depletion (AD), acidification (A), eutrophication (E), global warming (GW), ozone layer depletion (OLD) and photochemical oxidation (PO).

The functional unit chosen, namely the reference unit that expressed the function of the system in quantitative terms, was one ton of compost treated.

For the purpose of the current investigation, the system boundary of the model was: the processing of the pruning residues; the transportation of these materials to the plant; the collection of crop residues and its receipt; the construction of the capital equipment and infrastructures; the compost processing and the transport and distribution on the field of the compost produced.

The inventory of the data, associated to the studied systems, was collected in situ at the composting plant. Using a data collection sheet, information on the quantities of machinery, fuel, electricity and other items used were gathered.

During composting process many types of gaseous compounds can be emitted. Direct emissions of CH4, NH3 and N2O were not experimentally measured, but their values were taken from the available considering only researches similar to the present study for both composted materials and technologies used for the process.

1.1.2 Energy analysis

The energy analysis technique was used to calculate the energy involved in the production of 1 t of compost. The energy values, expressed as Mega Joule (MJ), was obtained by multiplying the amounts of each item (machinery, fuel, electricity, labour, etc.) by the relative conversion factors taken from the literature (Monarca et al., 2009; Page, 2009; Pimentel and Pimentel, 1979; Volpi, 1992).

1.1.3 Life Cycle Costing

The production costs of the compost were analyzed according to the Life Cycle Costing (LCC) methodology, through repayment of the capital cost of the facility (annualized over 20 years at an assumed 2% interest rate) plus operating and maintenance costs (van Haaren et al., 2010).

Assuming that the plant had a lifespan of 20 years, the cumulative costs of compost production were evaluated for each year taking into account expenses over the whole life cycle of the plant related to materials, labour, quotas and other duties. Materials included the cost of all non-capital inputs; labour included the cost of workers involved in farm production; quotas include machinery, equipment and depreciation costs (Pappalardo et al., 2013).

1.2 Results

1.2.1 Environmental aspects

Table 1 shows the total life cycle impacts in the reference period (20 years) and impacts per ton of compost produced by the two composting alternatives. Under our operative conditions, after 20 years of working, some processing steps (such as the construction of the facility, the collection of the bulking agent and the crop residues, the process, the transport of the compost to the field and its distribution) plus direct emissions produced during the decomposition phases could cause an abiotic depletion (AD) equal to 44 kg of Sb; a global warming potential (GWP) between 1,668,000 and 1,678,000 kg of CO2eq; an average ozone layer depletion (ODP) of 0.13 kg of CFC-11eq; a photochemical oxidation (PO) from 613 to 620 kg of C2H4eq; an air acidification (AA) between 14,558 to 14,581 kg of SO2eq; and an average eutrophication potential (EP) of about 5,965 kg of PO4 eq (Table 1).

The typology of materials composted during the lifespan of the plant will determine the type and the quantity of the compost produced and, consequently, the impacts per ton of compost, as showed in Table 1. The main differences between the two alternatives could be found in the collection of crop residues, in the composting process and in the final transport of the compost produced and in its distribution on the field.

Referring only to global warming (CARBON FOOTPRINT), under our operative conditions, the production of 1 ton of LC caused an emission of 250.02 kg of CO2eq; while the production of 1 ton of HC caused an emission of 198.90 kg of CO2eq. In both composting alternatives, the composting process was the item that emitted the largest amount of CO2eq (about 42%) followed by the construction of the plant (20%).

1.2.2 Energy consumptions

The energy analysis showed that the construction of the facility and its use for a period of 20 years could cause an energy consumption more than 15,500 GJ, that annually it could be approximately equal to 775 GJ (Table 2).

Differently from the environmental analysis, the energy analysis showed that in both composting alternatives the construction of the plant was the item that consumed the largest amount of energy used (38%), followed by the composting process (27%). In the first item, the concrete was the highest energy input representing 53% of the energy involved in the construction of the facility. On the contrary, in the composting process the highest energy input was shown by diesel fuel and lubricants used above all in the mixing of the bulking agent with compostable crop residues, in the creation of the pile and during the screening of the compost obtained.

1.2.3 Cumulative production costs

The cumulative total cost at the 20th year of plant working could be equal to 1,629,840 euro, if the compostable material is light, and equal to 1,314,768 euro if the crop residues are heavy (Table 3). This difference was due to differences in the quantity of crop residues to create piles (200 cubic meters per pile for LC versus 100 cubic meters per pile for HC) and in the transport and distribution of the compost produced (15 cubic meters per pile for LC versus 20 cubic meters per pile for HC).

With regard to the single cost, for LC around 40% of total costs (675,729 €) were related to the collection of crop residues, while for HC these costs were only the 27% (337,865 €). Indeed, for HC there was not a single major cost, but there were three most important costs: the composting process (27%), the collection of crop residues (26%) and the capital costs (20%).

The analysis of the distribution of the production factors in composting production suggested that in both alternatives, the highest cost was provided by labour (more than 40%) followed by electricity (about 30%). In this case, the quantity of the compost produced defined the cost per ton which was equal to 162 € t-1 for LC and 98 € t-1 for HC (Table 3). These results show how HC had a lower rate of overall costs than LC, and appeared to be twice as sustainable due to the lower costs in total and per yield.

Table 1. Results of the total life cycle impact assessment in the reference period (20 years) and per tonne of the Table compost produced

	Abiotic depletion		**Global warming potential**		**Ozone layer depletion**		**Photochemical oxidation**		**Acidification**		**Eutrophication**	
	kg Sb eq		**kg CO_2eq**		**kg CFC-11eq**		**kg C_2H_4eq**		**kg SO_2eq**		**kg PO_4---eq**	
	LC	**HC**	**LC**	**HC**	**LC**	**HC**	**LC**	**HC**	**LC**	**HC**	**LC**	**HC**
Costruction of the facility	2.31	2.31	333391.57	333391	0.01	0.01	77.14	77.14	1408.97	1408.97	325.12	325.12
Collection of bulking agent	1.54	1.54	244771.71	244771	0.02	0.02	69.37	69.37	1431.58	1431.58	411.72	411.72
Collection of crop residues	0.82	0.41	92356.82	46178	0.01	0.01	27.13	13.57	615.89	307.94	208.92	104.46
Composting process	38.21	38.25	724420.44	703937	0.07	0.07	369.60	366.84	9379.57	9213.91	4526.49	4490.34
Transport of the compost and its distribution	1.53	2.03	229458.36	305944	0.01	0.02	69.92	93.23	1489.50	1986.00	410.35	547.13
Direct emissions	0.00	0.00	43430.40	43430	0.00	0.00	0.00	0.00	232.96	232.96	84.66	84.66
Total at the 20th year	**44.41**	**44.54**	**1667829.31**	**1677653**	**0.13**	**0.13**	**613.16**	**620.14**	**14558.47**	**14581.36**	**5967.25**	**5963.43**
Impact per ton	**0.01**	**0.00**	**250.02**	**198**	**0.00**	**0.00**	**0.08**	**0.06**	**2.06**	**1.60**	**0.84**	**0.65**

Table 2. Energy consumption for the examined composting alternatives in the reference period (20 years) and per tonne of the compost.

	Energy consumptions (MJ)	
	LC	**HC**
Costruction of the facility	5,851,605	5,851,605
Collection of bulking agent	2,360,958	2,360,958
Collection of crop residues	1,225,719	612,860
Composting process	4,145,301	4,145,301
Transport of the compost and distribution	1,924,425	2,565,900
Total energy input at the 20 th year	**15,508,008**	**15,536,624**
Annual energy input	**775,400**	**776,831**
Energy consumption per tonne	**1,988**	**1,494**

Table 3. Production costs for the examined composting alternatives in the reference period (20 years) and per tonne of the compost.

	LC	**HC**
Capital costs (€)		
Costruction of the facility	232,772	232,772
Machines	29,934	29,934
Insurance and maintenance fees	928	928
Operation costs (€)		
Collection of bulking agent	230,606	230,606
Collection of crop residues	675,729	337,865
Composting process	364,679	356,635
Transport of the compost and its distribution	95,192	126,029
Total at the 20 th year	**1,629,840**	**1,314,768**
Annual Production Costs	**63,213**	**50,993**
Costs per tonne	**162**	**98**

2 LCA evaluation of field SOM managements

2.1 Methods

The assessment of the environmental and economic sustainability of proposed soil managements is started with the following steps:

- initial acquisition of rough data on field requirements for the different project sites and cropping systems;
- definition and outlining of comprehensive protocol for data collection
- acquisition of software GaBi 6
- data collection and elaboration for the first and second year of field cultivation (in progress).

Two PE INTERNATIONAL GaBi Databases were purchased. They are the largest internally consistent LCA databases on the market today and contain over 7,000 ready-to-use Life Cycle Inventory profiles. The first is “GaBi Professional” database: it is the standard database provided with the GaBi software. The GaBi professional database is regularly updated and is derived from industry sources, scientific knowledge, technical literature, and internal patent information creating a solid foundation for assessing your materials, products, services and processes.

The second is more agricultural oriented and it is called “Renewable raw materials” database. Renewable raw materials contains 140 processes: fertilizers, pesticides, tractors, agricultural equipment, industrial intermediate products, and different crops (corn, wheat, hemp, flax, rape seat, soybean, etc.)

System boundaries of the different crops were designed for each of the following productions: lettuce and Brassicacee on Grandi farm (Grugliasco TO), Grain maize on Tetto Frati UNITO experimental farm (Carmagnola TO), Maize on UNINA experimental farm (Castel Volturno) and BLA orchard system at Mellone farm.

The System boundaries of lettuce and Brassicacee contivated on Grandi farm is shown as example on Figure 1. Inside the boundaries were counted all the field operations, and all the impact generated to produce the input: fertilizer (PK), pesticide, seeds, fuel and lubrficants, bioplastic end one of the three fertilizer that characterizes the three different production technique: compost (humus anenzy®), digestate soild fraction or traditional fertilization (urea). Crop yield represent the output of the system.

System boundaries of compost production on new plant was also planned in order to calculate the impacts of the different process phases.

The scenarios and impact assessments were modelled and computed by GaBi 6 software by using ILCD (International Reference Life Cycle Data System), EDIP (Environmental Design of Industrial Products) method of impact assessment.

Impact assessment category selected are: Global Warming Potential (IPCC), Acidification Potential (EDIP), Freshwater Eutrophication Potential (RECIPE) and Terrestrial Eutrophication Potential (RECIPE).

In order to assess and compare the sustainability of different crops production three Function Units were selected. The impacts were reported per harvested production (product oriented), per cultivated land (surface oriented) and finally per crop revenues, in order to add the impact of different crops cultivated on the same land, during the same year.

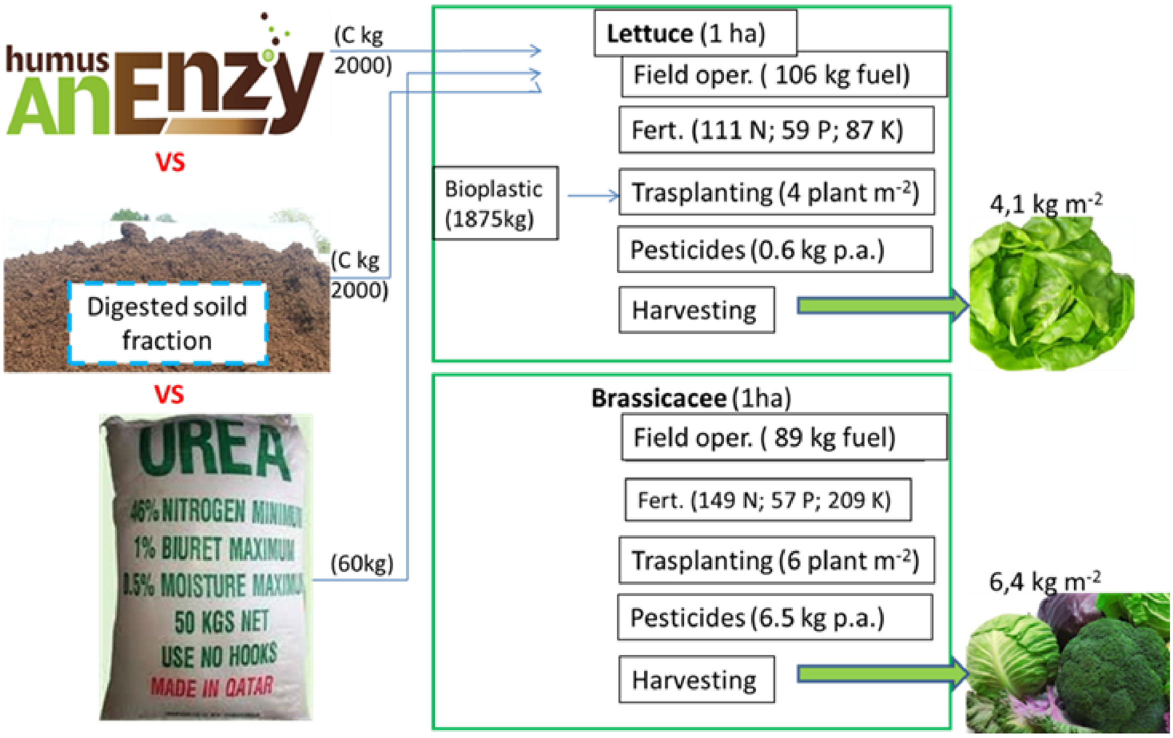


Figure 1. System boundaries for LCA calculations of lattuce and brassicacee production in Grandi farm.

2.2 Results

LCA analyses on maize cropping system was performed at the project sites of Tetto Frati (Piemonte) and Castel Volturno (Campania). The evaluation of main environmental impacts (Table 4) indicated that the compost application on maize systems, produced lower impacts than mineral fertilizers, for acidification (since urea emits more NH3), global warming potential (due to mineral fertilizers production), particulate matter (again due to emissions of NH3, precursor of PM), and terrestrial eutrophication. On the contrary, compost showed slightly higher impact than mineral fertilizers for freshwater eutrophication due to unbalanced supply of large amount of P into the manure-based organic materials.

The larger impact found for compost application on lettuce crops, (Table 5) may be related to two main factors: the industrial production of used compost, which have large energetic consume in respect to the on-fam approach and to the use of higher doses of organic matter addition. The larger dose of compost in fact correspond to an almost double quantity of nutrients (N and P) required by the crop uptake. However, at similar level of nutrient supply the compost has best performance than the mineral fertilizer.

Therefore, to evaluate the environmental impacts, it is necessary to combine the different approaches and targets. In organic depleted soils, with low fertility a large addition of humified compost may allow a fast increase of SOC content, thereby providing a suitable amounts of plant nutrients. On the other hand, in cropping systems with a suitable soil fertility a lower amount of compost will maintain an adequate level of SOM content and biodiversity, thereby allowing a decrease in the use of energetic inputs (mineral fertilizers, irrigation, chemicals).

Table 4 Environmental impacts of soil managements in maize cropping systems

	CastelVolturno		Tetto Frati		
	Compost	Mineral	Compost	Fresh digestate	Mineral
Acidification, accumulated exceedance [Mole of H+ eq.]	66.71	74.89	66.22	60.64	74.34
Ecotoxicity for aquatic fresh water, USEtox (recommended) [CTUe]	450.96	380.76	390.38	354.03	340.72
Freshwater eutrophication, EUTREND model, ReCiPe [kg P eq]	135.15	-2.05	90.96	41.48	6.79
Human toxicity cancer effects, USEtox (recommended) [CTUh]	0.00	0.00	0.00	0.00	0.00
Human toxicity non-canc. effects, USEtox (recommended) [CTUh]	0.00	0.00	0.00	0.00	0.00
Ionising radiation, human health effect model, ReCiPe [kg U235 eq]	25.04	28.80	17.67	12.86	24.76
IPCC global warming, excl biogenic carbon [kg CO2-Equiv.]	1013.50	1170.73	846.45	775.79	1072.52
IPCC global warming, incl biogenic carbon [kg CO2-Equiv.]	963.02	1158.72	816.64	769.28	1069.49
Marine eutrophication, EUTREND model, ReCiPe [kg N-Equiv.]	44.43	11.10	38.75	15.35	12.49
Ozone depletion, WMO model, ReCiPe [kg CFC-11 eq]	0.00	0.00	0.00	0.00	0.00
Particulate matter/Respiratory inorganics, RiskPoll [kg PM2,5-Equiv.]	1.82	1.92	1.73	1.60	1.84
Photochemical ozone formation, LOTOS-EUROS model, ReCiPe [kg NMVOC]	11.24	9.20	10.59	9.98	8.87
Resource Depletion, fossil and mineral, reserve Based, CML2002 [kg Sb-Equiv.]	0.01	0.01	0.01	0.01	0.01
Terrestrial eutrophication, accumulated exceedance [Mole of N eq.]	300.96	336.88	300.47	276.28	335.61
Total freshwater consumption, including rainwater, Swiss Ecoscarcity [UBP]	295597.60	294028.06	51262.08	50343.22	50184.20

Table 5Environmental impacts of soil managements in horticultural system at Grandi site

	1st crop (lettuce)			2nd crop		
	Compost	Fresh digestate	Mineral	Compost	Fresh digestate	Mineral
Acidification, accumulated exceedance [Mole of H+ eq.]	55.06	49.32	28.13	21.79	21.79	21.86
Ecotoxicity for aquatic fresh water, USEtox (recommended) [CTUe]	213.38	174.35	156.33	648.43	648.43	649.46
Freshwater eutrophication, EUTREND model, ReCiPe [kg P eq]	74.20	24.11	-6.34	23.01	24.63	27.97
Human toxicity cancer effects, USEtox (recommended) [CTUh]	0.00	0.00	0.00	0.00	0.00	0.00
Human toxicity non-canc. effects, USEtox (recommended) [CTUh]	0.00	0.00	0.00	0.00	0.00	0.00
Ionising radiation, human health effect model, ReCiPe [kg U235 eq]	13.66	8.82	13.74	16.48	16.48	16.49
IPCC global warming, excl biogenic carbon [kg CO2-Equiv.]	1800.29	1716.11	1779.98	1083.84	1083.84	1089.41
IPCC global warming, incl biogenic carbon [kg CO2-Equiv.]	1764.05	1703.32	1769.40	1078.63	1078.63	1084.16
Marine eutrophication, EUTREND model, ReCiPe [kg N-Equiv.]	46.64	33.66	14.35	21.49	26.40	45.21
Ozone depletion, WMO model, ReCiPe [kg CFC-11 eq]	0.00	0.00	0.00	0.00	0.00	0.00
Particulate matter/Respiratory inorganics, RiskPoll [kg PM2,5-Equiv.]	1.58	1.44	0.91	0.76	0.76	0.77
Photochemical ozone formation, LOTOS-EUROS model, ReCiPe [kg NMVOC]	10.49	9.66	6.75	8.77	8.77	8.86
Resource Depletion, fossil and mineral, reserve Based, CML2002 [kg Sb-Equiv.]	0.01	0.01	0.01	0.01	0.01	0.01
Terrestrial eutrophication, accumulated exceedance [Mole of N eq.]	236.02	210.95	113.65	98.02	98.02	98.38
Total freshwater consumption, including rainwater, Swiss Ecoscarcity [UBP]	135025.73	134070.24	133800.34	56495.23	56495.23	56509.42

The Economic and Energy budgets were calculated as the weighted average of all cropping cycles performed at the project sites. The economic data of maize fields are clearly affected by the lower yields found in the first year of new SOM managements applications (Table 6), with a lower performance in respect to mineral fertilizers. Conversely the results of lettuce (Table 7) confirmed the prompt positive response of horticultural crops to the use of organic amendments and fertilizers, not only for the SOC level but also for the agronomic aspects.

Table 6 Economic evaluation of soil management in maize systems

		Tetto Frati			Castel Volturno	
€/ha		Compost	Digestate	Mineral	Compost	Mineral
INCOME		2083	2056	2438	1948	2301
COSTS	Crop Management	862	852	844	872	863
	Fertilizers/pesticides	548	599	648	493	654
	Seeds	224	224	224	224	224
	PROFIT	449	381	721	358	560

Table 7 Economic evaluation of soil management in horticultural crops at Grandi site

		1st crop (lettuce)			2nd crop		
€/ha		Compost	Digestate	Mineral	Compost	Digestate	Mineral
INCOME		13977	12869	11349	8685	8098	8117
COSTS	Crop Management	4922	4733	4733	4333	4333	4333
	Fertilizers/pesticides	1755	1717	1646	542	525	542
	Seeds	2138	2138	2138	1705	1705	1705
	PROFIT	4661	4281	2833	2105	1535	1537

As explained in the report on field results (Annex 3), under application of a SOM management the soil resilience undergoes to a unavoidable transition period towards a new steady state, with a modified equilibrium of physical, chemical and biological parameters, and variation in crop yields. The target of the maintenance of crop productivity may be faced with tailored agronomic approaches based on a combination of different techniques and methodologies. In this respect the economic aspect is a key point of the recent Regional policies that started to support the adoption of sustainable managements in order to maintain the farmer’s income, promote the productive modification and the use of recycling in both by-products and processes, thereby conveying the agro-ecosystems into a more feasible approach requested by the circular economy

Positive response on the use of organic materials were shown by the evaluation of and Energetic balance, that revealed a saving energy process in all the analysed project sites (Figs. 2 and 3), as compared to conventional systems. Moreover, it has to be considered that the use of on-farm composting systems may further reduce the energy requirements associated to the industrial facilities

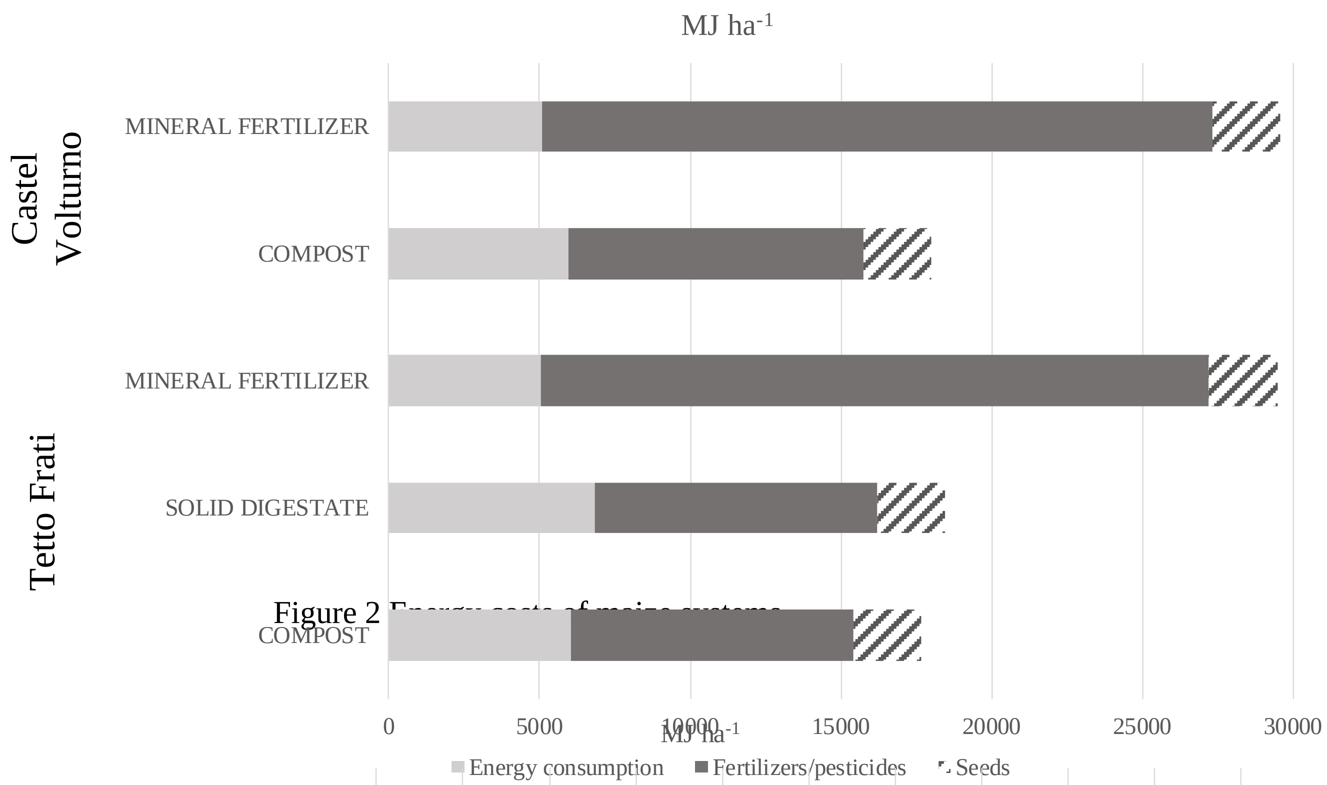


Figure 2 Energy costs of maize systems

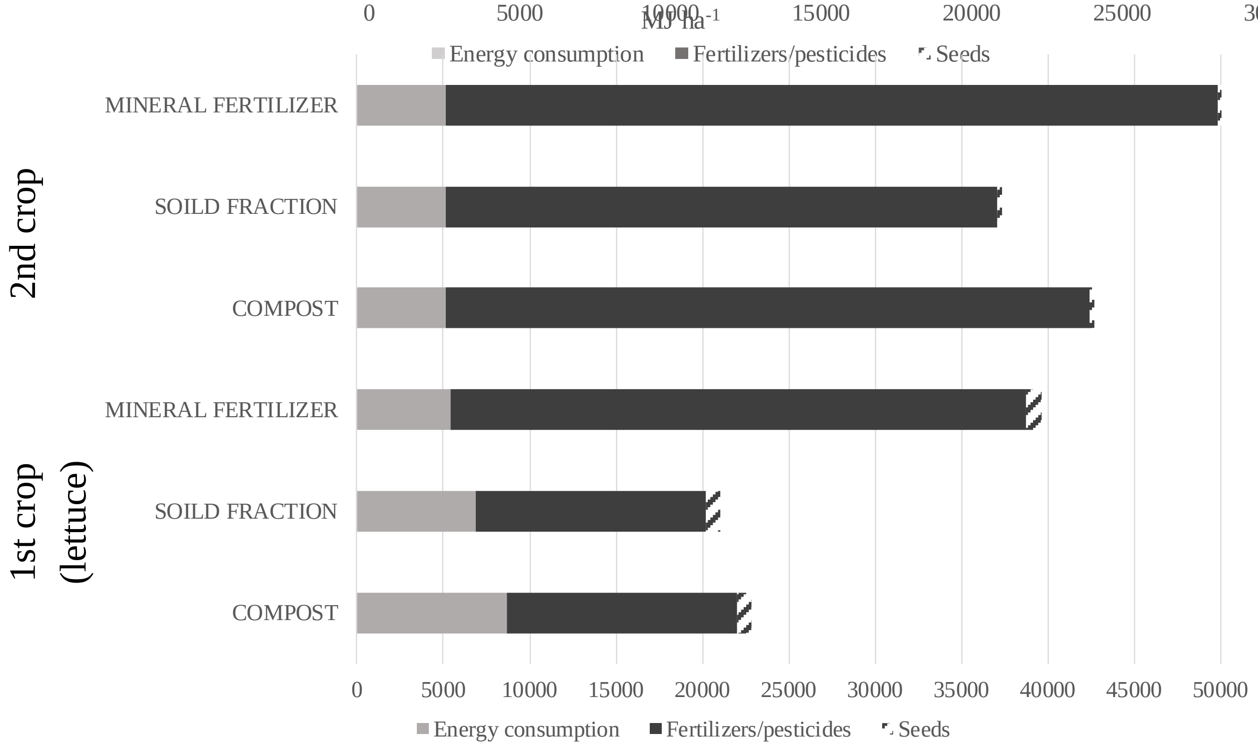


Figure 3 Energy costs at Grandi project site

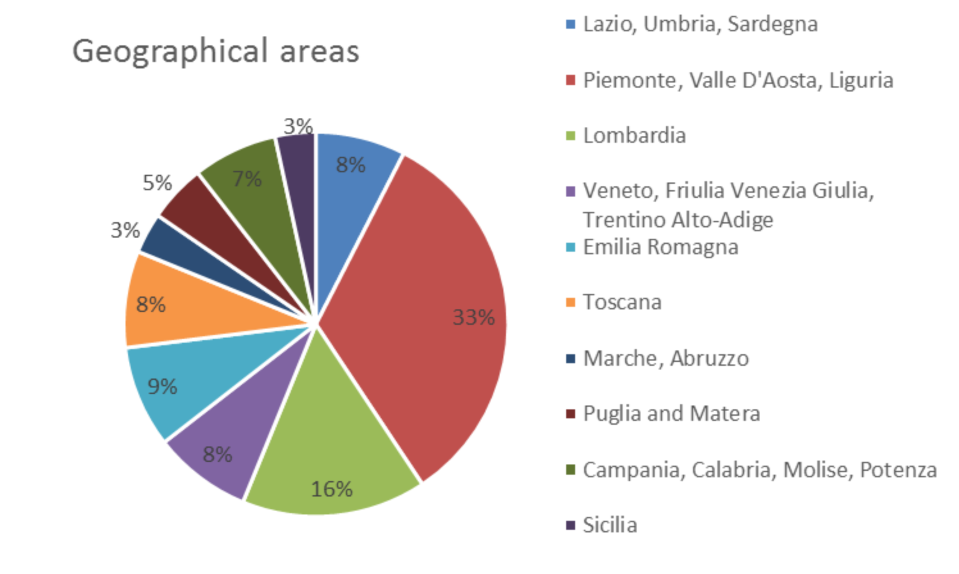
Annex 6 Closing report on C6 Action-

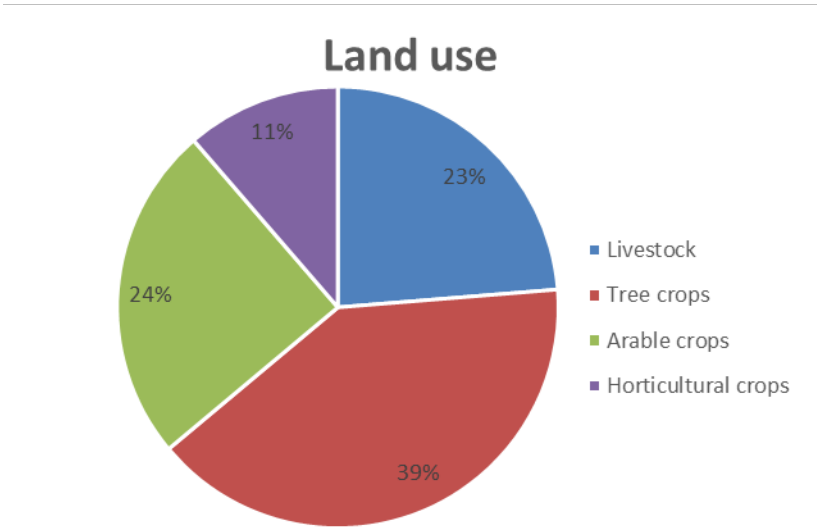
Monitoring the acquired awareness about available techniques for soil organic carbon stabilization and accumulation and bioresources valorisation

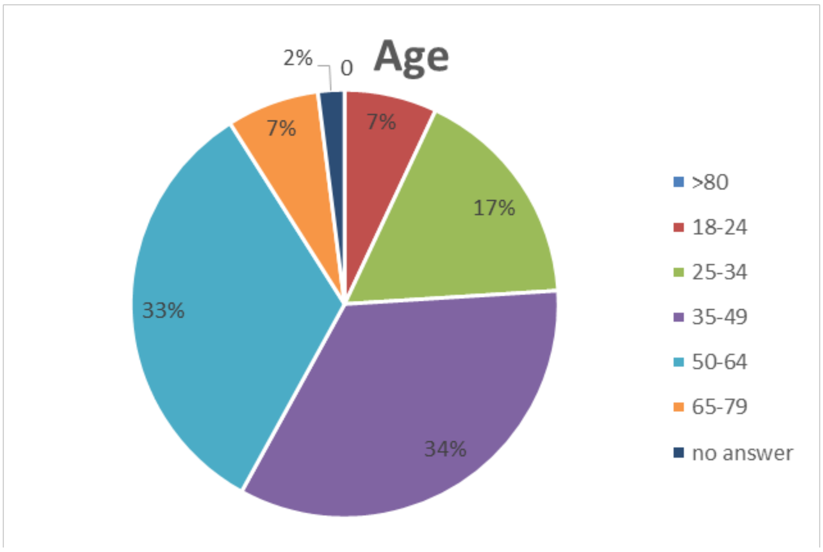
Beneficiary responsible AGROSELVIT DISAFA

Report for C.6 Action

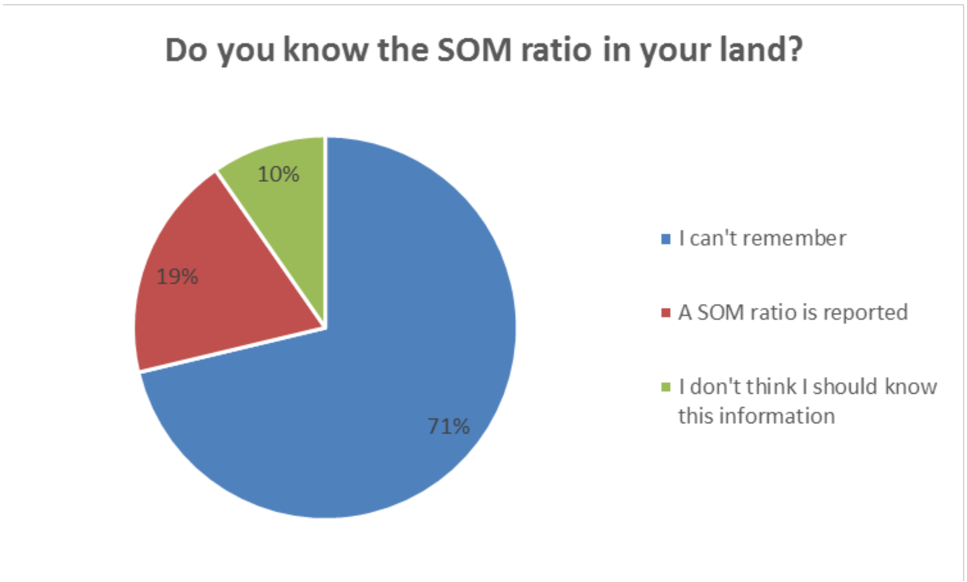
We obtained answers 705 questionnaires, quite homogeneously covering all the Italian regions, and different types of land use, and age of interviewed farmers.



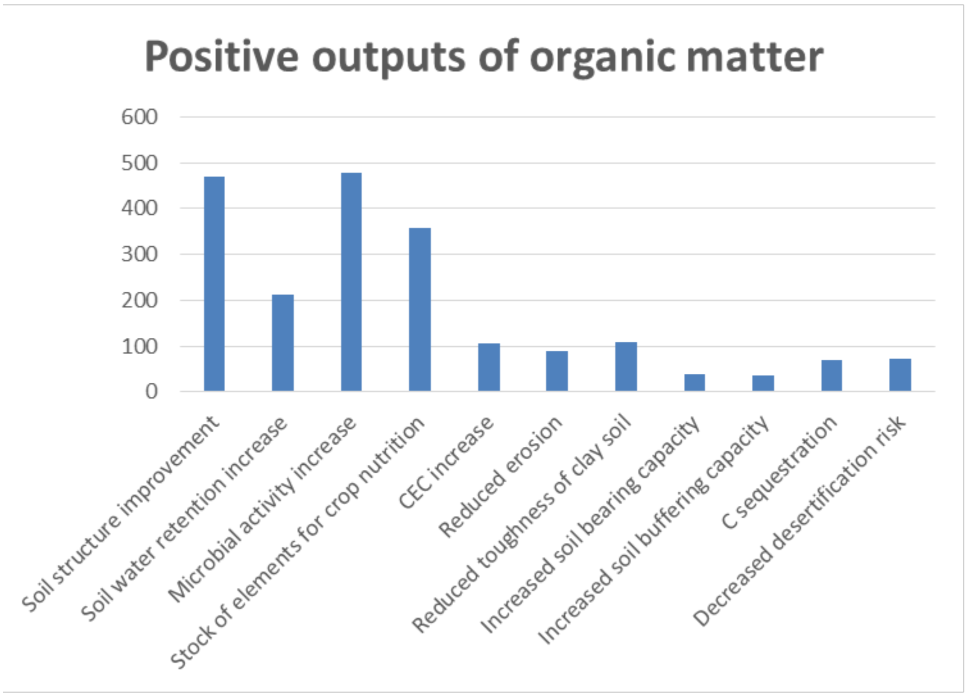




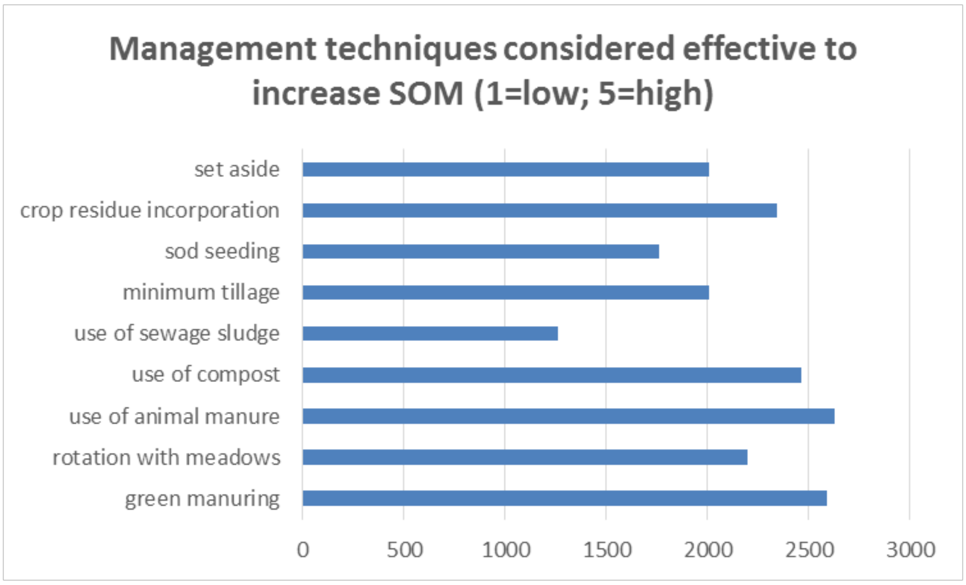
Only a minority of interviewees was able to report the mean SOC value of their soils, while more than 80% did not know the value or was even not interested to know it.



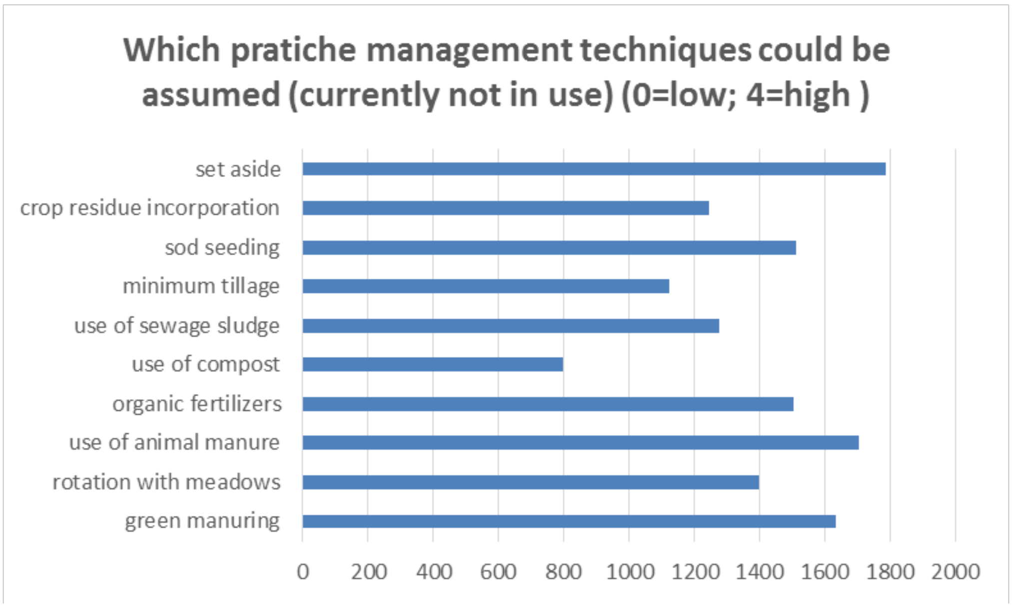
Despite this unawareness, farmers showed to be familiar with the positive outputs of SOM, especially those linked with the impovement of fertility (physical, chemical and biological), while aspects of environmental sustainability were more neglected.

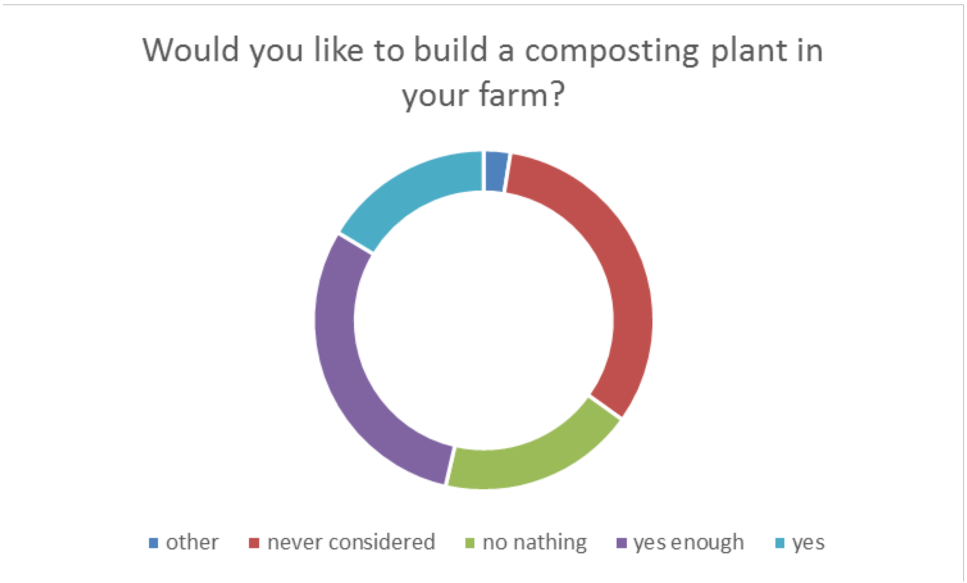


The use of compost is included among the techniques considered more effective for SOC increase.



Nevertheless, when asking the potential opportunity of using compost or building a composting plant, not more half of interviewees are available.


Which pratiche management techniques could be assumed (currently not in use) (0=low; 4=high)
set aside
crop residue incorporation
sod seeding
minimum tillage
use of sewage sludge
use of compost
organic fertilizers
use of animal manure
rotation with meadows
green manuring
0
200
400
600
800
1000
1200
1400
1600
1800
2000


Would you like to build a composting plant in your farm?
other
never considered
no nathing
yes enough
yes

After LIFE Communication plan

La continuazione delle azioni di diffusione e comunicazione relative alle attività del LIFE CarbOnfarm si basa su diversi approcci che combinano le specificità operative dei singoli Beneficiari e le indicazioni ricevute durante il decorso delle attività progettuali.

Approccio scientifico

I Beneficiari AGROSELVIT-DISAFA, CERMANU, CREA-OF, UNIBAS sono Dipartimenti Universitari e Centri di Ricerca pubblici, con una specifica orientazione scientifica. In questo ambito quindi un approccio estremamente valido per la diffusione dei dati del Progetto è rappresentato dalla pubblicazione dei risultati su rivista scientifiche internazionali
Sono stati individuate quattro tematiche principali nelle qauli sviluppare la pubblicazione di circa 12 articoli scientifici nei prossimi anni:

- analisi della sostenibilità delle metodiche affrontate dal progetto, con particolare riferimento agli impatti ambientali, energetici ed economici delle soluzioni proposte in termini di compostaggio, e metodiche di gestione della sostanza organica dei suoli

I risultati relativi alla valutazione LCA (Life Cycle Assessment) e alla valutazione degli aspetti economici del compostaggio "on-farm" e dei sistemi fruttiferi, sono stati pubblicati su due articoli disponibili presso il sito web del progetto: *Journal of Cleaner Production 142 (2017) 4059-4071; Journal of Cleaner Production 172 (2018) 3969-3981*)

- due articoli riguarderanno le valutazioni relative alla qualità dei compost utilizzati nel progetto con il dettaglio delle informazioni sulle caratteristiche chimiche e molecolari, sulle proprietà biologiche, biostimolanti e suppressive
- altre tre pubblicazioni saranno focalizzate sui risultati delle analisi riguardanti la carattrizzazione della trasformazione della sostanza organica e le valutazioni delle emissioni di gas serra in pieno campo, suti siti progettuali localizzati in Piemonte e in Campania;
- infine, verranno pubblicati i dati riguardanti la comparazione delle produzioni agronomiche ottenute con i diversi approcci gestionali dei sistemi colturali utilizzati nei diversi siti progettuali; la prima pubblicazione riguardante l'adozione delle tecniche di rilevamento EMI e ESAP per la valutazione della qualità dei suoli, adoperata nell'azienda Mellone è disponibile sul sito web del progetto (*SoftwareX 6 (2017) 107–117*)

Questa parte delle attività di comunicazione riguarderà anche la presentazione dei risultati in Converni nazionali e internazionali delle società scientifiche (e.g. *Società Italiana di Chimica Agraria* SICA; *Società Italiana di Agronomia* SIA, *International Society of Humic Substances* IHSS; *Convegno annuale sulla Biodiverstià*; *Società Italiana di patologia Vegetale SiPAV*; *SOC conferences* FAO)
Al fine di coinvolgere altre categorie interessate agli sviluppi dei risultati progettuali, quali agricoltori, professionisti e tecnici, delle sintesi lavori verranno proposta su riviste specifiche a diffusione nazionale. Attualmente sono stae attivati contatti col la rivista dell'Agenzia della regione Basilicata *ALSIA-Agrifoglio*, e con la pubblicazione "*L'Informatore Agrario*" per le presentazione dei dati relativi alla realizzazione dell'impianto di compostaggio on farm presso l'azienda Prima Luce

- Eventi di comunicazione

L'organizzazione e la partecipazione a giornate dimostrative inerenti le tematiche progettuali proseguirà secondo le modalità già utilizzate durante lo sviluppo del progetto

- Visite ai siti progettuali (Tetto Frati, CastelVolturno Prima Luce) con gli studenti dei corsi di Scienze Agrarie saranno organizzate dai Dipartimenti delle Università di Torino, Napoli e Basilicata coinvolte nel progetto nell'ambito delle attività didattiche. Il *Manuake sul compostaggio aziendale* e il *Manuale per l'auto valutazione della qualità del suolo* saranno adottati come strumenti didattici integrativi
- Viene altresì prevista l'organizzazione di una nuova Summer school per Dottorandi e giovani ricercatori sulle tematiche del ruolo della sostanza organica nelle pratiche della agricoltura sostenibile
- I contatti attivati con le aziende, agricoltori, associazioni di produttori, scuole superiori e istituti agrari saranno mantenuti ed ampliati con lo svolgimento di incontri e giornate dimostrative.
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After LIFE Communication plan

The extension of the communication of backgrounds, targets and results of LIFE CarbOnFarm has been conceived following the guidelines already activated during the project course. Therefore, the main activities will hence ground on the specific expertise of associated Beneficiaries and on the indications acquired in the operative dissemination performed in these years.

➢ Scientific approach

Four associated beneficiaries (AGROSELVIT-DISAFA, CERMANU, CREA-OF, UNIBAS) are University departments and Research centres with a scientific oriented activity. In this respect the more useful and viable way to communicate the results to a large audience of scientific and technical communities, is represented by the publication of the array of acquired data on the Implementation and Monitoring actions.

Four main topics have been individuated for the scientific articles, with a planned publication of about 12 comprehensive works in the specific international journals:

- four planned publications are focused on the analyses of practical sustainability of proposed approaches, regarding the evaluation of energetic and environmental impacts of on-farm composting facilities, composting process and applied Soil Organic Matter management; this latter will include also the practical results of the interviews on the awareness of regional farms on the project topics. Two articles related to the results of the Life Cycle Assessment and economic evaluation of composting process and on the development of LCA evaluation in orchard systems, have been already attained and are available on the download page of project website (*Journal of Cleaner Production 142 (2017) 4059-4071; Journal of Cleaner Production 172 (2018) 3969-3981*)
- two articles will deal with the analyses of compost qualities, including the chemical and molecular characterization, biological essays, metagenomic approach, suppressivity and bio-stimulation activities
- three publications have been programmed for the results of soil organic matter analyses and the data of GHG emission; given the array of applied managements and analysed data two papers will be dedicated to the comparison of the soil treatments at Tetto Frati and Grandi project sites; one additional article will combine the results obtained in the field sites of Campania region
- three publication will focus on the agronomic results of applied management in different project sites; one publication involving the application of EMI and ESAP methodologies for the check of soil characteristics used in Mellone farm has been already published and is available in the download page of project website (*SoftwareX 6 (2017) 107–117*)

As already carried out during the project, this part of communication activity will include also the presentation of main project results in specific National and International meetings in the next two years (e.g. *Società Italiana di Chimica Agraria* SICA; *Società Italiana di Agronomia* SIA, *International Society of Humic Substances* IHSS; *Convegno annuale sulla Biodiverstià*; *Società Italiana di patologia Vegetale SiPAV*; *SOC conferences* FAO)

In order to involve the specific national/regional audience of farmers, technician, agronomists, a synthesis of the main results of LIFE CarbOnFarm, as previously highlighted, will be displayed also in technical journals dedicated to the Agricultural sector. Currently two main tools have been individuated: the monthly publication of the Agricultural Sector of Regione Basilicata *ALSIA-Agrifoglio*, will dedicate specific articles to the results; a first communication is planned for the number of December 2018 with the overview of project activities. A specific contact has been also activated with the Journal "*L'Informatore Agrario*" (reference contact Dr. V. Marcantonio) with a wide and large national audience in the agricultural sector; a first appointment is set for January 2019 with an interview to the responsible of the Project site Prima Luce and the description of the on-farm composting prototype.

These activities will be activated either without additional costs or with the current funds used for the Departments and Research centres for the own dissemination programme

- Dissemination events

Following the experience acquired in the project activities the main envisaged operative lines will regard the involvement of students and of specific stakeholders (Farmer association, Agronomist, Regional Institution)

- the visit of the project sites (Tetto Frati, CastelVolturno Prima Luce) will be continued in the Agricultural Departments of University of Torino, Napoli, Salerno and Basilicata, with at least one annual event for each involved university, within the usual field experiences foreseen in the teaching plans. The produced *Manual for on-farm composting* and the *Kit and manual for self-soil evaluation* will be adopted as operative official recognized tool for the specific teaching courses of master students
- given the previous positive feedback, an attempt will be provided by the CERMANU, supported by CREA-OF, AGROSELVIT, and University of Salerno (UNISA) to organize additional Summer School for young researchers on the topics of LIFE CarbOnfarm; the costs will be covered by fees registration, scientific sponsors and the available specific funds of University of Napoli within the Erasmus programme
- the contact activated with regional Higher schools of Agriculture will be further maintained with the organization of dedicated info-days on the recycle of organic biomass and the use of the kit for self-soil evaluation. An initial event is programmed for the 19th November 2018 within the premises of ALSIA with 10 Higher schools with the distribution of kits (auto produced by ALSIA) and the pdf copy of manual
- contact activated with farm associations, already involved in the project activities, will be further implemented with the co-organization of dedicated workshops on organic farming and the use of the methodologies for self-soil evaluation, at the premises of selected farms, starting with the new realized on-farm composting facilities.

  In this respect a starting event was organized in collaboration with the National organization of Biological producers "rete Humus" (www.retehumus.it), already involved in the project activities, within the Meeting on Manure (26-28 Luglio Modena http://www.modenatoday.it/eventi/festoval-letame-bio-slow-serramazzoni-27-28-29-luglio-2018.html). An additional event is planned on the 12th November 2108 at the premises of Regione Basilicata with the presentation of LIFE CarbOnFarm results and dissemination products, the participation of the regional Coucilor for Agriculture and the representatives of National association Rete Humus and ConProBIO (Annex 7.3.2a Programme event 12 November)
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