Elsevier Editorial System(tm) for Soil & Tillage Research
Manuscript Draft

Manuscript Number:

Title: Soil quality, microbial functions and tomato yield under cover crop mulching in the Mediterranean environment

Article Type: Research Paper

Section/Category: Other

Keywords: lacy phacelia; white mustard; hairy vetch; mulching; microbial biomass; microbial functional diversity.

Corresponding Author: Dr. Roberto Mancinelli, Ph.D.

Corresponding Author's Institution: University of Tuscia

First Author: Sara Marinari, Ph.D.

Order of Authors: Sara Marinari, Ph.D.; Roberto Mancinelli, Ph.D.; Paola Brunetti, Ms.; Enio Campiglia, Ms.

Abstract: An experiment concerning the biological and chemical responses of soil to cover crop mulching was carried out in two adjacent experimental fields (2012 and 2013) under different climatic conditions in the Mediterranean environment (Central Italy). The monthly aridity index was calculated in order to verify the relationship between soil properties and climatic factors under three different cover crop mulches: Vicia villosa Roth (HV), Phacelia tanacetifolia Benth. (LP), and Sinapis alba L.(WM). A conventional management was also included in the experimental fields as control (C). Soil samples were collected at 0-20 cm depth after the transplanting and the harvesting of tomato (May and August, respectively), in order to assess the initial and residual effects of mulching on soil quality. In the two experimental years, the amount of precipitation from May to August was 110 mm in 2012 and 172 mm in 2013. The average values of AI were 18 and 49 in 2012 and 2013, respectively. LP mulching was sensitive to low precipitation levels in terms of higher aboveground decomposition rate (from May to August 2012 the variation of dry matter was -53% in LP, 64% in HV and 69% in WM) and a lower tomato yield compared to the control in 2012 (4.2 kg m-2 in LP and 5.2 kg m-2 in C). WM mulching was sensitive to low precipitation in terms of soil nutrient storage (from May to August 2012 the variation of soil carbon was 19% in WM., 6% in C, -5 % in LP and 10% in HV; the variation of soil nitrogen was 44% in WM, 2% in C, -2% in LP and 13% in HV). Soil microbial activity and functional diversity were strongly affected by the climatic conditions in all mulching treatments. In particular, precipitation influenced soil carbon availability, which enhanced microbial functional diversity. In short, the effects of lacy phacelia, white mustard and hairy vetch mulching on soil quality, microbial functions and tomato yield were influenced by summer precipitation and temperature in the Mediterranean environment

Suggested Reviewers: Mary Cathleen Savin PhD
University of Arkansas, Fayetteville, USA
msavin@uark.edu

Carmen Trasar Cepeda

Instituto de Investigaciones Agrobiológicas de Galicia-Consejo Superior de Investigaciones Científicas (Spain)
ctrasar@iiag.csic.es

Graziana Masciandaro
ISE-CNR Pisa (Italy)
grazia.masciandaro@ise.cnr.it

Emilio Benitez
Departamento de Agroecología y Protección Vegetal, Estación Experimental del Zaidín, CSIC (Spain)
emilio.benitez@eez.csic.es

Jeffrey S. Buyer
USDA ARS Beltsville Agricultural Research Center, Beltsville, United States
jeffrey.buyer@ars.usda.gov

Cover Letter

Click here to download Cover Letter: Cover Letter.doc

UNIVERSITÀ DEGLI STUDI DELLA Tuscia

DAFNE
DIPARTIMENTO DI SCIENZE E TECNOLOGIE PER L'AGRICOLTURA, LE FORESTE, LA NATURA E L'ENERGIA

Via S. Camillo de Lellis s.n.c. 01100 – Viterbo

Direzione: Tel. 0761 357581
Amministrazione: Tel. 0761 357437-554 - Fax 0761 357434

Dr. Roberto Mancinelli

Viterbo May 22nd, 2014

Dear Editor,

I am sending you the manuscript titled "**Soil quality, microbial functions and tomato yield under cover crop mulching in the Mediterranean environment**" to be submitted for possible publication in Soil & Tillage Research. I hereby declare that the present Ms. in not either being submitted to or already published in another journal. All co-authors agree to submit the present Ms. version to Soil & Tillage Research.

The present Ms. aimed to verify the effects of cover crop mulching on soil quality and microbial functions under fluctuating climatic factors occurring during the two-year study period in the Mediterranean environment.

The main evidence provided in the study is that in the Mediterranean environment the effect of different cover crop mulching on soil microbial biomass, its activity and functional diversities were strongly affected by the climatic conditions that influenced the decomposition rate of mulch and soil carbon availability, as K2SO4 extractable-C, which enhanced microbial functional diversity.

Yours sincerely

Roberto Mancinelli

www.dafne.unitus.it ♦ e-mail: s_dafne@unitus.it; direzione.dafne@unitus.it

Highlights

- Tomato yield and soil responses to cover crop mulching were assessed
- Two fields experiment (years 2012 and 2013) were carried out in a Mediterranean area
- Cover crop mulches were: hairy vetch HV, lacy phacelia LP, and white mustard WM
- LP mulch showed higher decomposition rate and lower tomato yield under arid conditions
- The effects of mulches on soil quality and Tomato yield depend on summer weather

Soil quality, microbial functions and tomato yield under cover crop mulching in the Mediterranean environment

Marinari S. [b], Mancinelli R. [a*], Brunetti P. [a], Campiglia E. [a]

[a] Department of Science and Technology for Agriculture, Forestry, Nature and Energy, Via S. Camillo de Lellis, 01100 Viterbo, Italy

[b*] Department for Innovation in Biological, Agro-food and Forest systems, Via S. Camillo de Lellis, 01100 Viterbo, Italy

[*] Corresponding Author Roberto Mancinelli Tel.: +39 0761 357556, e-mail address: mancinel@unitus.it

Abstract

An experiment concerning the biological and chemical responses of soil to cover crop mulching was carried out in two adjacent experimental fields (2012 and 2013) under different climatic conditions in the Mediterranean environment (Central Italy). The monthly aridity index was calculated in order to verify the relationship between soil properties and climatic factors under three different cover crop mulches: *Vicia villosa* Roth (HV), *Phacelia tanacetifolia* Benth. (LP), and *Sinapis alba* L.(WM). A conventional management was also included in the experimental fields as control (C). Soil samples were collected at 0-20 cm depth after the transplanting and the harvesting of tomato (May and August, respectively), in order to assess the initial and residual effects of mulching on soil quality. In the two experimental years, the amount of precipitation from May to August was 110 mm in 2012 and 172 mm in 2013. The average values of AI were 18 and 49 in 2012 and 2013, respectively. LP mulching was sensitive to low precipitation levels in terms of higher aboveground decomposition rate (from May to August 2012 the variation of dry matter was -53% in LP, 64% in HV and 69% in WM) and a lower tomato yield compared to the control in 2012 (4.2 kg m^{-2} in LP and 5.2 kg m^{-2} in C). WM mulching was sensitive to low precipitation in terms of soil nutrient storage (from May to August 2012 the variation of soil carbon was 19% in WM., 6% in C, -5 % in LP and 10% in HV; the variation of soil nitrogen was 44% in WM, 2% in C, -2% in LP and 13% in HV). Soil microbial activity and functional diversity were strongly affected by the climatic conditions in all mulching treatments. In particular, precipitation influenced soil carbon availability, which enhanced microbial functional diversity. In short, the effects of lacy phacelia, white mustard and hairy vetch mulching on soil quality, microbial functions and tomato yield were influenced by summer precipitation and temperature in the Mediterranean environment

Keywords: Lacy phacelia; white mustard, hairy vetch; mulching; microbial biomass, microbial functional diversity.

1.Introduction

In the last century, humans have intensely started cultivating land for producing plants for food thus causing a depletion of natural resources and environmental degradation (Pankhurst et al., 1997). Recently people have become more and more environmentally friendly towards environmental pollution, food quality and strategies for sustainable agriculture in order to preserve non-renewable natural resources such as soil. Soil is a dynamic, living, natural body which is vital for the correct functioning of terrestrial ecosystems and it represents a unique balance between physical, chemical and biological factors (Pankhurst et al., 1997; Shukla and Varma, 2011). It is important to establish sustainable agriculture, environmental quality, plant, animal and human health in order to maintain soil quality and health (Pankhurst et al., 1997; Doran and Zeiss, 2000). Karlen and Mausbach (1997) define soil quality as the "capacity of soil to function". Since it is difficult to measure and quantify this capacity, it is useful to examine related properties to biological processes and environmental quality. There are some measures which are suitable for quantifying soil quality by means of soil biological characteristics (e.g microbial biomass and its activities), soil chemical (e.g. soil organic matter), and physical characteristics (e.g. water infiltration rates or bulk density) (Campbell et al., 2001). Among all soil properties, microbial biomass and soil enzyme activities prove to be sensitive indicators of soil quality as they are measurements which rapidly respond to changes due to different management and environmental factors (Alvear et al., 2005). Soil perturbations, such as tillage, can alter microbial processes, biogeochemical nutrient cycles (overall C stored) (Janzen et al., 1997) thus modifying the structural and functional diversities of the soil microbial communities (Lienhard et al., 2012). In fact, physical disturbance exposes Soil Organic Matter (SOM) to biological activity, thus facilitating the penetration of water at greater depths and accelerating its decomposition. Tillage modifies the physical and chemical environment of the soil and affects soil water content, soil temperature, aeration and the contact between SOM and mineral particles, globally influencing the soil microbial population which in turn effects the physical/chemical environment of the soil (Kladivko, 2001; Bünemann et al., 2006; Pascault et al.,

2010). Modern and intensive agriculture, using large amounts of mineral nitrogen and water, affects soil microbial biomass and its activity in different ways (Bünemann et al., 2006) just as deep soil tillage causes a faster oxidation of SOM (Mancinelli et al., 2008). The advantages of no tillage for improving soil organic matter are greatly enhanced by growing cover crops and mulches (Franzluebbers, 2005). Cover crop is any living ground cover which is planted into or after a main crop and then killed before planting the next crop. In the Mediterranean environment, cover crops are generally planted in the autumn after harvesting the summer crop and they grow during the winter. There are numerous benefits of using cover crops for enhancing soil health. They prevent soil erosion, protect water quality, improve yields by enhancing soil health (soil structure and tilth), cut fertilizer costs by fixing the atmospheric Nitrogen, conserve soil moisture, and reduce the need for herbicides and other pesticides (Hartwig and Ammon, 2002). Cover crops can be cut and left on the soil surface as dead mulches (Bond and Grundy, 2001). For instance, straw mulching and no-till technologies have improved grain yields in the 250-mm-rainfall wheat belt of the northern Negev region of Israel (Landau et al., 2007). In the Mediterranean environment dead mulches are generally cut during spring, just before cultivating the main crop and are left on the soil until the end of the growing season, thus causing a reduction in soil water evaporation, an increase in soil water content, a decrease in daily soil temperature excursion (Dahiya et al., 2007) and weed control thus enhancing the yield of the main crop (Campiglia et al., 2014). The total surface area used for cultivating processing tomato in Italy is about 75.525 ha (ISTAT, 2012), representing the most important vegetable crop. Conventional practices are often used such as deep tillage, plastic mulching and chemical fertilization in order to obtain high tomato yields (Carrera et al., 2007). On the other hand, sustainable farming in tomato cultivation can be used to improve organic matter in the soil (green manuring of cover crops), to reduce synthetic inputs and environmental pollution caused by chemical fertilizers and pesticides, and reduce crop losses caused by diseases and pests thus enhancing environmental characteristics (Briar et al., 2007). However, until recently little was known about the biological, chemical, and physical responses of soil to cover crop mulching. Since

seasonal fluctuations of soil microbiological processes can be caused by variable climatic conditions (Manzoni and Porporato, 2009; Mancinelli et al., 2013), the aim of this study was to verify the effects of cover crop mulching on soil quality and microbial functions under fluctuating climatic factors occurring during the two-year study period in the Mediterranean environment (Central Italy).

2. Materials and Methods

2.1. Experimental Site and design

The research was carried out over a two-year period (2011-2013) in two adjacent and homogeneous fields established in September 2011 at the experimental farm of the University of Tuscia (Viterbo) located approximately 80 km North of Rome (45°25'N, 12°04'E). The climate of the area is typical of the Mediterranean environment with a mean annual precipitation of 760 mm, mostly concentrated during the autumn and spring seasons, minimum temperatures a little below 0 °C in the winter and maximum temperatures of about 36 °C in the summer. The monthly aridity index (AI) was calculated according to the following equation (1) to verify the differences between the months of the cover crop and main crop (tomato) growing seasons during the two-year study (De Martonne, 1926):

$$AI = \frac{Pi}{Ti + 10} \quad (1)$$

where *AI* = aridity index; *Pi* = monthly precipitation amount; *Ti* = monthly mean air temperature (Mancinelli et al., 2013). The aridity index was used to verify the relationship between the soil properties and climatic factors.

Chemical and physical analyses were carried out on three soil samples collected from the experimental fields before starting the trials (autumn) in order to verify the homogeneity of each field. The soil of the experimental field is of volcanic origin classified as a *Typic Xerofluvent*. Physicochemical characterization was carried out using the official methods of analysis (MiPAF, 2000). The particle size distribution analysis indicated that the textural class of the surface horizon

(0–20 cm depth) fell within the sandy-loam USDA classification with 63% sand, 22% silt, and 15% clay; pH of 6.9 in 2012 and 7.2 in 2013 (1:2.5 w:v) (Table 1). The soil nitrogen content in the two fields was similar, while the organic carbon was higher in 2013 compared to 2012. The amount of carbonates also varied between the two fields since it was five times higher in 2013 compared to 2012 (Table 1).

2.2 Experimental field and treatments

In both experimental years, the fields were arranged in a randomized block design with three replications. In autumn three cover crops, *Vicia villosa* Roth (HV), *Phacelia tanacetifolia* Benth. (LP), and *Sinapis alba* L. (WM), were sown; they were then cut in spring to be used as mulches. The three cover crop mulches were compared with a control treatment without mulching (C). In May the tomato seedlings were transplanted into the mulches. The tomato plants were irrigated with 100% of potential evotranspiration and they were left unfertilized.

The area of the experimental plots was 4400 m^2 (55m x 80 m) which makes it possible to carry out all farming operations with agricultural machinery. Soil tillage was carried out before sowing the cover crops in September, while soil tillage in control plots was also carried out in May. In May a particular machine was used to cut the cover crops and arrange the mulch in strips over the soil surface. The tomato plants were then manually transplanted onto the mulch rows. The tomato yield was harvested at the end of August.

In order to study the effects of mulching on soil quality, soil samples were collected at the initial and final steps of the main crop (tomato) cycle: (i) after transplanting (30^{th} May 2012 and 24^{th} May 2013) and (ii) after harvesting (23^{th} August 2012 and 30^{th}August 2013). The soil samples were collected at a depth of 0-20 cm in each plot, after removing the litter layer. The soil samples were sieved (<2mm) and preserved at 4°C until they were analyzed.

2.3.: Aboveground biomass, C and N content of cover crop mulches

In both experimental fields, samples of mulch biomass were collected in a 0.5m^2 central area of each plot before tomato transplanting (May) and following tomato harvesting (August). The samples of the mulch biomass were dried at 70 °C until constant weight in order to determine their dry weight and the carbon and nitrogen content were determined by means of an elementary analyzer (Thermo Soil NC—Flash EA1112). The samples of dried mulch were homogenized with a mill before analyses. The variations which occurred from May to August of the dry aboveground biomass, soil carbon and nitrogen input were expressed as percentages of the initial value (Δ%).

2.4. *Soil chemical and biochemical properties*

Soil pH was determined in both solutions, water pH_{H2O} and KCl 1 N pH_{KCl} (1:2.5 w:v). The total organic carbon (Corg) and nitrogen (Ntot) contents were determined using the elemental analyzer (Thermo Soil NC—Flash EA1112). 20 mg of minced soil were weighed in Ag-foil capsules, then 40 µl of HCl solution (10%) were added to eliminate carbonates and the procedure was repeated again after a night of rest. After 4 hours, the Ag-foil capsules were placed on a hotplate at the temperature of 65 °C for 3 hours. The samples were left to cool and then closed to be analyzed with the elemental analyzer. The variations of the chemical properties of the soil from May to August were expressed as percentages (Δ%). Moreover, the microbial biomass carbon (Cmic) was determined with the fumigation-extraction method (Vance et al., 1987). Two portions of soil (20 g), were conditioned at 55% of water holding capacity (WHC). The first portion was not fumigated and immediately extracted with 80 ml of 0.5 M K_2SO_4 for 30 min by oscillating at 200 rpm and then filtered (Whatman no. 42). The second portion was fumigated for 24 h at 25 °C with ethanol-free $CHCl_3$ and then extracted as described above. Organic C and N in the extracts were determined with the TOC-V CSN and TNM-1 analyzer (Shimadzu). The extractable carbon obtained from non fumigated soil samples was described as soluble carbon form (Cext). The microbial biomass was calculated as follows: biomass C = EC:k_{EC}, where EC is the difference between organic C extracted from fumigated soils and organic C extracted from non-fumigated soils and k_{EC} = 2.64; microbial

biomass N = EN:$k_{EN,}$ where EN is the difference between organic N extracted from fumigated soils and organic N extracted from non-fumigated soils and k_{EN}= 2.22.

2.4.1 Enzymatic Activity

The enzymatic activities were determined with microplate assay and fluorogenic substrates, according to the method described by Marx et al.(2001) and Vepsäläinen and al. (2001), based on the use of fluorogenic methylumbelliferyl (MUF)-substrates. The soils were analyzed for β-cellobiohydrolase (EC 3.2.1.91), N-acetyl-β-glucosaminidase (EC 3.2.1.30), β-glucosidase (EC 3.2.1.21), α-glucosidase (EC 3.2.1.20), acid phosphatase (AP, EC 3.1.3.2), arylsulfatase (EC 3.1.6.1), xylosidase (EC 3.2.2.27) and butyrate esterase (EC 3.1.1.1) using 4-MUF-β-D-cellobioside, 4-MUF- N-acetyl-β-glucosaminide, 4-MUF-β-D-glucoside, 4- MUF-α-D-glucoside, 4-MUF-phosphate, 4-MUF–sulphate, 4-MUF-7-β-D-xyloside and 4-MUF–butyrate as substrates, respectively. 2 g of soil were weighed inside a sterile jar and 50 ml of water was added. Soil suspension was obtained by homogenizing it with an Ultra Turrax at 9600 rpm for three minutes. Aliquots of 50 µl were withdrawn and dispensed into a 96 well microplate (in three analytical replicates). Finally, 50 µl of Sodium Acetate buffer 0.5M pH 5.5 and 100 µl of 1 mM substrate solution were added thus obtaining a final substrate concentration of 500 µM. Fluorescence (excitation 360 nm, emission 450 nm) was measured with an automatic fluorimetric plate-reader (Fluoroskan Ascent) and readings were taken after 0, 30, 60, 120 and 180 min of incubation at 30 °C (Marinari et al., 2013; Pignataro et al., 2012).

The synthetic enzyme index (SEIc) was calculated using the values of the enzymatic activities involved in the C cycle (β-glucosidase, α-glucosidase, xylosidase, cellobiohydrolase) which release the same reaction product (MUF) (Dumontet et al., 2001).

The soil functional diversity was determined using *Shannon*'s diversity index calculated by Eq. (2) (Bending et al., 2002)

$$H' = -\sum pi \log_2 pi \quad (2)$$

where *pi* is the ratio of the activity of one enzyme to the sum of activities of all enzymes.

2.5. Data analysis and statistics

The analysis of variance (ANOVA) was carried out for data regarding the aboveground mulch, soil C, soil N and soil C/N ratio of the cover crop mulches, soil organic carbon, soil total nitrogen, soil C/N ratio of the 2 years and 2 months (May and August) considering the year or the month as a repeated measure across time. The tomato yield was analyzed for each year by one way ANOVA. The analysis of variance (ANOVA) was carried out for data regarding the soil microbial biomass carbon and the microbial C/N ratio, synthetic enzyme index (SEIc) and microbial functional diversity (Shannon's index H') of the two sampling dates (May and August) as a repeated measure across time. Fisher's protected least significant differences (LSD) at the 0.05 probability level ($P<0.05$) were used for comparing the main and interaction effects. All statistical analyses were performed using the JMP 9.0 statistical software package (SAS Institute, Cary, NC).

Pearson's correlation coefficients were computed for the correlation matrix between soil chemical and biochemical properties.

The principal component analysis (PCA) was performed with the JMP 9.0 statistical software package. The soil treatments were grouped using soil chemical and biochemical properties as variables.

3. Results

3.1. Climatic conditions

The daily minimum and maximum temperatures and rainfall throughout the periods of study in 2012 and 2013 are shown in Figure 1. In the two experimental years, the amount of precipitation from May to August was 110 mm in 2012 and 172 mm in 2013. The monthly aridity index (AI) differed between the two experimental years and was generally higher in 2013 (on average 18 and 49 in 2012 and 2013, respectively) (Figure 1). In particular in the period before the sampling dates,

AI was 36 and 40 in May; while it was 0 and 9 in August 2012 and 2013, respectively. The temperatures in 2013 were generally lower than in 2012, while the precipitation levels were higher. The temperatures from May to August, showed different trends in the two experimental years, they were usually higher in May 2012 than 2013. Overall in the second half of May there were several peaks around 5 °C and 16 °C for the minimum and maximum temperatures, respectively. The difference in soil moisture between the two years was more evident in May than in August. Soil moisture after tomato transplanting in 2013 was 66% of WHC while it was 93% of WHC in 2012 due to a rainy season which occurred in May of both years. There were no precipitations in August 2012 and very few in August 2013, therefore soil moisture content was 34% and 40% of WHC in August 2012 and 2013, respectively (Figure 1).

3.2. Aboveground biomass of mulches and tomato production

The aboveground biomass produced by the cover crops differed among the three species at both sampling dates (Table 2). In 2012 the dry matter produced by HV was significantly higher than that produced by LP and WM, 785 *vs.* 365 and 439 g m^{-2} after cover crop suppression (May), 284 *vs.* 111 and 204 g m^{-2} after tomato harvesting (August). In 2013 a greater amount of dry matter was obtained from HV and LP compared to WM, 564 and 525 *vs.* 365 g m^{-2} after cover crop suppression (May), 214 and 136 *vs.* 106 g m^{-2} after tomato harvesting (August). The carbon content of the aboveground biomass was higher in May compared to August, even if the only significant decrease was observed in WM mulch (44% *vs.* 42% and 42% *vs.* 35% in 2012 and 2013, respectively). Moreover, the residual mulch biomass (August) showed a slight yet not significant enrichment of nitrogen compared to the initial biomass (May). In accordance with dry matter production and elemental composition of mulch biomass, the soil C and N inputs differed significantly among treatments. Soil C inputs after cover crop suppression were WM=LP<HV and WM <LP=HV in 2012 and 2013, respectively. The residual amount of C inputs in August, after tomato harvesting were HV<LP<WM in both 2012 and 2013 (Table 2). The C/N ratio of LP, MW and HV biomass

after tomato transplanting (May) and harvesting (August) in 2012 differed significantly (in May 45, 33 and 14; in August 37, 26 and 17, respectively). In 2013 a significant difference of the C/N ratio was observed only between HV and the other two cover crops at both sampling dates (May and August).

Finally, the yields of tomato were significantly lower in WM and higher in HV mulching than in the control in both years (2012 and 2013); while the tomato yield was lower in LP mulching than in the control only in 2012 (Figure 2).

3.3. Soil Carbon and Nitrogen content

The cover crop mulching affected the soil carbon content in 2013 at both sampling dates (Table 3). The soil carbon content of the control treatment was significantly lower than LP and WM (25%) after cover crop suppression (May) and HV (17%) after tomato harvesting (August). The soil C content was significantly higher in 2013 than in 2012 in all treatments at both sampling dates with the exception of the conventional soil in May. The variation of the soil carbon content during the tomato growing season was negative in LP 2012 (Δ=-5%) and in WM 2013 (Δ=-1%), while a significant increase was observed for WM (Δ=19%), HV (Δ= 10%) in 2012 and for the control treatment (Δ=28%), HV (Δ=22%) in 2013. Moreover, soil nitrogen in 2012 was positively affected by cover crop mulching only after tomato harvesting (August). On the other hand, in 2013 Ntot was significantly higher in LP, WM and HV compared to the control treatment at both sampling dates (May and August). Similarly to soil carbon, the soil nitrogen was significantly higher in 2013 than 2012 for each treatment and the best performance of nitrogen storage was observed in WM 2012. The soil C/N ratio, after cover crop suppression reached its highest values in LP 2012 (10.5), in WM and HV 2013 (11.7 and 11.4, respectively). On the other hand after tomato harvesting, the lowest soil C/N ratio was observed in WM 2012 and 2013 (8.5 and 9.7, respectively). On average the C/N ratio showed the highest values in the second experimental year.

3.4. Soil microbial biomass, its activity and functional diversity

The results obtained concerning the biochemical properties of the soil showed significant differences between the two experimental years; in 2013 the soil microbial biomass was more sensitive to mulch treatments than in 2012. In particular, before tomato transplanting in 2012, WM, LP and HV mulching caused a general increase of Cmic compared to the control treatment (38%, 80 % and 44%, respectively). The same mulching treatments in 2013 showed a greater increase of Cmic than in 2012 especially under WM mulching (122%, 90% and 98%, respectively) (Figure 3A). In 2012, there were significant differences between May and August in the control treatment and HV, thus creating a residual effect with a higher Cmic content in August than in May. In 2013 the residual effect differed significantly compared to the initial effect in the control treatment and LP mulching. Among the cover crops, LP showed the highest Cmic content in May and there was HV with higher amount of Cmic compared to the control treatment and WM mulching. The C/N ratio of microbial biomass ranged from 3 to 6 in 2012 and from 4 to 12 in 2013 (Figure 3 B). In May, after tomato transplanting in both years, microbial C:N ratio values were in the following order C<LP=HV<WM. Therefore, the highest value of the microbial C/N ratio was observed in soil under WM mulching at both sampling dates (May and August), while there was a significant decrease in the microbial C/N ratio after tomato harvesting (August) in soil under HV mulching (Figure 3 B).

The activities of enzymes involved in the carbon cycle expressed as SEIc differed in the two experimental years (Figure 3 C). In 2012 SEIc was lower in WM than in the other treatments as initial and residual effects (May and August). Moreover, in August a significant increase of SEIc was observed in HV compared to the other treatments (Figure 3 C). In 2013 a general increase of SEIc was observed in all mulching treatments (HV, WM and LP) compared to the control treatment as the initial effect (May) while only WM was still significant in August as residual effect (Figure 3 C).

The microbial functional diversity measured using various enzymatic activities, expressed as Shannon's index, was lowest in LP and WM as initial effect (May) and in WM as residual effect (August) in 2012. On the other hand, in 2013 an increase of microbial functional diversity was observed as residual effect (August) of all mulches (LP, WM and HV) (Figure 3 D). The Shannon's Index (H') was negatively correlated to total organic carbon, total nitrogen, soil C/N ratio and microbial biomass carbon. Conversely, a positive correlation was found between H' and the soluble extractable carbon pool (Table 4). The extractable C was only higher in soil under LP mulching after tomato transplanting, a higher extractable C value was observed under WM and HV mulching at the end of tomato season only in 2013 (Figure 4). The analysis of the principal components of the variables analyzed in the four mulching treatments (Figure 5) showed that the first and second components accounted for 63.9% of the total explained variance. The enzymatic activity involved in the C cycle (SEIc) and Shannon's index are the two parameters which differed most in the soils under investigation. The score plots indicate that the samples can be divided into two groups, 2012 and 2013, which suggest that climatic condition of the two years had a strong effect on microbial activity and functional diversity. The parameters observer were more variable (i.e. spread out across the ordination diagram) within the 2012 group than within the 2013 group. Moreover, a third group of soil samples (WM collected in August) was separated from the others. In this group the most variable properties were soil C and N contents.

Discussion

The cover crop mulching usually has positive effects on soil properties (e.g. moisture, nutrient content), weed control and main crop production, but the results are strongly affected by cover crop species. In this study the results obtained concerning soil quality and microbial functions differed in two experimental years, which was probably due to the different climatic conditions, AI in 2012 was lower than in 2013 therefore soil moisture was generally higher in 2013. The cover crop aboveground biomass production was also affected by the different climatic conditions, the LP

aboveground biomass production and its decomposition rate was negatively affected by the lower AI value recorded in 2012 than in 2013. It is known that, primary productivity and nutrient cycling are directly influenced by the amount and seasonal distribution of precipitation and temperatures (Mazzarino et al., 1998; Noy-Meir, 1973). In this study the distribution of precipitation and temperatures during the growth of the cover crops, expressed by the monthly AI, effected both the quality and the amount of LP aboveground biomass. In fact, the biomass production of LP increased and its C/N ratio decreased when AI was greater than before. As far as the cover crop biomass decomposition rate is concerned, previous studies reported that when Phacelia is suppressed at flowering, it can be relatively more decomposable in soil than some other cover crops (Thompson, 1996). In 2012, the decomposition rate of LP mulch was significantly lower than the WM and HV mulches (from May to August the Δ of aboveground biomass was 53% *vs.* 64% and 69%), probably because the initial C/N ratio of LP aboveground biomass in this year was significantly higher than the C/N ratio of WM and HV. Moreover, the LP aboveground biomass lost between May and August was not recovered by the soil, since no significant variation of Corg was observed in the soil. Although the rate of biomass decomposition depends on both its composition and environmental conditions, cover crop residues usually trigger soil microbial biomass and its activities when they are incorporated into the soil (Elfstrand et al., 2007; Lagomarsino and Marinari, 2008). However, the use of cover crops as residues mulched and left on the soil surface (Teasdale and Mohler, 1993) tends to produce a greater amount of fungi compared to residue incorporation into the soil (Holland and Coleman, 1987); (Elfstrand et al., 2007; Ramos-Zapata et al., 2012). In this study, the increase of the soil microbial C/N ratio under mulching may suggest the shift of the microbial population versus fungal species, which generally have greater C/N ratios in their biomass (relative to bacteria). Moreover, a positive effect of cover crop mulching on soil microorganisms was observed, showing increases of both microbial biomass and enzymatic activities especially after cover crop suppression (May) in the year with more abundant precipitation (2013). According to previous studies (Manzoni and Porporato, 2009; Mancinelli et

al., 2013), the interaction between cover crop species and climatic conditions was found to have an effect on soil quality and microbial functions. In particular, among the three cover crop species, the LP was strongly affected by the different climatic conditions which occurred in 2012 and 2013, which had an effect on both soil quality and tomato yield. On the other hand, among the cover crop species, the WM was the least affected by climatic conditions since it was able to reduce the soil C/N ratio while it increased of the C/N ratio of microbial biomass in both years, thus improving nitrogen storage yet hindering carbon storage in soil. Moreover, the tomato yield obtained under different mulching species showed that the WM caused a reduction in tomato yield probably because the nutrients (e.g. nitrogen) were less available. In fact, in this treatment the increase of soil nitrogen storage may have occurred during mulching probably due to a minor availability of nitrogen to plant and soil microorganisms as suggested by the increase observed in the microbial C/N ratio. Finally, among the adopted mulches, the HV was the most effective for increasing the tomato yield in both experimental years, but the effect of HV mulching on soil quality and microbial functional diversity, similarly to LP, was strongly affected by the climatic conditions. According to previous studies, the monthly aridity index may be a useful tool for agronomists and soil scientists which represents an interesting new approach for studying the combined effects of temperature and precipitation on soil quality and processes (Mancinelli et al., 2013), although AI can also be used for weather-crop production model building (Oury, 1965) under specific agricultural practices such as mulching. In this study, the low monthly aridity index in 2012 may accelerated LP mulch decomposition, causing a depletion of tomato yield as final effect. Microbial activity was also affected by climatic conditions, probably because precipitation influenced soil extractable carbon availability which promoted microbial functional diversity as shown by the increase in Shannon's index.

Conclusion

In conclusion the effects of mulching on soil quality and microbial functions were caused by the interaction between cover crop species and climatic conditions. In the Mediterranean environment the cover crop species were influenced by climatic conditions: lacy phacelia mulching in terms of aboveground decomposition rate and tomato yield production, white mustard mulching in terms of soil carbon and nitrogen storage. The effect of different cover crop mulching on soil microbial biomass, its activity and functional diversities were strongly affected by the climatic conditions in all treatments. In particular, precipitation and temperatures influenced the decomposition rate of mulch and soil carbon availability, as K_2SO_4 extractable-C, which enhanced microbial functional diversity.

Acknowledgements

This research is a part of Paola Brunetti's Ph.D. thesis entitled "Organic mulching impacts on vegetable crop production". The authors would thank Rosita Marabottini, Ph.D. and Ruxandra Papp for the support in the laboratory analyses. The authors are grateful for the technical support in the experimental fields of Claudio Stefanoni and Fulvia Gatti. The authors thank Mrs. Eleanor Margaret Lea for checking the English text. This work was funded by the University of Tuscia (Italy).

Bibliography

Alvear, M., Rosas, A., Rouanet, J.L., Borie, F., 2005. Effects of three soil tillage systems on some biological activities in an Ultisol from southern Chile. Soil Tillage Res. 82, 195–202.

Bending, G.D., Turner, M.K., Jones, J.E., 2002. Interactions between crop residue and soil organic matter quality and the functional diversity of soil microbial communities. Soil Biol. Biochem. 34, 1073–1082.

411 Bond, W., Grundy, A., 2001. Non-chemical weed management in organic farming systems. Weed
412 Res. 41, 383–405.
413 Briar, S.S., Grewal, P.S., Somasekhar, N., Stinner, D., Miller, S. a., 2007. Soil nematode
414 community, organic matter, microbial biomass and nitrogen dynamics in field plots
415 transitioning from conventional to organic management. Appl. Soil Ecol. 37, 256–266.
416 Bünemann, E.K., Schwenke, G.D., Van Zwieten, L., 2006. Impact of agricultural inputs on soil
417 organisms—a review. Aust. J. Soil Res. 44, 379.
418 Campbell, C.A., Selles, F., Lafond, G.P., Biederbeck, V.O., Zentner, R.P., 2001. Tillage - fertilizer
419 changes: Effect on some soil quality attributes under long-term crop rotations in a thin Black
420 Chernozem. Can. J. Soil Sci. 81, 157–165.
421 Campiglia, E., Radicetti, E., Brunetti, P., Mancinelli, R., 2014. Do cover crop species and residue
422 management play a leading role in pepper productivity? Sci. Hortic. (Amsterdam). 166, 97–
423 104.
424 Carrera, L.M., Buyer, J.S., Vinyard, B., Abdul-Baki, a. a., Sikora, L.J., Teasdale, J.R., 2007. Effects
425 of cover crops, compost, and manure amendments on soil microbial community structure in
426 tomato production systems. Appl. Soil Ecol. 37, 247–255.
427 Dahiya, R., Ingwersen, J., Streck, T., 2007. The effect of mulching and tillage on the water and
428 temperature regimes of a loess soil: Experimental findings and modeling. Soil Tillage Res. 96,
429 52–63.
430 De Martonne, E., 1926. Une Nouvelle fonction climatologique. L'Indice d'aridité. Impr. Gauthier-
431 Villars.
432 Doran, J., Zeiss, M., 2000. Soil health and sustainability: managing the biotic component of soil
433 quality. Appl. Soil Ecol. 15, 3–11.
434 Dumontet, S., Mazzatura, A., Casucci, C., Perucci, P., 2001. Effectiveness of microbial indexes in
435 discriminating interactive effects of tillage and crop rotations in a Vertic Ustorthens. Biol.
436 Fertil. Soils 34, 411–416.

437 Elfstrand, S., Hedlund, K., Mårtensson, A., 2007. Pulse-labelling a cover crop with 13C to follow
438 its decomposition in soil under field conditions. Appl. Soil Ecol. 36, 70–82.
439 Franzluebbers, A., 2005. Soil organic carbon sequestration and agricultural greenhouse gas
440 emissions in the southeastern USA. Soil Tillage Res. 83, 120–147.
441 Hartwig, N., Ammon, H., 2002. Cover crops and living mulches. Weed Sci. 50, 688–699.
442 Holland, E., Coleman, D., 1987. Litter placement effects on microbial and organic matter dynamics
443 in an agroecosystem. Ecology 68, 425–433.
444 ISTAT, 2012. Tavola CPOM□ : Superficie (ettari) e produzione (quintali): pomodoro, pomodoro da
445 industria. Dettaglio per regione - Anno 2012 -
446 http://agri.istat.it/jsp/dawinci.jsp?q=plCPO0000010000012000&an=2012&ig=1&ct=418&id=
447 15A|18A|28A (accessed 4.8.14).
448 Janzen, H.H., Campbell, C.A., Ellert, B.H., Bremer, E., 1997. Soil organic matter dynamics and
449 their relationship to soil quality, in: Developments in Soil Science, Volume 25. Elsevier.
450 Karlen, D.L., Mausbach, M.J., Doran, J.W., Cline, R.G., Harris, R.F., Schuman, G.E., 1997. Soil
451 quality: a concept, definition, and framework for evaluation (a guest editorial). Soil Sci. Soc.
452 Am. J. 61, 4–10.
453 Kladivko, E.J., 2001. Tillage systems and soil ecology. Soil Tillage Res. 61, 61–76.
454 Lagomarsino, A., Marinari, S., 2008. Microbial biomass and activity of soil under organic
455 management, in: Marinari, S., Caporali, F. (Eds.), Soil Carbon Sequestration Under Organic
456 Farming in the Mediterranean Environment. earch Signpost, Transworld Research Network.
457 Landau, S., Schoenbaum, I., Barkai, D., 2007. Grazing, mulching, and removal of wheat straw in a
458 no-till system in a semi-arid environment. Crop Pasture Sci. 58, 907–912.
459 Lienhard, P., Tivet, F., Chabanne, A., Dequiedt, S., Lelièvre, M., Sayphoummie, S., Leudphanane,
460 B., Prévost-Bouré, N.C., Séguy, L., Maron, P.-A., Ranjard, L., 2012. No-till and cover crops
461 shift soil microbial abundance and diversity in Laos tropical grasslands. Agron. Sustain. Dev.
462 33, 375–384.

463 Mancinelli, R., Di Felice, V., Di Tizio, A., 2008. Carbon dioxide emission in agricultural soils, in:
464 Marinari, S., Caporali, F. (Eds.), Soil Carbon Sequestration Under Organic Farming in the
465 Mediterranean Environment. Transworld Research Network.
466 Mancinelli, R., Marinari, S., Di Felice, V., Savin, M.C., Campiglia, E., 2013. Soil property, CO2
467 emission and aridity index as agroecological indicators to assess the mineralization of cover
468 crop green manure in a Mediterranean environment. Ecol. Indic. 34, 31–40.
469 Manzoni, S., Porporato, A., 2009. Soil carbon and nitrogen mineralization: theory and models
470 across scales. Soil Biol. Biochem. 41, 1355–1379.
471 Marinari, S., Bonifacio, E., Moscatelli, M.C., Falsone, G., Antisari, L.V., Vianello, G., 2013. Soil
472 development and microbial functional diversity: Proposal for a methodological approach.
473 Geoderma 192, 437–445.
474 Marx, M., Wood, M., Jarvis, S., 2001. A microplate fluorimetric assay for the study of enzyme
475 diversity in soils. Soil Biol. Biochem. 33, 1633–1640.
476 Mazzarino, M., Bertiller, M., Sain, C., Satti, P., Coronato, F., 1998. Soil nitrogen dynamics in
477 northeastern Patagonia steppe under different precipitation regimes. Plant Soil 202, 125–13.
478 MiPAF, Ministero delle Politiche Agricole e Forestali, 2000. Metodi di analisi chimica del suolo,
479 in: Violante, P., Sequi, P. (Ed.), Collana Di Metodi Analitici per l'Agricoltura. Franco Angeli,
480 Milano, pp. 1–474.
481 Noy-Meir, I., 1973. Desert ecosystems: environment and producers. Annu. Rev. Ecol. Syst. 4.1, 25–
482 51.
483 Oury, B., 1965. Allowing for weather in crop production model building. J. Farm Econ. 47, 270–
484 283.
485 Pankhurst, C., Doube, B., Gupta, V., 1997. Biological indicators of soil health.
486 Pascault, N., Nicolardot, B., Bastian, F., Thiébeau, P., Ranjard, L., Maron, P.-A., 2010. In situ
487 dynamics and spatial heterogeneity of soil bacterial communities under different crop residue
488 management. Microb. Ecol. 60, 291–303.

Pignataro, A., Moscatelli, M.C., Mocali, S., Grego, S., Benedetti, A., 2012. Assessment of soil microbial functional diversity in a coppiced forest system. Appl. Soil Ecol. 62, 115–123.

Ramos-Zapata, J., Marrufo-Zapata, D., Guadarrama, P., Carrillo-Sánchez, L., Hernández-Cuevas, L., Caamal-Maldonado, A., 2012. Impact of weed control on arbuscular mycorrhizal fungi in a tropical agroecosystem: a long-term experiment. Mycorrhiza 22, 653–661.

Shukla, G., Varma, A., 2011. Soil Enzymology, Soil Enzymology, Soil Biology. Springer Berlin Heidelberg, Berlin, Heidelberg.

Teasdale, J., Mohler, C., 1993. Light transmittance, soil temperature, and soil moisture under residue of hairy vetch and rye. Agron. J. 85, 673–680.

Thompson, R., 1996. Pulse-labelling a cover crop with 13C to follow its decomposition in soil under field conditions. Plant Soil 180, 49–55.

Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biol. Biochem. 19, 703–707.

Vepsäläinen, M., Kukkonen, S., 2001. Application of soil enzyme activity test kit in a field experiment. Soil Biol. Biochem. 33, 1665–1672.

Figure captions

Figure 1: Daily minimum [– – –] and maximum [—] temperatures (°C), and rainfall [▄] (mm) throughout the periods of study in 2012 and 2013. The values of monthly aridity index are reported below.

Figure 2: The tomato yield in the two mulching experimental years (2012 and 2013). Values without common letters are statistically different according to LSD ($P < 0.05$) (C= Control, LP= lasy phacelia, WM= white mustard, HV= hairy vetch).

Figure 3: Soil microbial biomass carbon (A) and microbial C/N ratio (B), Synthetic Enzyme Index – SEIc (C),microbial functional diversity - Shannon's index H' (D) after tomato transplanting (initial effect) and harvesting (residual effect) in the two years of experiments (2012 and 2013). Values belonging to the same year without common letters are statistically different according to LSD ($P < 0.05$), in upper case letter between the two months and in lower case letter among the treatments. (C= Control, LP= lasy phacelia, WM= white mustard, HV= hairy vetch).

Figure 4: Soil extractable carbon after tomato transplanting (initial effect) and harvesting (residual effect) in the two years of experiments (2012 and 2013). (C= Control, LP= lasy phacelia, WM= white mustard, HV= hairy vetch). Values belonging to the same year without common letters are statistically different according to LSD ($P < 0.05$)

Figure 5: Principal component analysis. Symbols are: Circle = Control; Triangle = lasy phacelia; Square = white mustard ; Rhombus = hairy vetch . Empty = 2012; Full = 2013. The solid lines distinguish the two groups of the different years; the dotted line distinguishes the soil properties under WM mulching in August.

Table

Click here to download Table: Mancinelli et al Tables.docx

1 Table 1: Soil properties of the two experimental fields (2012 and 2013).

	pH_{H_2O}	*Corg*	*Ntot*	*C/N*	*Clay*	*Silt*	*Sand*	*Carbonates*	*Texture*
		mg g^{-1}			%				
2012	6.9	11.0	1.0	9.7	15	22	63	1.3	Sandy-loam
2013	7.2	11.9	1.1	10.6	15	22	63	6.3	Sandy- loam

2

3

Table 2: Soil inputs from mulching after tomato transplanting (initial effect) after tomato harvest (residual effect), and variations during tomato growth season (Δ%). Dry matter production, input of carbon and nitrogen, C/N ratio of aboveground biomass. In rows values belonging to the same years without common letters are statistically different according to LSD ($P < 0.05$), among the cover crops (lower case letter) and between years 2012 and 2013 (upper case letter).
(C= Control, LP= lasy phacelia, WM= white mustard, HV= hairy vetch).

	Year	LP	WM	HV
		Aboveground mulch of cover crop mulching (g m^{-2})		
Initial	2012	439Bb	365Ab	785Aa
	2013	525Aa	365Ab	564Ba
Residual	2012	204Aa	111Ab	284Aa
	2013	136Aab	106Ab	214Aa
Δ%	2012	-53Aa	-69Ab	-64Aab
	2013	-74Ba	-71Aa	-61Aa
		Soil C input from cover crop mulching (kg C ha^{-1})		
Initial	2012	1724Ab	1605Ab	3327Aa
	2013	2074Aa	1514Ab	2337Ba
Residual	2012	811Ab	455Ac	1152Aa
	2013	540Bb	372Ac	848Ba
Δ%	2012	-53Aa	-71Ab	-65Aab
	2013	-74Ba	-75Aa	-63Aa
		Soil N input from cover crop mulching (kg N ha^{-1})		
Initial	2012	33Bb	49Ab	207Aa
	2013	73Ab	56Ab	214Aa
Residual	2012	22Ab	21Ab	66Aa
	2013	22Ab	17Ab	58Aa
Δ%	2012	-32Aa	-56Ab	-67Ab
	2013	-70Ba	-70Aa	-72Aa
		Soil C/N input from cover crop mulching		
Initial	2012	45Aa	33Ab	14Ac
	2013	28Ba	27Ba	11Ab
Residual	2012	37Aa	26Ab	17Ac
	2013	25Ba	23Aa	15Ab
Δ%	2012	-18Ab	-20Ab	27Aa
	2013	-12Ab	-17Ab	38Aa

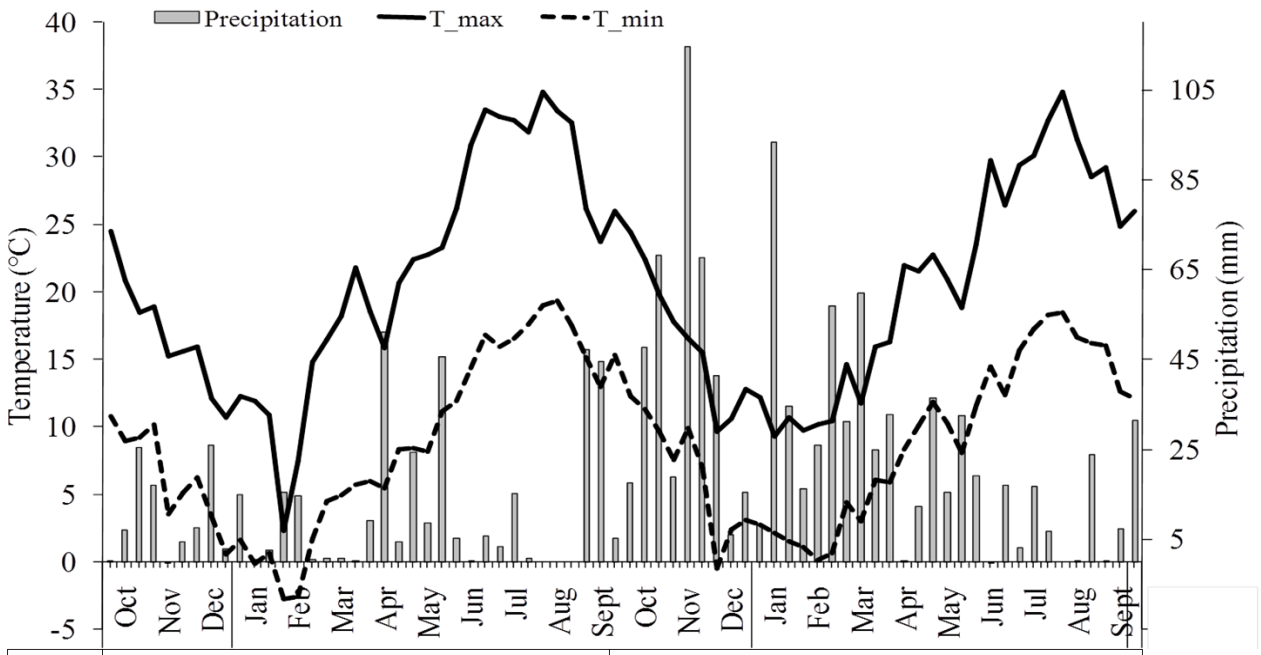
Table3: Soil organic carbon, total nitrogen content, C/N ratio after tomato transplanting (initial effect) harvesting (residual effect), and variation during tomato growth season (Δ%), in the two years of experiment (2012 and 2013). In rows values belonging to the same years without common letters are statistically different according to LSD ($P < 0.05$), among the cover crops (lower case letter) and between years 2012 and 2013 (upper case letter). (C= Control, LP= lasy phacelia, WM= white mustard, HV= hairy vetch).

	Year	**C**	**LP**	**WM**	**HV**
		Soil organic Carbon (mg Corg g^{-1})			
Initial (May)	2012	10Aa	13Ba	10Ba	11Ba
	2013	12Ab	16Aa	16Aa	15Aab
Residual (August)	2012	11Ba	12Ba	12Ba	12Ba
	2013	15Ab	17Aab	16Ab	18Aa
Δ (%)	2012	6Bab	-5Ab	19Aa	10Aab
	2013	28Aa	4Ab	-1Bb	22Aa
		Soil total Nitrogen (mg Ntot g^{-1})			
Initial (May)	2012	1.03Aa	1.19Ba	0.98Ba	1.09Ba
	2013	1.12Ab	1.47Aa	1.40Aa	1.30Aa
Residual (August)	2012	1.05Bb	1.15Bb	1.40Ba	1.23Bab
	2013	1.35Ab	1.59Aa	1.66Aa	1.61Aa
Δ%	2012	2Ab	-2Ab	44Aa	13Ab
	2013	21Aa	8Aa	19Ba	25Aa
		Soil C/N ratio			
Initial (May)	2012	9.7Bb	10.5Aa	10.1Bab	9.9Bab
	2013	10.6Ab	11.1Aab	11.7Aa	11.4Aa
Residual (August)	2012	10Ba	10.3Aa	8.5Bb	9.7Ba
	2013	11.3Aa	10.6Aab	9.7Ab	11.2Aa
Δ%	2012	3Aa	-3Aab	-16Ab	-2Aab
	2013	7Aa	-4Ab	-17Ac	-2Aab

Table 4: Pearson correlation coefficient between soil chemical and biochemical properties.

	Shannon's Index (H')	*Soil C/N ratio*	*Extractable N*
Corg	-0.59 **		
Ntot	-0.43 **		
Soil C/N ratio	-0.55 **		
Exractable C	0.47 **	-0.42 **	-0.53 **
Microbialbiomass C	-0.70 ***	0.59 **	0.33 *
Microbial C/N ratio	-0.64 ***		0.39 **

Figure 1



	Cover and tomato crops cycle - Field 2012												Cover and tomato crops cycle - Field 2013											
Monthly AI	16	12	24	14	30	1	35	36	4	7	0	39	60	128	46	100	76	71	23	40	15	10	8	16
Mean	18												49											

Figure 2

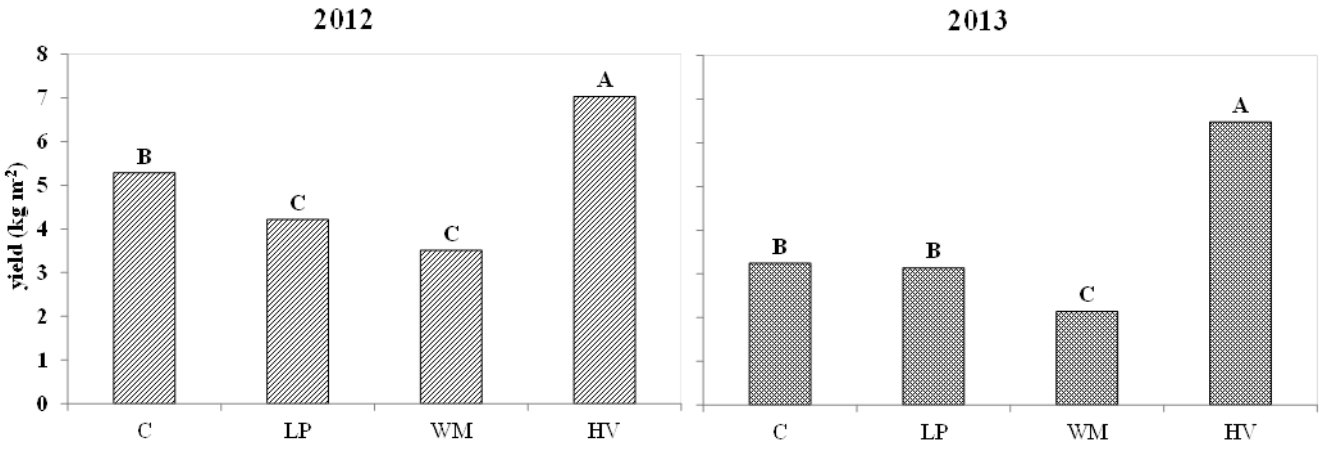


Figure 3

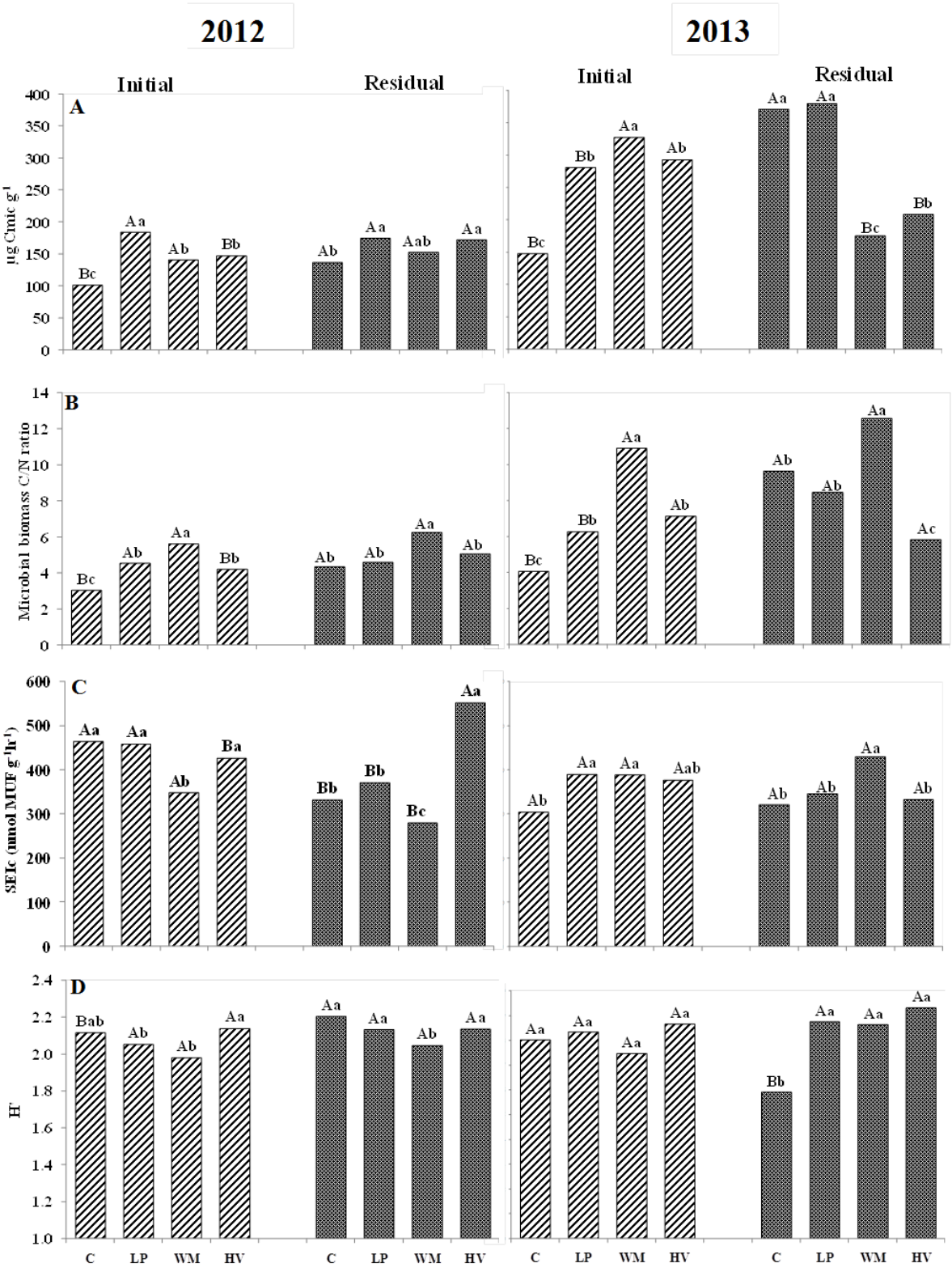

2012
2013
Initial
Residual
Initial
Residual
A
B
C
D
µg Cmic g-1
Microbial biomass C/N ratio
SEIc (nmol MUF g-1h-1)
H'
C
LP
WM
HV

Figure 4

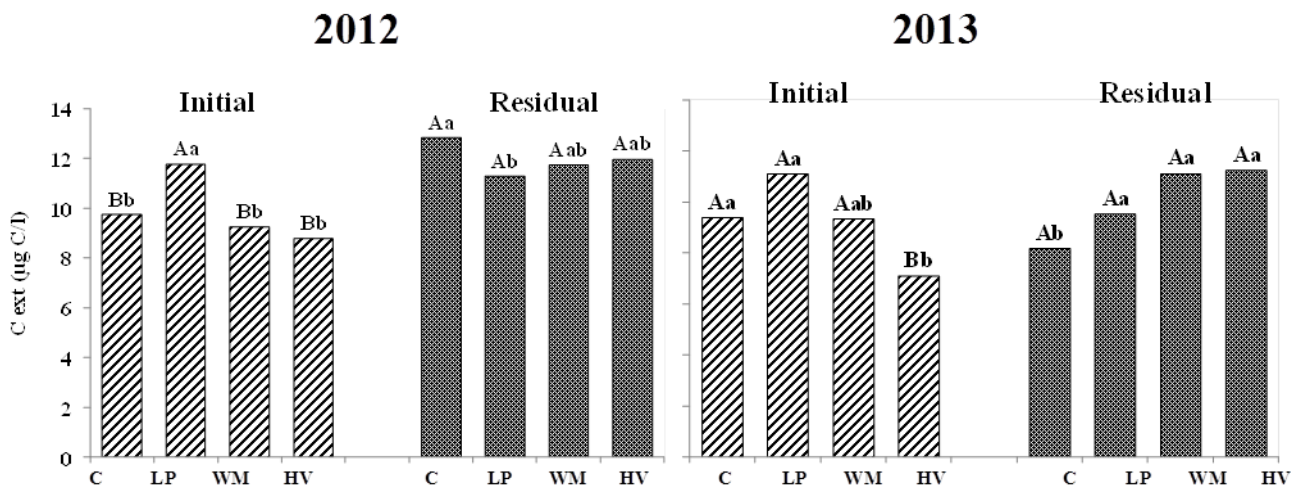
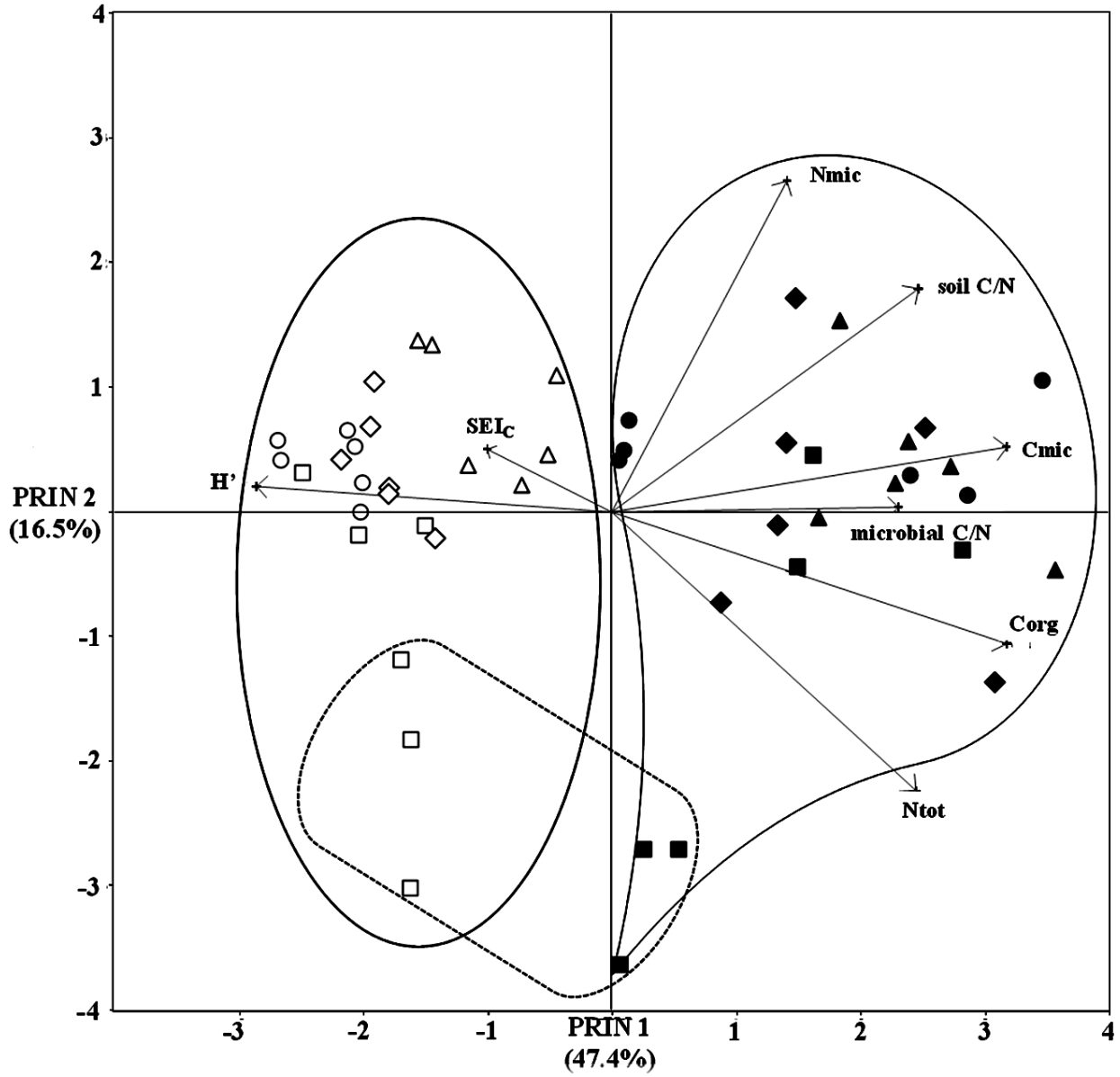

2012
2013
Initial
Residual
Initial
Residual
C ext (ug C/l)
0
2
4
6
8
10
12
14
Bb
Aa
Bb
Bb
Aa
Ab
Aab
Aab
Aa
Aa
Aab
Bb
Ab
Aa
Aa
Aa
C
LP
WM
HV
C
LP
WM
HV
C
LP
WM
HV
C
LP
WM
HV

Figure 5


Nmic
soil C/N
Cmic
microbial C/N
Corg
Ntot
SEIC
H'
PRIN 2
(16.5%)
PRIN 1
(47.4%)
4
3
2
1
-1
-2
-3
-4
-3
-2
-1
1
2
3
4