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Organic mulching impacts on vegetable crop production

AGR/02

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ABSTRACT

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A sustainable approach seems to be the better way to resolve the sustainability issue of human activities. Among all the anthropogenic activities that have had negative impact on the environment, the agriculture results to be the main sector in changing land use, depleting earth's resources or polluting the environment. The sustainable agriculture seems to be the solution to resolve some issues, such us high use of mineral nitrogen and/or decreasing of organic matter by the deepest soil tillage, etc.

At the experimental farm of University of Tuscia, from 2011 to 2013, several studies were carried out to compare yield productivity of different sustainable agronomic techniques with conventional management (C) on C stock dynamics and CO2 fluxes, soil microbial activity and chemical properties, and weeds control. The obtained results showed different interesting aspects.

The hairy vetch (HV) showed the highest positive influences on the tomato yield productivity compared to other cover crop species and to the conventional treatment adopted in the trials.

As far as the soil CO_2 fluxes resulted that were influenced by climatic conditions and by soil volume water content and soil temperature. The polynomial regression model evidenced the optimum of soil temperature and water content different between treatments in comparison [21.5 °C as average in barley straw (BS), C and HV, and 18.3 °C in white mustard (WM); soil water content of 28% as average in BS, C and HV, 55% in WM, and 87% in lacy phacelia (LP)]. About to the net balance of C soil, the hairy vetch seems to be most advantageous: despite the soil output by means the soil respiration was higher compared to other treatments; the total soil C input (given by cover crops and tomato biomass, fruits excluded), was higher too. It is possible resume that the use of hairy vetch mulch on no-tilled soil, slight reduction of irrigation water (-25%) and a rationalized use of N fertilizer could cause a positive effect on C storage in the soil, and consequently better benefits on the agroecosystem.

Concerning to the microbial activity and functional diversity, results showed that they were strongly affected by the climatic conditions in all mulching treatments. In particular, precipitations controlled soil carbon availability, which promoted microbial functional diversity. In the two years of the experiment, the amount of precipitation from May to August was 110 mm

in 2012 and 172 mm in 2013. The average values of aridity index were 18 and 49 in 2012 and 2013, respectively. LP mulching was sensitive to low precipitation in terms of aboveground decomposition rate and tomato yield with lower production with respect to control in 2012.WM mulching was sensitive to low precipitation in terms of soil nutrients storage. In conclusion, the effects of LP, WM and HV mulching on soil quality, microbial functions and tomato yield were regulated by precipitation and temperature of the summer in Mediterranean environment.

Regarding the weed control, cover crop residues placed in strips suppressed weeds more effectively than incorporated residues. A better nitrogen nutrition and weed control led to an increase in pepper productivity cultivated in vetch mulch strips. Therefore combining legume cover crops and a strip mulching technique to manage cover crop residues, could contribute to effectively increasing the crop productivity and consequently the yield of the following pepper crop.

Than it seems that an organic management where were used hairy vetch as cover crop during the winter and mulch during the summer, results to be better respect to a conventional management both as regarding the tomato yield productivity than for the net C soil balance and soil microbial biomass. Moreover the mulching techniques could be considered to be a profitable option for organic and conventional growers seeking a way to reduce the agronomical inputs in a winter cover crop–pepper sequence.

RIASSUNTO

Brunetti, P., 2014. *Impatto della pacciamatura organica sulle produzioni vegetali*. Tesi di Dottorato dell'Università degli studi della Tuscia, Viterbo, Italia. 127pag

L'approccio sostenibile sembra essere la strada giusta per risolvere il problema della sostenibilità delle attività umane. Tra tutte le attività antropogeniche che hanno avuto un impatto negativo sull'ambiente, l'agricoltura risulta essere il principale settore nel cambiamento dell'uso del suolo, incidendo notevolmente sulla diminuzione delle risorse terrestri e sull'inquinamento ambientale. L'agricoltura sostenibile può essere d'aiuto per risolvere alcuni problemi, come ad esempio l'eccessivo uso di N minerale e/o la diminuzione della sostanza organica causata dalle lavorazioni profonde.

All'azienda sperimentale dell'Università degli studi della Tuscia,dal 2011 al 2012, diversi studi sono stati condotti per confrontare la produzione orticola di alcune tecniche agronomiche sostenibili con una gestione invece convenzionale (C) analizzando le dinamiche degli stock di C e i flussi di CO_2, l'attività microbica e le proprietà chimiche del suolo, e il controllo delle infestanti. I risultati ottenuti, mostrano differenti aspetti interessanti.

Tra tutti i trattamenti adottati nell'esperimento, la veccia (HV)ha mostrato alte influenze positive sulla produzione del pomodoro, in confronto alle altre colture di copertura e al trattamento convenzionale adottate nell'esperimento.

Per quanto riguarda i flussi di CO_2 dal suolo, questi sono risultati essere influenzati sia dalle condizioni climatiche e sia dal contenuto volumetrico di acqua e dalla temperatura del suolo. Il modello di regressione polinomiale ha evidenziato un optimum di temperatura del suolo e il suo contenuto di acqua differente tra i trattamenti [21.5 °C di media nella paglia di orzo (BS), C and HV, and 18.3 °C nella senape (WM); contenuto volumetrico di acqua di 28% di media in BS, C and HV, 55% in WM, and 87% nella facelia (LP)]. Riguardo al bilancio netto del C nel suolo, la Veccia sembra essere la specie più vantaggiosa utilizzata sia come coltura di copertura che come pacciamatura: infatti nonostante gli output dovuti alla respirazione siano i più alti rispetto alle altre cover, anche gli input di C nel suolo sono i più alti, con un bilancio netto totale in favore del C stoccato nel suolo. E' quindi possibile riassumere che, l'uso della veccia come pacciamatura su suolo non lavorato, una leggera riduzione di irrigazione (-25%) e un uso razionale dei fertilizzanti azotati possono causare un effetto positivo sul C stoccato nel suolo, e conseguentemente creare migliori benefici all'agroecosistema.

Riguardo all'attività microbica e la diversità funzionale del suolo, i risultati hanno mostrato che sono state influenzate nettamente dalle condizioni climatiche, in tutti i trattamenti studiati. In particolare le precipitazioni hanno controllato la disponibilità del C, il quale promuove la diversità microbica funzionale. Nei due anni di sperimentazione, la quantità di precipitazioni da Maggio ad Agosto è stata di 110 mm nel 2012 e di 172 mm nel 2013. Il valore medio dell'indice di aridità è stato di 18 e di 49 nel 2012 e nel 2013 rispettivamente. La pacciamatura di LP è risultata essere sensibile alle basse precipitazioni, in termini di decomposizione della biomassa e di produzione del pomodoro con basse produzioni rispetto al controllo nel 2012. La pacciamatura di WM, invece risulta essere sensibile alle basse precipitazioni in termini di stoccaggio di nutrienti nel suolo. Per cui, in ambiente Mediterraneo, gli effetti della pacciamatura di LP, WM e HV sulla qualità del suolo, sulle funzioni microbiche e sulle produzioni pomodoro sono fortemente regolate dalle precipitazioni e dalle temperature avvenute durante l'estate.

Per quanto riguarda il controllo delle infestanti, i residui delle colture di copertura, posizionati in strisce hanno un maggior effetto di soppressione delle infestanti rispetto ai residui incorporati nel suolo. Una migliore fertilizzazione azotata ed il controllo delle infestanti hanno incrementato la produzione del peperone nei campi coltivati con la pacciamatura di Veccia in strisce. Perciò combinando l'uso delle colture leguminose di coperture con la tecnica della pacciamatura in strisce, potrebbe contribuire efficacemente all'incremento della produzione orticola come il peperone.

Da tutto ciò si evince che una gestione biologica composta dall'uso della Veccia come coltura di copertura durante l'inverno e pacciamata durante l'estate, risulta essere migliore rispetto alla gestione convenzionale sia per quanto riguarda la produzioni di pomodoro e peperone sia per quanto riguarda il bilancio netto del C nel suolo e la biomassa microbica. Inoltre la tecnica di pacciamatura potrebbe essere considerata un'opzione remunerativa per tutti quei coltivatori, siano essi biologici che convenzionali, che cercano di diminuire gli input agronomici, come gli erbicidi.

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Organic agriculture is worth studying
because it is more complex, challenging and
knowledge intensive than conventional
horticulture (IFOAM).

1. INTRODUCTION

Since the end of World War II, agriculture has changed dramatically. Food and fiber productivity has risen because of new technologies, mechanization, increased use of chemicals, specialization and government policies.

Even though these changes have had many positive effects, there have also been significant costs. The most important issues have been: topsoil depletion, groundwater contamination, soil organic matter decrease, decline of family farms, increase of production costs, and disintegration of economic and social conditions in rural communities.

During recent years the term sustainable has begun to mean many all things to different people. Now, there are several hundred definitions but the earliest one suggests that sustainable development should imply that no generation in the future will be worse off than the present generation(Pearce et al., 1990). The general idea is that sustainability is too often seen as a result of something that exists, such as sustainable farming system, rather than a process of change. Sustainable agriculture can produce abundant food without depleting the earth's resources or polluting its environment. It follows the principles of nature to develop systems able to improve crop growth and livestock production. Sustainable agriculture is a method of farming that is not only humane and socially ethical, but can sustain itself. One might say that organic agriculture is the real right way to resolve the sustainability issue of human activities.

In this study, after examining the evolution of land use change and anthropogenic impact on CO_2 emissions, three main aspects related to crop management system will be investigated: the soil C cycle, soil quality and microbial biomass, and weed management. This Ph.D. dissertation provides information regarding ecological approaches of these aspects on tomato and pepper crops through field experiments and laboratory analyses. Field experiments were planned to evaluate how tillage, cover crops and their residues can be managed to increase soil fertility (organic carbon and microbial biomass) and to reduce weed germination. The results of this research are included in the dissertation as chapters and presented in the form of manuscripts submitted to international journals and that are already published.

2. LAND USE CHANGE

As the human population grows, humans are using increasing amounts of Net Primary Production (NPP) for their own purposes, thus greatly influencing the C cycle (Rojstaczer et al., 2001).The land use change that had a greater impact on the C cycle was the expansion of agriculture (Houghton, 1999).Over the past four centuries, the Earth's surface intended for grazing and agriculture has increased from negligible values to 30% of the total area, mainly at the expense of forests and grasslands (Figure 1).

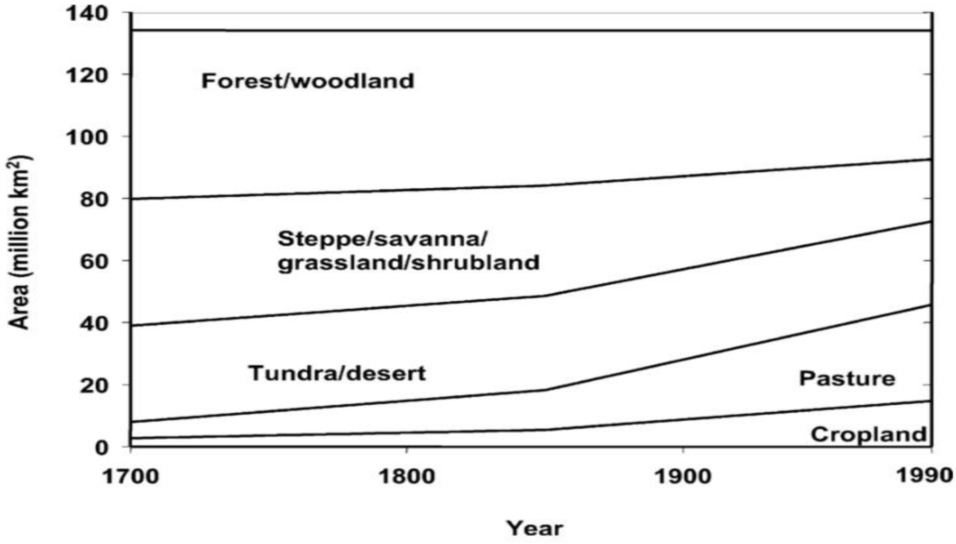

Figure 1: expansion of areas for grazing and agriculture, since 1700. Source:(Janzen, 2004)**.**

According to Ramankutty and Foley (1999), the net loss in forested area was 11.4 million km^2 and the net loss of savannas, grasslands and steppes was 6.7 million km^2. Generally, this change in land use has caused a depletion of the C stored in the environment. Indeed, the C stored in an ecosystem is a function of both photosynthesis (input) by the NPP and heterotrophic decomposition (output) (Figure 2). When the inputs of photosynthesis outweigh the losses, C is accumulated; on the contrary, when the losses are greater than inputs, C obviously decreases. During the early developmental stages of agroecosystems, the inputs of C predominated so that the C gradually accumulated an equilibrium balanced by decomposition (Janzen, 2004). During the last 100 years, humans have cultivated lands for their food needs, causing major changes in the amount of C stored (Janzen et al., 1997). In fact, the physical disturbance of the soil has exposed more of the topsoil Organic Matter (OM)to increased biological activity, thus accelerating its decomposition.

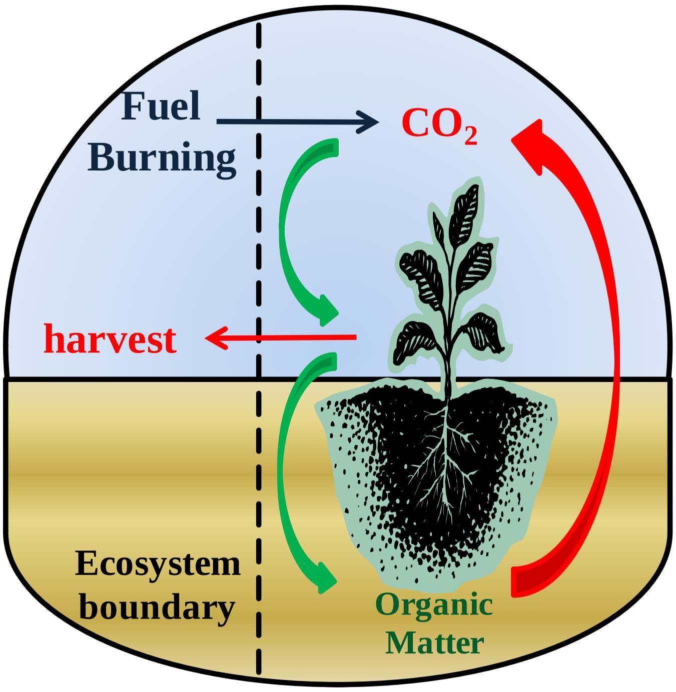

Figure 2: C cycle in the agroecosystem. Source (Janzen, 2004)**.**

Immediately after initiating crop cultivation, it is estimated that the soils of grasslands quickly lost a large amount of C stored: around 20-30 % of the C of the Earth's surface(Janzen, 2004). In the forests, however, where C is mainly stored in the plant biomass, the losses were higher. Indeed, C global losses from plant biomass were two times greater than those of the only soil compartment (Houghton, 1995). At the global level, C loss steadily increased starting from the second half of the last century (Figure 3), mainly because of the deforestation of tropical forests. The cumulative emissions of CO_2 resulting from the change of land use, from 1850 to 2000, were approximately 156 Pg C (Houghton, 2003).

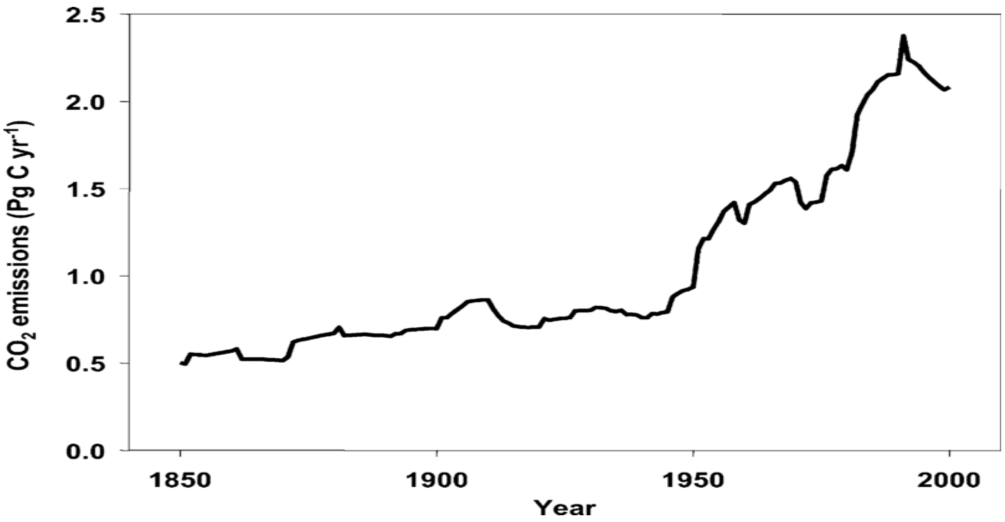

Figure 3: CO_2 emissions from changes in land use (Source: Houghton and Hackler, 2002)

2.1 ANTHROPOGENIC IMPACT ON SOIL CO_2 FLUXES

The annual exchange of carbon dioxide between the soil and the atmosphere is generally quantified by changes in the levels of organic carbon in the soil (Lal, 2004a). In this particular environment, the different cycles are all interconnected to each other so that any dangerous anthropogenic interference with the nutrient cycles (carbon, nitrogen, phosphorus and sulfur), energy balance and water cycle can affect the C pool in the soil, increasing atmospheric C.

The anthropogenic actions that negatively affect the soil C stock in favor of the atmospheric pool are (Figure 4): 1) deforestation and fires and all activities related to the conversion of natural areas into agroecosystems; 2) tillage and other disturbances to the soil; 3) drainage of wetlands; 4) growing crops on organic soils; 5) use of biomass for fuel production, feed and other uses; 6) soil erosion (Lal, 2004a).

Agricultural activities causes higher C loss rate in croplands than pasture and, in general, there will be greater loss from soils starting with a higher C content (Lal, 2004a).

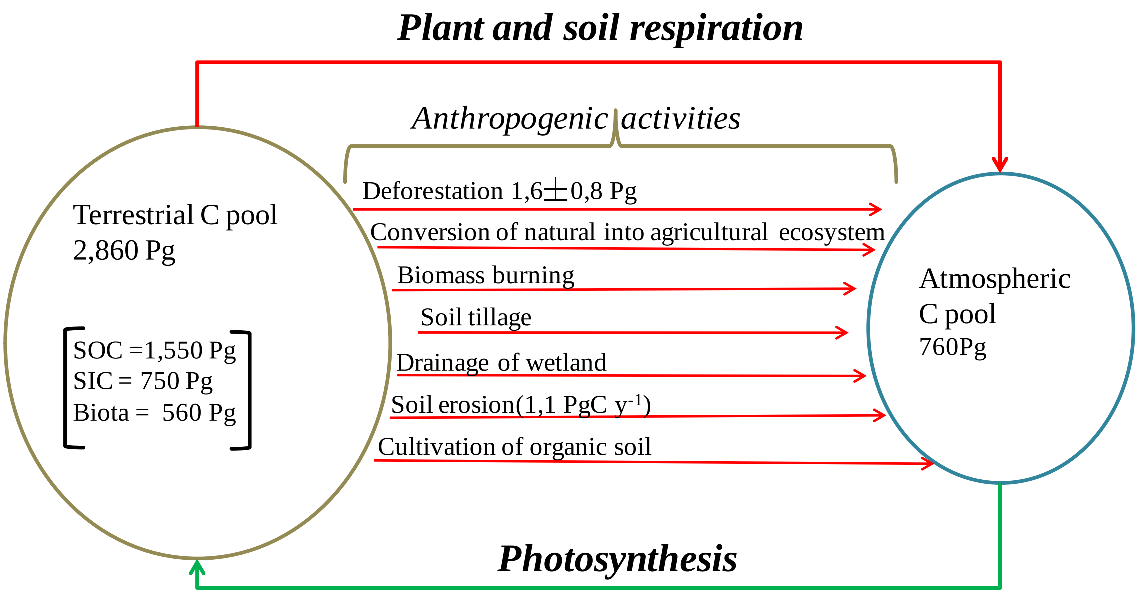

Figure 4:anthropogenic emissions affecting the C emission from land pool to atmospheric pool. The direction of arrows indicates the C flow from a pool to another. Photosynthesis and respiration of soil and plants are natural flows. All other activities are related to human activity that causes emissions of CO_2 (and other gases) from the Earth to the atmosphere. Source:(Lal, 2004a)**.**

2.1.1 IMPACT OF ANTHROPOGENIC ACTIVITIES IN AGROECOSYSTEMS

The total area devoted to agriculture is 35%more than all of the other human activities and it occupies about 11% of the Earth's surface (Betts et al., 2007).Major greenhouse gas emissions related to agriculture are generally associated with a kind of simplified and intensive management (IPCC, 2007).

Modern and intensive agriculture uses large amounts of mineral nitrogen and water, and through the deepest soil tillage causes more rapid oxidation of organic matter. Phenomena such as desertification (D'Angelo et al., 2000), salinization (Sarah, 2004; Herrero and Pérez-Coveta, 2005), overgrazing (Margaris et al., 1996) and cultivation of invasive alien plants (McNeely et al., 2003)have significant negative impacts on ecological systems, greatly reducing the productivity of the land (Mancinelli et al., 2008).

Alternative techniques to conventional farming can capture more atmospheric C in the soil, resulting from less soil disturbance and / or the increased production of crop residues, promoting the conversion of CO_2 into plant residues. In other words, the carbon dioxide can be sequestered into soil organic C by reducing soil disturbance and / or the increase of crops (Paustian et al., 1998). It has been calculated that the use of the best management practices (BMPs)in agriculture contributes to the mitigation of emissions from soil to the atmosphere of about 5.5-6 Gt CO_2per year (IPCC, 2007), promoting soil C accumulation, OM content and soil respiration. To apply “alternative techniques” is necessary to change from the linear approach of farming into a cyclic view of the agroecosystem, where the system is able to self-sustain and ensure productivity without excessive use of external energy. To do this, first it is necessary to diversify the farm. It means to give more autonomy to the whole farm by the integration of biotic and abiotic components of the agroecosystem. If the agroecosystem is more diversified - by the different crops cultivated in different fields, by the presence of livestock, by the presence of natural vegetation (hedges)- there is more functional integration among its compounds and that greater autonomy will mean lesser reliance on external inputs (energy use) and lesser environmental impacts (Caporali, 2003). Diversification of the farm means to integrate animals with plant breeding by the transfer and recycling of the materials in the whole farm (some crops for animal feed and animal manure for fertilizing crops). For example, some fodder and grain legumes could be cultivated as protein support for the animal diet. At the same time, the cultivation of legumes allows less use of fossil energy, because legumes use solar energy to fix atmospheric N in the soil; thus, the C cycle improves by means of grazing and detritus chain. The system, in this way is self-sustainable, ensuring a balanced productivity.

The C cycle and the SOM content are generally affected by soil conservation measures and minimum tillage practice. To reduce the negative effects of soil degradation, such as erosion, water instability, floods, eutrophication and water pollution, proper use of the hydrological cycle is necessary everywhere but especially in those areas of the world (like the Mediterranean region) where the growing season corresponds to the long period of drought. It is necessary reduce as much as possible the soil disturbance to avoid C loss from the soil. Infact plowing, tilling, disking, harrowing, seedbed preparation, planting, mechanical weed control, and other similar agronomic practices, can directly and indirectly affect CO_2 emissions (Mancinelli et al., 2008). Lal (2004b) found that the contribution of direct emissions of fossil fuel use for soil tillage amounted to 35.3 kg C ha^{-1}:emissions could be drastically reduced by the adoption of minimum or no-tillage.

Other alternative techniques have been defined to reduce the external inputs in sustainable cropping systems. Among these, the most important are: crop rotation, intercropping and cover cropping, fertilization with manure and organic composted materials or green manuring and no chemical weed and pest disease control.

If it is true that a sustainable approach is the better way to maintain the resistance and resilience of the agroecosystem, it is difficult to grow vegetable crops without the support of non-renewable energy inputs. Therefore, the introduction and application of some alternative agronomical techniques (e.g. BMPs) in these types of cropping systems are essential to improve the sustainability, i.e. soil fertility, weed control, biodiversity, etc.

2.2 CARBON CYCLE IN AN AGROECOSYSTEM

The pools directly and actively involved in the carbon cycle are the atmosphere, the biota (the vegetation), the soil (with organic matter) and the ocean (IPCC, 2001). Even though the greatest amount of C is stocked by the oceans (equal to 39,ooo PgC), the majority of this C is not actively cycled because of ocean depth (Halvorson et al., 2006; Falkowski, 2000) (Figure 5).

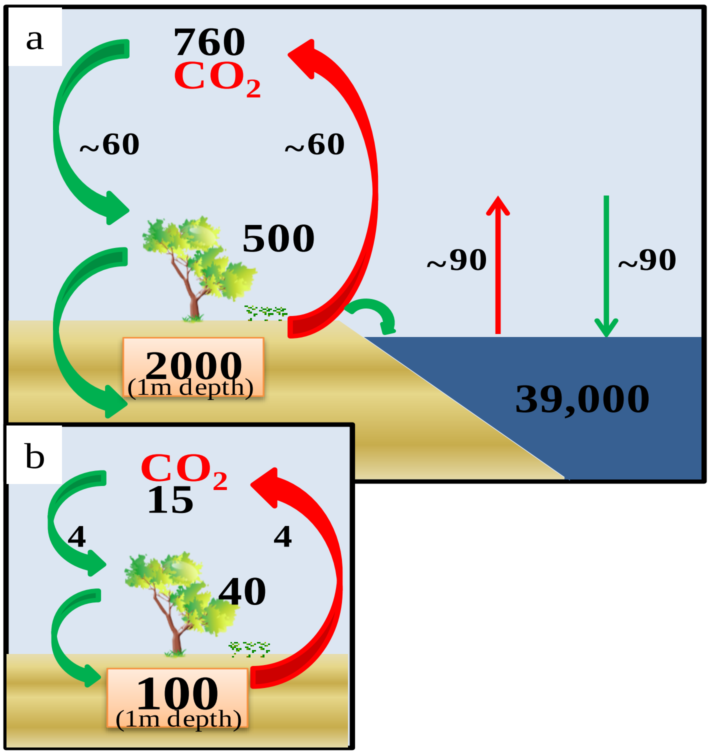

Figure 5: (a) An overview of the global C cycle, as it was in 1990. All the storage sites of the C are in units of PgC, and flows are in units of PgC year^{-1}. (b) Stocks and global flows of C, expressed as an average per hectare of the earth's surface. The values are expressed in units of Mg C ha^{-1} or Mg C ha^{-1} per year. Source:(Janzen, 2004).

The atmosphere, with a concentration of CO_2equal to 380 ppmv (Keeling and Whorf, 2005), contains about 785 Pg C in CO_2 form, equivalent to 15MgCha^{-}1of terrestrial surface (Janzen, 2004). The biota consists of about 400-600 Pg of C stored, although this amount is not certain or readily determinable (Ipcc. 2001; Smil 2002): about 75% is stored in forests (Table 1), while algae stock less than 1% (Falkowski, 2002; Körner, 2000; Smil, 2002).The soil pool accounts for a total of about 3000 Pg C and it is comprised of two distinct components: soil organic carbon (SOC) and soil inorganic carbon (SIC).

All these C storage pools(oceans, atmosphere, vegetation and soil) are connected to each other. The atmospheric CO_2 captured by plants through photosynthesis (approximately 120 PgC per year) is defined as GPP (gross primary production).About 50% of GPP is released through plant respiration as CO_2into the atmosphere, such that NPP is reported to be 60 PgCyear^{-1}. This amount is temporarily stored in plant tissues and part of it enters into the soil compartment through senescence of plant tissues.

Table 1: C stocks. Source:**(Janzen, 2004)**

Biome	**Area (10^9 ha)**	**Global carbon stock (Pg C)**			**NPP (Pg C per year)**
		Plants	Soil	total	
Tropical forest	1.76	212	216	428	13.7
Temperate forest	1.04	59	100	159	6.5
Boreal forest	1.37	88	471	559	2.2
Tropical savannas and grasslands	2.25	66	264	330	17.77
Temperate grasslands and shrublands	1.25	9	295	304	5.3
Deserts and semi-deserts	4.55	8	191	199	1.4
Tundra	0.95	6	121	127	1.0
Croplands	1.60	3	128	131	6.8
Wetlands	0.35	15	225	240	4.3
Total	15.12	466	2,011	2,477	59.9

Heterotrophic respiration (mainly implemented by soil microorganisms) and some special events such as fires return to the atmosphere an amount approximately equal to 60 PgC per year, closing the cycle. Averaged over the total area of continents, these C inputs and losses amount to about 4MgCha^{-1} per year(Janzen, 2004).

Among all terrestrial ecosystems, the soil is the component that stores the most C. At a depth of 1 m, the soil contains about 1,500-2,000 Pg C in different organic forms: the most recent litter to the oldest formations such as coal and some humified compounds (Amundson, 2001). Other forms of organic C and carbonates are found at depths greater than one meter, but it is assumed that they do not participate actively in the carbon cycle.

About one-third of the soil organic carbon is captured in the forests, another one-third is in grasslands and savannahs, and the remainder is divided among wetlands, cultivated fields and other biomes.In the soil, the OM is the most important C stock: it holds more than twice the C content of the atmosphere and it is 4.5 times larger than the aboveground biomass (Delgado and Follett, 2002). The OM is defined as the sum of the plants and animals residues at different levels of decomposition, cells and tissues of soil organisms and substances well-decomposed

(Brady and Weil, 1999). Despite the fact that living organisms are not considered in this definition, they are very important for OM building. The plants roots and wildlife (e.g.rodents, worms and mites) all contribute to the movement and to the decomposition of the organic material in the soil by means of respiration (autotrophic by plants and roots; heterotrophic by microbial population). Root respiration accounts for 50% of all the soil respiration, while the CO_2 released from the decomposition of OM is primarily (99%) released by soil microflora(Singh and Gupta, 1977). Four are the main process, carried out by soil microorganisms, involved in the soil OM cycling: 1) decomposition of inorganic compounds2) mineralization of nutrients, 3) transfer of organic carbon and nutrients from OM pool to another4) continuous release of CO_2as a result of respiration and microbial oxidation chemistry (Figure 6).The three main pools of OM, defined by ability to decomposition are: ACTIVE (1-2 years), SLOW (15-100 years) and PASSIVE (500-5000 years) (Brady and Weil, 1999).

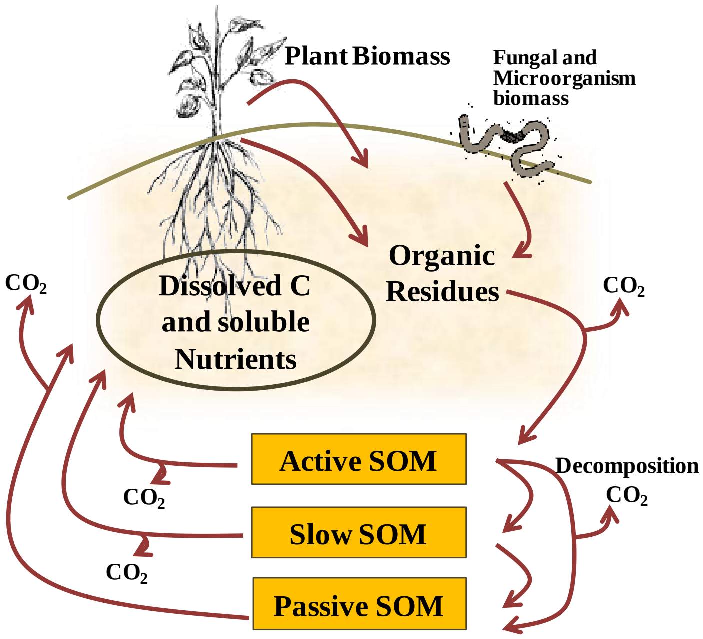

Figure 6: Cycle of soil organic matter (Source: Brady and Weil, 1999)

The first two pools are biologically active since they are continuously decomposed by microorganisms and therefore releasing many nutrients that were organically linked, such as N, P, and other essential nutrients, back into soil solution. The *active* OM is mainly composed of fresh residues of plants and animals and is decomposed fairly quickly. The OM compounds not completely decomposed pass into the OM pools underlying. The OM *slow* pool is mainly composed by detritus (decomposed cell and tissue) and is partially resistant to microbial decomposition and will remain in the soil longer than the OM active pool. The *passive* OM, also called humus, is not biologically active and is responsible for certain chemical and physical properties associated with the quality of the soil and the OM. The humus is defined as a complex organic mixture modified from the original organic tissues, synthesized by various soil organisms and resistant to further microbial decomposition (Power and Prasad, 1997).

The OM content is equal to the net difference between the amount of OM accumulated and that mineralized. The literature highlights how many factors can affect the decomposition of OM, its accumulation and emission of CO_2 from the soil: soil texture, temperature, moisture, pH, available C (OM lability), root density, structure and size of the microbial community, soil N content, physical and chemical properties of the soil, vegetation type, soil drainage, and last but not least the human pressure and agricultural practices (Raich and Tufekciogul, 2000).

In past centuries the human population has increased about six-fold, reaching nearly six billion inhabitants and exerting a strong influence on the C cycle, mainly due to the combustion of coal, thus implementing a major change in land use. Soil organic carbon lost due to agriculture, can be 30 to 60 $Mgha^{-1}$, depending on the initial pool, climate, land use and the management of the system (Lal, 2001). Through human activities, about 25% of $CO_2$is released into the atmosphere, 20% of these emissions of greenhouse gases comes from agricultural ecosystems (Rosenzweig and Hillel, 2000). Lal (2004a) summarized the C dynamics of an ecosystem in Figure 7: if the total losses (emissions, erosion and leaching) are less than the gains (photosynthesis and deposition), there will be C sequestration.

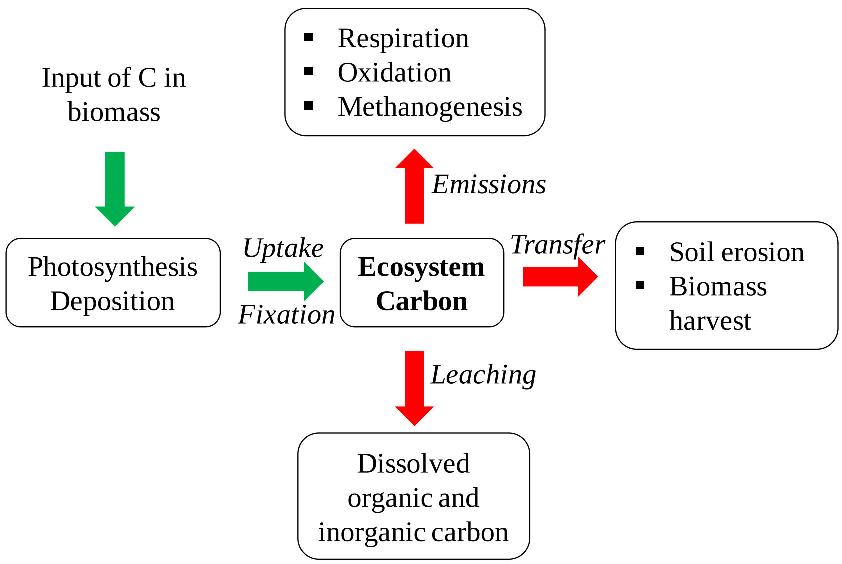

Figure 7: Dynamics of ecosystem carbon. Source: (Lal, 2004a)**.**

Agricultural practices that can be used to enhance C sequestration in croplands are:

- *Conservation tillage*: conventional plowing increases mineralization and CO_2 emissions, favoring soil erosion. On the contrary, conservation tillage, reduce the risks of soil erosion and the C losses (Lal, 2001;Lal, 2004b);
- *Mulch farming*: in comparison to the bare soil, mulches are able to reduce soil erosion (reducing raindrop impact, increasing water infiltration, increasing surface storage, decreasing runoff velocity, improving soil structure and porosity, and improving the biological activity in the soil) and decrease C losses(Lal, 1997). Application of mulch to cultivated soil increases SOC concentration (Saroa and Lal, 2003; Blanco-Canqui and Lal, 2007);
- *Integrated nutrient management*: the use of organic fertilizers, as manure or compost, has positive effects on SOC pool: a part of produced biomass returns to the soil, improving C, soil aggregation and its humification (Lal, 2004b);
- *Precision farming*: nitrogenous fertilizers have hidden C costs of 0.86 kg C/kg N (Lal, 2004b): judicious use of fertilizers with a high use efficiency through precision farming (Matson et al., 1998) is an efficient means of SOC sequestration;
- *Growing cover crops*: the summer fallow practice can decrease the SOC pool from a minimum of 320 to a maximum of 530 kg C $ha^{-1}yr^{-1}$. Some cover crops can be used rather than summer fallow to enhance SOC and soil quality (Lal, 2001);
- *Crop rotation and forage crops*: scientists agree on the benefits of the use of crop rotations in managing SOC and soil quality. Differences in plant species composition contribute to differential retention of SOC content. Growing legume cover crops can also lead to increased biological N fixation and biodiversity. Moreover some cover crops can be grazed, adding extra value for the farmers (Lal, 2001);
- *Integrated pest management*: the use of multiple tactics can optimize the control of all classes of pests (insects, pathogens, weeds, vertebrates) in an ecological and economical way (Prokopy, 2003): pesticides have hidden C costsat least 5 times higher than fertilizers (Lal, 2004b).

2.3 SOIL QUALITY AND MICROBIAL BIOMASS IN AGROECOSYSTEMS

In the last two decades the role of soil in ecosystem functioning has gained interest since it is considered a non-renewable natural resource that is constantly endangered by human activities. Soil has two important characteristics that sustain its dynamics with consequences on the plant-animal-human system: quality and health. Soil quality is often defined as an abstract feature, difficult to define because it depends on external factors such as the type of agricultural practices, interactions with the environment and the ecosystem, etc. (Pankhurst et al., 1997). Karlen and Mausbach (1997) define the soil quality as its "capacity to function". The major soil functions are:

i) to provide a suitable substrate for plant growth and the biological activity;

ii) to adjust the flow and retention of water in the environment;

iii) to establish the right conditions that would buffer the formation and destruction of chemical synthesis of compounds with high environmental impact (Larson and Pierce, 1994).

In an ecological approach, soil quality is often associated with soil health even though the latter refers more to the biotic components of the soil, the maintenance of soil organisms and their proper functioning as regulators of nutrient cycling (Anderson and Domsch, 2010), the continuous capacity to maintain fertility and ecological functions throughout time.

There are some measures suitable to quantify soil quality by soil biological, chemical, and physical characteristics(Campbell et al., 2001). Pankhurst (2004) presented a set of synthetic list of these indicators (Figure 8).The biological indicators are more sensitive to biotic or abiotic stressand, therefore, are more reliable for quantifying and comparing anthropic influences. Among all the measures suitable to quantify the soil capacity to function, microbial biomass can be considered a potential indicator of soil quality because it responds rapidly to changes resulting from different management and environmental factors (Alvear et al., 2005).

The complexity of the soil ecosystem represents 95% of total diversity on Earth, but only 5% of it has been classified (Pignataro et al., 2012).

INDICATOR	Relevance to: *Soil quality*	Relevance to: *Soil health*
Physical indicators		
Mineral composition	+	-
Texture	+	-
Depth	+	-
Bulk density	+	+
Water holding capacity	+	+
Porosity	+	+
Chemical indicators		
pH	+	+
Electrical condictivity	+	+
Cation exchange capacity	+	+
Organic matter	+	+
Major elements	+	+
Heavy metals	+	+
Biological indicators		
Microbial biomass	+	+
Soil respiration	+	+
Mineralizable N	+	+
Enzyme activity	+	+
Abudance of microflora	+	+
Abudance soil fauna	+	+
Root disease	+	+
Soil biodiversity	-	+
Food web structure	-	+
Plant growth	+	+
Plant biodiversity	-	+

Figure 8: Soil physical, chemical and biological indicators (-, of little or no relevance; +, relevant); Source (Pankhurst, C.E., 1994).

In the soil there is a community of different species: insects, mites, protozoa, nematodes, fungi and bacteria. One common classification scheme follows the relative size of organisms: there are microflora, microfauna, mesofauna, macrofauna and megafauna(Swift et al., 1979), and every environmental or anthropic disturbance can modify the community's composition. The microflora represents the majority of the soil populations and it is defined as the living component of soil organic matter (with the exclusion of macrofauna and roots). Microflora is made up of five main groups: Viruses, Bacteria, Actinobacteria, Fungi and Algae (Paul, 2006). Viruses are acellular and the smallest micro-organism (0.01-1 µm): they are specific internal parasites of plants, animal and bacteria and sometimes fungi. Bacteria (0.3-3 µm) are the most numerous component: 1 g of soil contains billions of them. The actinobacteria are filamentous bacteria (0.5-1 µm) living in basic environmental conditions (Lavelle and Spain, 2001). Fungi are the second more numerous group after the bacteria and their biomass is very important since they represent 70-80% of the microbial biomass weight in the soil (Miller and Lodge, 1997). Algae are very important for the ecological role that they play: they are primary colonizers and sensitive to soil fertility, pollutants, and pH modifications (Lavelle and Spain, 2001). The microbial biomass is concentrated in the top fewcentimeters of the soil (Pankhurst et al., 1997) and it plays a crucial role in terrestrial ecosystems because it is responsible for OM decomposition, mineralization and immobilization of nutrients, formation and stabilization of soil aggregates (Nannipieri and Badalucco, 2003).

The soil microbial biomass structure and activity can be affected by several ecological factors, such as C and energy source, nutrient and water content, temperature, pressure, atmospheric composition, pH, redox potential, microbial genetics and the interaction between them and the vegetation type on the soil (Nannipieri and Badalucco, 2003; Rutigliano et al., 2004). The microbial community structure is heterogeneous and changes both in space and in time. In the vertical distribution it is interesting to note how the population densities of most micro-organisms decrease regularly with depth: maximum density occurs close to the surface and the rate of decrease may vary depending on the species. Geological factors and plant distributions influence the horizontal distribution of soil micro-organisms in soil (Lavelle and Spain, 2001).

Rapid changes in environmental conditions, as in a wet soil after a sudden rainfall, can activate the microbial activity that was latent in the dry soil. In this way, the microbial population densities and biomass can mirror seasonal climatic changes. On the contrary, the organic matter decomposition can have effects on micro-decomposer populations over longer timescales (Lavelle and Spain, 2001). Therefore, when measuring the microbial biomass content of soils it is important to define the sampling depth and time, and if we are operating in tillage or no-tillage conditions (Pankhurst et al., 1997).

By its relatively fast rate of turnover, the microbial biomass can be considered as a sensitive indicator of soil process changes, especially as compared to the total SOM.

Some of the functions related to the microbial community are:

- nutrient availability for plant uptake by the decomposition of SOM (Zaman et al., 1999; Tu et al., 2003, 2006);
- mycorrhizal associations;
- nitrogen fixation;
- reduction in plant disease by antibiosis and competition for nutrients (Whipps, 1997; Garbeva et al., 2004);
- alteration of soil properties influencing plant growth(Lagomarsino and Marinari, 2008).

It is necessary to maintain a large and active soil microbial biomass for soil productivity. Since it is well known that microbial biomass and organic matter decline are related to land disturbance caused by the tillage and fertilizers (Carter and Rennie, 1982; Srivastava and Singh, 1991; Weigand et al., 1995), a sustainable approach is required for the maintenance of viable, diverse populations and functioning microbial communities in the soil (Kennedy and Smith, 1995). In comparison to conventional management, an ecological approach promotes SOM content enhancement, favoring both microbial biomass turnover and SOM degradation (Tu et al., 2006a). The use of crop rotation, adds diversity on the farm, and can increase soil quality and fertility by means of residue management approach that can include green manuring (Stark et al., 2007). In organic farming, the exclusion of chemical inputs (e.g. pesticides and fertilizers), causes the positive dependence of plant nutrition and pest control on microbial processes (Watson et al., 2002). Soil microbes play an important role in nutrient cycles (N, P and S), functioning as a nutrient sink and releasing them for plant nutrition. The complex system of a sustainable farming approach has positive effects on a nutrient cycle, overall increasing N availability for plants, because of high net N mineralization rates associated with enhanced soil microbial biomass and activity (Tu et al., 2006b). The relationship between C and soil biomass is well known: less soil C means fewer soil microbes (Smith and Paul, 1990); lower microbial biomass in soils from conventional agroecosystem management is often a result of reduced organic C inputs into the soil (Fließbach and Mäder, 2000). Organic management enhances the activities of some enzymes (in the N and P cycles, for example), positively influencing the soil nutrient supply (Melero et al., 2006). The sustainable system, by a better and efficient resource utilization, has more positive effects on flora, fauna and microbial diversity than conventional systems (Mäder et al., 2002).

2.4 WEED AND WEED MANAGEMENT IN AGROECOSYSTEM

The main goal of cropping system management is to optimize yield and product quality with the lowest costs. One of the most important problems that limit the achievement of this goal is weed growth. Weed definitions are abundant because it is not easy to define them. The European Weed Science Society defines weeds as "any plant of vegetation, excluding fungi, interfering with the objectives or requirements of people". Weeds are undesirable and unwanted plants which play havoc with human activity (Rao, 2000): considering this meaning, all plants can be weeds, depending on the human target. In a wider sense, it is possible to take into account the biological and ecological aspects of weeds, such as those belonging to the agroecosystem dynamic: from this point of view weed populations evolve together with the crops and human activities (Aldrich and Kremer, 1997). Crawley(1997) defines weeds by their abundance and their damage on the main crop. On the contrary, non-crop plants may be considered valuable for numerous interactions with other organisms and the improvement of the agroecosystem functioning: the more biodiversity there is, the more efficient the agroecosystem will be. Weeds represent a biodiversity index because they are a source of food and shelter for many animal species, in particular those useful for pest control (Gibbons et al., 2006; Hawes et al., 2009). Moreover, weeds provide continuous sources of pollen and nectar thus favoring insects populations (Landis et al., 2000). Another important aspect is that related to the erosion risk: covering the ground, weeds reduce soil loss caused by water and wind (Hartwig and Ammon, 2002).

Despite all these benefits on the agroecosystem, weeds are considered undesirable plants because they compete with crop for nutrients, water and light. The weeds' power resides in their seeds, particularly in their quantity, small size and dormancy that inhibit germination even under favorable condition. With respect to crops, weeds germinate earlier, grow, flower and mature faster. Other harmful impacts can be considered at agroecosystem level. Naylor (2002) listed the main effects and mechanisms of weeds as follows:

- reduction of crop yield: interfering with access to plant growth resources of light, water and nutrients;
- reduction of crop quality: mixtures containing contaminating seeds in arable crops; contamination of vegetable crops;
- delay of harvest: conservation of moisture may delay ripening and increase crop moisture level when harvested;
- interference with harvest: climbing plants and late-growing weeds make combine operation more difficult;

- interference with animal feeding: plants with spines or thorns inhibit animal foraging;
- alteration of animal products: impart undesirable flavor;
- action as plant parasites: some plants are not able to do photosynthesis and derives some or all of its nutritional requirements from another living plant (e.g.: *Cuscuta* spp, *Orobanche* spp.)
- reduction of crop health: alternate or alternative hosts for pests and diseases;
- reduction of animal (and human) health: intermediate hosts or a vehicle for ingestion of pest and parasites;
- contribution to safety hazard: roadside view reduction and fire risk increases under electricity lines;
- reduction of wool quality: hooked seeds reduce value of fleeces;
- prevention of water flow: plant masses block ditches and irrigation channels;
- allelopathic inhibition: release of substances toxic to crop plants;
- impact on crop establishment: vegetation prevents establishment of young trees.

The most common techniques of weed control are summarized in Table 2.

Since the end of World War II, agriculture has changed dramatically towards the maximization of yield and reduction of costs. This modification was favored by the mechanization and a new approach to weed control. Herbicides became the answer to new goals that modern agriculture aimed to reach. Chemical substances are able to suppress weeds, interrupting their growth with minimum efforts in terms of labor and money. Thanks to pre-emergence herbicides, it is possible to control weeds early with positive effects on the main crop yield. Decreasing weed competitiveness, less fertilizer and water amounts are required by the crops. Moreover, selective herbicides allow an effective weed control, minimizing soil and plant disturbance. Despite their numerous advantages, chemicals can also have negative effects on environmental and human health, requiring caution when used. The consequences can be direct, such as those on persons who handle the herbicides, and indirect, referring to the environmental contamination caused by persistent molecules (Zimdahl, 2013). The contamination together with natural evolution and the existing interactions in the environment have led to herbicide resistance in some weed species which is occurring all over the world (Vila-Aiub et al., 2003).

Method		Application	Details
Chemical weed control (only in conventional farming)		Herbicides	chemicals used to kill or interrupt the growth of weeds
Non-chemical weed control (sustainable farming)	Indirect (in absence of main crop)	Crop rotation	Rotating crops with different life cycles can disrupt the development of weed-crop associations
		Primary tillage	controls weeds by burying a portion of germinable seeds
		Secondary tillage	is used for carrying out the false seedbed technique
		Mulching	organic mulching black or clear plastic(solarization) cover crops
	Direct (in presence of main crop)	Cultivation tillage	to achieve a shallow tillage which loosens the soil and controls weeds
		Mulching	living mulches cover crops
		Flaming	use of high temperatures to damage weeds
		Agronomic choice of main crops	crop competition cultivar selection time of seedling fertilization

Table 2: most common techniques of weed control

In comparison with other weed management and control options, the herbicides success is results from marked improvements in crop productivity and farm labor efficiency (Bastiaans et al., 2008). However, in the long-term, chemical weed control has failed and has induced farmers to adopt other strategies, increasing costs (Mortensen et al., 2000; Weber and Gut, 2005). The negative aspects have found voice in the agro-ecological approach, where alternative weeds management strategies try to join environmental and human health to quantity and quality of food production. The renewing interest in no-chemical weed management has contributed to the improvement and development of new techniques in weed control. Generally, the most common techniques of no-chemical weed control that can be used in organic or low-input farming are classified based on the presence or absence of the main crop.

Some tillage practices are used as mechanical control of the development of weeds, but in some cases they can decrease soil organic matter content and fertility. Primary tillage, for example, is often aggressive when carried out at a considerable depth by the use of mould-board ploughs, disc ploughs, diggers, and chisel ploughs(Leblanc and Cloutier, 2001). On the contrary, secondary tillage conducted with harrows (disc, spring tine, radial blade, and rolling) and power take-off machines (Cloutier et al., 2007) is preferred in sustainable farming because it is less aggressive and causes less soil disturbance and results in less SOM decrease. Finally, cultivation tillage is carried out after crop planting by means of cultivators used to uproot and break the weed root contact with the soil (Kurstjens and Perdok, 2000).

The agronomic choice of main crops represents the method that favors the competitive ability of the crop against weeds (Paolini, 2001); it is a zero-cost choice for farmers because it is based on a decision that optimizes the factors listed below (Blachsaw et al., 2007). Cultivar selection, row spacing, fertilization management, seed rate and time of seeding or transplanting are the more efficient agronomic factors that are manipulated to increase crop competitiveness (Blachsaw et al., 2007).

In recent years, interest in cover crops has increased; however, they have been used to improve soil, yield of subsequent crops and for weed management since antiquity (Caamal-Maldonado et al., 2001). A cover crop is any living ground cover that is planted into or after a main crop and is usually killed on the surface (*organic or dead mulches*) or incorporated into the soil before they mature (*green manure*). If a cover crop is able to retrieve available nutrients still in the soil after an economic crop, preventing nutrient leaching over the winter, it is called *catch crop.* When the cover crop is planted before or with a main crop and grows together with it, is called *living mulch* (Hartwig and Ammon, 2002).

Cover crops have multiple influences on an agroecosystem (Sarrantonio and Gallandt, 2003). They are able to intercept radiation, influenzing the temperature of the environment and biological activity both at canopy level and in the soil. They can also store C and nutrients, affecting their avaibility (Chalker-Scott, 2007) reduce rain impact on the soil, erosion risk and distribution of the moisture in the soil (Teasadale et al., 2007). Living mulch plays a crucial role in weed management: there is a negative correlation between cover crops and weed biomass (Teasadale et al., 2007). Moreover, weed growth can be inhibited by some allelopathic interactions with living cover mulches (Hartwig and Ammon, 2002). Dead cover crops affect weed growth in different ways: the compact and thicker mulch layer on the soil surface decreases soil temperature fluctuation, light penetration and releases phytotoxins, inhibiting germination and establishment of many weeds (Sarrantonio and Gallandt, 2003). Furthermore, some predators of weed seeds can find in dead mulches a hidden retreat from the soil surface and significantly reduce the weed seedbank(Gallandt et al., 2005).

From a holistic point of view, where it is necessary to intregate environmental and human health with crop yield in agroecosystem management, the introduction of cover crops for multiple reasons that include sustainable weed control represents a fundamental agronomic choice.

3. ORGANIC MULCHES

Briefly, mulches could be defined as "materials that are applied to, or grow upon the soil surface" (Chalker-Scott, 2007). Many materials can be used as mulches (black plastic, geotextiles, fine-textured organic mulches, sheet mulches,mulches with waxy components, gravel, stones, living mulch, etc.) but, in our study, we consider just some organic, dead mulches. Organic mulches have been demonstrated to be effective in different aspects of soil and crop management; some of those are summarized below.

Soil moisture - The organic mulches influence the soil water cycle by increasing retention and percolation and reducing evaporation. The bare soil is more vulnerable to wind, soil radiation and rain activity. Mulches improve soil absorption capacity because they decrease the rain compacting force. Moreover the absence of a ground cover increases soil evaporation, with consequent water loss and supplemental irrigation needs (Chalker-Scott, 2007).

Soil erosion and compaction- the application of a mulch prevents wind and water erosion, soil compaction and consequently improves root and plant health (Teasadale et al., 2007). The more or less thick layer of organic material disperses the pouring rain force, enhancing soil aggregation and porosity (Oliveira and Merwin, 2001).

Soil temperature- The organic barrier on the soil has the capacity for the maintenance of optimal soil temperatures, working to buffer against temperature extremes (Long et al., 2001). This buffer capacity will depend on layer thickness: thicker applications of organic mulches moderate temperature better than thin layers (Teasadale et al., 2007). Moreover, organic mulch promotes a less stressed plant establishment and the optimal use of solar energy for plant growth (Goulet, 1995).

Soil nutrition- The organic mulch quality can affect nutrient cycle and soil microbial biomass. Environmental conditions can quicken the rate of organic material decomposition, releasing nutrients into the soil for root uptake (Chalker-Scott, 2007).

Salinization and pesticide contamination- The evaporation reduction caused by organic mulch can affect soil salinization processes (Chalker-Scott, 2007). Many researchers found decreasing salt toxicity for plants grown on mulched soils (Ansari et al., 2001;Landis, 1988; Yobterik and Timmer, 1994). Moreover, the positive effect of mulch application on microbial populations and activity can increase pesticide degradation (Gan et al., 2003; Smith and Skroch, 1995).

Plant establishment and growth- The water retention and weed control regulated by organic mulches are positively correlated with seed germination and transplanting success, seedling survival and root development. All of those effects depend on quality and thickness of the material left on the soil (Fausett and Rom, 2001).

Diseases-The reduction of diseases takes into account different aspects. First, there is a mechanical impediment for spore dissemination by a reduction of the rain drop impact (Chalker-Scott, 2007). Furthermore, the better conditions of the mulched soil in terms of moisture, temperature, porosity and aggregation, improve plant health and increase tolerance to pathogens (Turchetti et al., 2003). All those beneficial effects of organic mulching allow a reduction of the use of insecticides and fungicides during crop growth (Chalker-Scott, 2007).

Weed control- The ability of organic mulch to control weeds is principal because of its capacity to reduce light penetration on the soil surface, inhibiting seed germination and stressing existing weeds. In other cases weed suppression is achieved by the leaching of allelopathic chemicals that occur naturally in the organic material (Chalker-Scott, 2007).

The study was carried out in two experimental fields: the first one grew tomato crops (*Solanum lycopersicum*L.), while the second one the pepper crop (*Capsicum annuum* L.). The mulches used were from winter cover crops *Vicia villosa* Roth (Hairy Vetch), *Phacelia tanacetifolia* Benth. (Lacy Phacelia), *Sinapis alba* L. (White Mustard) and *Barley straw* in the first experimental field, while *Vicia villosa* Roth., var. Capello (Hairy vetch) and *Avena sativa* L. var Donata (oat) in the second field.

Hairy vetch is a vigorous winter growing forage legume, and is well known its capacity to fix nitrogen (Hartwig and Ammon, 2002). Hairy vetch can accumulate a large amount of nitrogen during the growing period (Anugroho et al., 2009; Seo and Lee, 2008; Choi and Daimon, 2008), supplying N for the following summer crop (Brandsæter et al., 2008). In sustainable agriculture it possible use hairy vetch as dead mulch for its ability to suppress weeds through allelopathic interference(Hill et al., 2007)with low regrowth after mowing(Curran et al., 1994). In Mediterranean environment, Hairy vetch has been used successfully for weed control to supply N (Campiglia et al., 2010a;2010b;2012;Mancinelli et al., 2013).

Lacy Phacelia is not a native plant in central Italy but is indigenous to America, and is usually used by apiarists. It produces large quantities of pollen and nectar(Hickman and Wratten, 1996;Holland and Thomas, 1996; Baggen et al., 1999), thereby beckoning a lot of insects, which is an important quality to enhance environmental biodiversity. Moreover, pollen can also be a useful marker, particularly if the plant is not native to the area (Landis et al., 2000). In addition reductions of aphid populations were found by the use of Lacy Phacelia around cereal fields (Hickman and Wratten, 1996). Because the common use of honey production in central Italy and its high aboveground biomass, Lacy Phacelia has been used as cover crop and mulch in the experimental fields (Wyland et al., 1996).

White mustard is an annual plant which belongs to the *Brassicaceae* family and is used as fodder for cattle and as fertilizer for fields. White mustard can establish quickly and suppress weeds during the autumn months because it contains allelopathic compounds that inhibit weed germination and growth (Cloutier et al., 2007). In the past, a species belonging to the same family (*Brassica napus* L. -rape), has been used successfully in the Mediterranean environment (Radicetti et al., 2013). White mustard use as mulch is not very common in central Italy, even though produces abundant dry matter (Talgre et al., 2011). For this reason, white mustard is tested in experimental fields, as a winter cover crop and consequently as summer mulch.

Use of straw as mulch provides an attractive and an environmental friendly option in Italy, where the land use for winter cereal grains it is about 2,163,951 ha with a total annual production of 8,533,421 t (ISTAT). Straw mulching reduces maximum soil temperature and helps in conserving soil moisture(Bhatt and Khera, 2006) and virus disease control in various crops (Döring et al., 2005).

Oat is a winter cover crop well adapted to the mild winters of the Mediterranean environment, producing a large amount of aboveground biomass (Campiglia et al., 2012). Oat mulch shows a consistent reduction in weed species richness (Campiglia et al., 2012) and its residues negatively affect weed density and weed aboveground biomass(Campiglia et al., 2010b; Murungu et al., 2011).

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5. MANUSCRIPTS

CHAPTER 5.1

Mancinelli, R., Marinari, S., Brunetti, P.,Radicetti, E., Campiglia, E.

Soil CO_2 emission and C storage as affected by organic mulching, irrigation and fertilization of vegetable crop in the Mediterranean environment

Submitted to *Agriculture Ecosystems & Environment*

CHAPTER 5.2

Marinari, S., Mancinelli, R., Brunetti, P., Campiglia, E.

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

Submitted to *Soil Tillage Reseach*

CHAPTER 5.3

Campiglia, E., Radicetti, E., Brunetti, P.,Mancinelli, R.

Do cover crop species and residue management play a leading role in pepper productivity?

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5.1 CHAPTER

Soil CO_2 emission and C storage as affected by organic mulching, irrigation and fertilization of vegetable crop in the Mediterranean environment

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Abstract

Nowadays there is a growing interest concerning the role of agriculture in mitigating GHGs resulting from the significant release of GHGs. Since most of the C in agricultural systems is stored in the soil, carbon stock and CO_2 emissions in agriculture are highly affected by the management of applied practices in arable farms, such as fertilizer use, irrigation, soil tillage, cover crop management, etc. This study evaluated the effects of various organic mulches, nitrogen fertilization and irrigation levels on soil CO_2 fluxes, soil carbon sequestration and processing tomato production in the Mediterranean environment. The field experiment was carried out with five main treatments, three cover crops of hairy vetch (HV), lacy phacelia (LF) and white mustard (WM) transplanted in autumn and cut in May to be used as mulches, plus barley straw mulch (BS) and conventional (C). After tomato transplanting, the main plots were split into two nitrogen fertilization treatments (0 and 100 kg N ha^{-1}) and the sub-plots were then split again into three irrigation levels(irrigation water 100%, 75%, 50% of evapotranspiration).In all treatments, a general effect was observed in the temporal fluctuations of soil CO_2 emissions throughout the observation period which were significantly influenced by soil temperature and also by volume water content. The temporal fluctuations of the soil CO_2 fluxes were attributed to climatic conditions and the peaks achieved optimal conditions of soil temperature and water content for soil respiration. A polynomial regression model highlighted the best conditions of soil temperature and water content in the various treatments in comparison (21.5 °C as average in BS, C and HV, and 18.3 °C in WM; soil water content of 28% as average in BS, C and HV, 55% in WM, and 87% in LP). A larger amount of TOC was observed in the mulching treatments than in the control after tomato harvesting, probably due to the residual biomass of the cover crops and a greater growth of the tomato. Although the soil carbon output as cumulated CO_2 emissions did not show statistically significant differences between the treatments, the soil carbon balance enabled us to estimate the highest net carbon contribution to the soil in HV determined by inputs and input/output ratio. However, except for the BS in 2013, the input/output ratios were >1 in all mulch treatments. In the Mediterranean environment, agronomical practices, such as the use of hairy vetch mulch on no-tilled soil, a slight reduction of irrigation water (-25%) and a rationalized use of N fertilizer potentially could shift the C balance in favor of soil C accumulation.

Keywords: soil CO_2 emission; soil carbon, organic mulching; irrigation; fertilization; tomato production.

Introduction

It is a well-known fact that the intensification of anthropic human activities increase greenhouse gas (GHGs) in atmosphere with a consequent influence on climate (Canadell et al 2007). The agriculture sector contributes significantly to the release of greenhouse gases into the atmosphere (Paustian et al., 2004; Smith et al., 2008) which although it is only responsible for 6.9% of Italy's total emission (Binatti et al., 2013), it plays an important role in mitigating strategies for reducing GHG emissions. Consequently, there is a growing interest concerning the role of agriculture in mitigating GHGs (Paustian et al., 1998; Mancinelli et al. 2008; Brahim et al. 2009). In agriculture the carbon (C) stocks and the GHG emissions are highly affected by the management of the agronomic practices applied in arable farms, such as fertilizer use, irrigation, soil tillage, cover crop management, etc. (Mosier et al., 1998; Triberti et al., 2008; Kallenbach et al., 2010). In agricultural cropping systems, most of the C is stored in the soil (Freibauer et al., 2004), therefore by means of various soil practices, such as tillage, fertilization, mulching, crop residue incorporation and irrigation (Lal 2004; Sainju et al., 2005; Gesch et al., 2007; Álvaro-Fuentes et al., 2008) it is possible to increase soil organic matter (SOM) and then soil carbon content which is also one of the principle parameters of soil quality (Andrews et al., 2002; Reicosky, 2008).

SOM plays an important role in the physical, chemical and biological properties of the soil (Manlay et al., 2007) and represents the largest terrestrial pool in the C biogeochemical cycle (Houghton et al., 2001). The increase of organic soil carbon stock is due to a positive budget between C inputs (above and belowground biomass) and C outputs (heterotrophic soil organism and soil erosion) (Kuzyakov, 2006).120 Pg of atmospheric C-CO_2 are annually transformed into carbohydrates and other organic molecules by plants with the natural process of photosynthesis and during the decomposition process sequestered by SOC. The choice of the species and nutrient application can positively affect the photosynthetic process thus increasing the net primary production and consequently the carbon pools (Lal, 2006).The reduction of the number and intensity of soil tillage directly (with less use of fossil fuel) and indirectly (with less alteration of soil characteristic and fauna) affects soil CO_2 emissions and consequently soil C stock (Reicosky, 2008; West and Marland, 2002). The results of several long-term experiments showed that the use of no-till (NT) compared to conventional tillage (CT) can sequester nearly 60 g C m^{-2} yr^{-1} and soil tillage with moldboard accelerates the SOM decomposition (West and Marland, 2002). Other studies in a corn–soybean rotation showed that the use of less intensive tillage systems and better management of crop residues are effective in reducing CO_2 emission and improving soil C (Al-Kaisi and Yin, 2005). The reduction of soil CO_2 emissions can be achieved by using agronomic practices that favor soil C input and reduce the mineralization of soil organic matter, such as reduced soil tillage and cropping system intensification which maximize water and nutrient use thus increasing production (Paustian et al., 2000).

It is known that soil moisture, temperature and nutrient contents affect soil respiration and organic matter decomposition (Kirschbaum, 2004). How soil moisture by irrigation and temperature affect soil CO_2 emission in various herbaceous cropping systems is still an uncertain issue. A correct use of irrigation water can promote both aboveground and root biomass production, favoring and improving soil organic carbon concentration and decreasing C lost through erosion (Sauerbeck, 2001).

Nitrogen fertilization is one of the classical agronomic practices used in conventional cropping systems with the aim of increasing yield and productivity. Concerning the soil CO_2 emissions and soil C decrease, some studies show that nitrogen fertilization can negatively affect soil carbon stock (Al-Kaisi et al., 2008; Mulvaney et al., 2009) while other studies show that fertilization has no direct effect on soil carbon (Halvorson et al., 2002; Liang et al., 2012).

The introduction of cover crops in a crop rotation is an agronomic practice that are generally adopted for addressing the improvement of cropping system sustainability and therefore soil quality (Campiglia et al., 2009; Campiglia et al., 2011). A appropriate management of crop residues and the use of winter cover crops can have a positive effect for limiting the leaching of residual Nitrogen and increasing the nitrogen supply to the following main crop as well as improving both the functions and properties of soil physical, chemical and biological, water movement, infiltration and runoff (Clark et al., 1998;Liebig et al., 2002; Sainju et al., 2007; Mancinelli et al., 2013; Campiglia et al., 2014). Specifically, the cover crop mulch residues left on soil surface can positively affect various aspects of the cropping system such as the increase of water infiltration due to the decrease of runoff, reduction of soil erosion, increase of moisture retention, weed control and reduction of soil temperature in summer (Reicosky, 2008; Campiglia et al., 2012; Campiglia et al., 2014).

Although various studies have been carried out in order to evaluate the influence of soil tillage, irrigation and N fertilization on some crops, little is known about their effects on soil CO_2 emissions and vegetable crop production. Concerning vegetable cropping systems in Italy and in the Mediterranean environment, to our knowledge no research has yet been carried out regarding the impact of organic mulching, N fertilization and different irrigation levels on soil CO_2 emissions, soil carbon content and processing tomato production yield.

It is assumed that, in the Mediterranean environment, a good combination of agronomical practices such as the organic mulching of cover crops with irrigation and N fertilization can be profitably used for growing vegetable crops in sustainable and/or organic agriculture. In order to find and evaluate the best method for using organic mulching, irrigation and N fertilization on soilcarbon emissions and vegetable crop production yields in the Mediterranean environment, the processing tomato was grown on organic mulch made from winter cover crops and barley straw.

The main objectives of this study were: (1) to evaluate the effects of various organic mulches, nitrogen fertilization and irrigation levels on soil CO_2 emissions; (2) to understand the impact of soil management with various organic mulches, nitrogen fertilization and irrigation levels on carbon sequestration; (3) to verify which type of organic mulching and which levels of nitrogen fertilization and irrigation are the most suitable for applying in processing tomato production in the Mediterranean environment

2. Materials and Methods

2.1. Study site and soil characteristics

The study was carried out in 2011/2012 and 2012/2013 in two adjacent experimental fields established in September 2011 and in September 2012 at the experimental farm of the University of Tuscia (Viterbo) located approximately 80 km North of Rome (45°25'N, 12°04'E).

The daily minimum and maximum temperatures and the rainfall during the study period were collected by means of a meteorological station located 100 m from the study site. The climate of the area is typical of the Mediterranean environment, generally characterized by cool, moist winters and warm, dry summers. The long-term (last 30 years) mean annual amount of precipitation is 752 mm of which 183 mm falls between June and September. The long-term mean annual average temperature is 14°C. The highest mean monthly temperatures occur between July and August when the monthly maximum values are 31°C and the monthly minimum values are 17°C.

The soil of the experimental fields was classified as a *Typic Xerofluvent*, of volcanic origin. The physicochemical characterization and sub-sequent classification analyses were carried out using the official methods of analysis (MiPAF, 2000). The soil surface horizon (0–25 cm depth) was classified as sand-loam USDA classification by the particle size distribution analysis, with 63% sand, 22% silt, 15% clay. In the two adjacent experimental fields, the total organic C and N content was on average 1.07 and 0.12, respectively (C/N ratio 9.2), pH(H_2O) of 7.1.

Chemical and physical analyses were carried out with twelve soil samples collected from the two experimental fields (four soil samples in each block) before starting the trials (in September of both years), in order to verify the field homogeneity. The soil properties did not differ within the three blocks. In the year prior to the experimentation, barley (*Hordeum vulgare* L.)was grown in both adjacent experimental fields.

2.2. Experimental field and treatments

The study focuses on the period of the growth of the tomato crop following a winter cover crop.

The area of the experimental field was 4400 m^2 (55 x 80) which made it possible to carry out all farming operations with agricultural machinery. The experimental field was structured in five main treatments arranged in a randomized block design with three replications. Three cover crops [hairy vetch (*Vicia villosa* Roth, var. Villana) (hereinafter called HV), lacy phacelia (*Phacelia tanacetifolia* Benth, var. Boratus) (hereinafter called LF) and white mustard (*Sinapis alba* L., var. Emergo) (hereinafter called WM)] were sown in autumn and cutin spring to be used as mulches. The other two main treatments consisted in barley straw mulch [hereinafter called BS] and conventional (bare soil) [hereinafter called C].

In May the tomato plants were transplanted onto the cover crop mulches, barley straw mulch and in the conventional plots. After tomato transplanting, the main plots were split into two nitrogen fertilization treatments applied as: i) 0 kg N ha^{-1} of nitrogen fertilization (hereinafter called N0); and ii) 100 kg N ha^{-1} of nitrogen fertilization (hereinafter called N100). The sub-plots were then split again into three levels of irrigation: the amount of irrigation water ranging from 50% (hereinafter called irr050), 75% (hereinafter called irr075) and 100% (hereinafter called irr100) of the total evapotranspiration in the tomato crop.

The experimental design was a split-split-plot with three replications, where the main plots were represented by the mulches [size 108 m^2 (18 m by 6 m)], the sub-plots were the nitrogen fertilization treatments [size 54 m^2 (18 m by 3 m)], and the sub-sub-plots were the levels of irrigation [size 18 m^2 (6 m by 3 m)].

In September of each year of the experiment, all plots were ploughed in at a depth of 30 cm, fertilized with 100 kg of P_2O_5 ha^{-1} as a triple superphosphate and then harrowed in at a depth of 10 cm in order to prepare the soil for the sowing of the cover crops. The cover crop species were sown on September 26th, 2011 and September 30th, 2012 at seed rates of 60, 25 and 35 kg ha^{-1} for HV, LP, and WM, respectively. While, the soil of the other two treatments were kept bare and weed-free throughout the cover crop growing season by chemical means (glyphosate applied when the weed seedlings started to emerge).On May 8th, 2012 and May 14th, 2013, the cover crop aboveground biomass was mowed using a hay-conditioner farm machine which cut the biomass to a width of 180 cm and arranged the residues in mulch strips about 80 cm wide and 100 cm apart. In the treatment with barley straw mulch (no-tilled soil), the transplanting beds were prepared placing the straw in strips at the rate of 400 g m^{-2} of dry matter similar to those realized with cover crop aboveground biomass.In the conventional treatment, the transplanting bed was prepared with plough and rotary harrow at cover crop suppression. On May 18th, 2012 and May 27th, 2013, the tomato seedlings (*Lycopersicon esculentum* Mill.) cv Ronco were hand-transplanted in paired rows into the mulch layer at a distance of 40 cm between one another and a distance of 140 cm between the paired rows at a density of 3 plants m^{-2}.

The drip irrigation tape, with 30-cm-spaced emitters, was installed over the mulch on each row of tomato seedlings. The amount of water input was applied according to the irrigation treatment in order to reintegrate 50%, 75% and 100% of maximum evapotranspiration estimated by a class A evaporimeter and adjusted by crop coefficients (Allen et al. 1998). In the tomato plots where nitrogen fertilization was foreseen, 50 kg N ha^{-1} of ammonium nitrate was applied twice on June 15th and July 4th in the first year and on June 24th and July 9th in the second year. The weeds were controlled whenever required by means of a rotary hoe between the paired rows and by hand inside the paired rows.

The tomato was harvested on August 19th, 2012 and on August 20th, 2013.

2.3. Soil CO_2 emission, temperature and water content

In each of the 2 years, CO_2 emission from soil was measured weekly in each plot in the tomato-growth period. In the event of rain the CO_2 emissions were measured at least 48 h after rainfall. The measurements of soil respiration (SR) were carried out using a portable dynamic closed-chamber infra-red gas analyzer system (Pumpanen, 2004). The CO_2 flux was measured between 8 am and 12 am in order to reduce diurnal variation in the CO_2 emitted without exception (Adviento-Borbe et al., 2007). The non-steady-state through-flow chamber (SRC-1, PP Systems, Stotfold, UK; volume 1334 cm^3, cover area 78.5 cm^2) had only one opening to the soil and it was placed on a small permanent PVC frame. The collars were placed in a central area of each plot between the paired rows of the tomato crop.

The aboveground parts of the weeds were cut off before inserting the collars and clipped whenever necessary. The increase of CO_2 within the chamber was monitored by means of a sensitive infra-red gas analyzer instrument (EGM-4, PP Systems, Stotfold, UK).The closure time ranged between 30 and 180 seconds depending on the respiration rates of the soil. The SR was calculated automatically by fitting a quadratic equation to the relationship between the increasing CO_2 concentration and elapsed time. Each measurement took about 3 minutes.

The soil temperature close to the chamber was measured simultaneously to the measurement of CO_2 at a depth of 5 cm by using the “STP-1 Soil Temperature Probe” connected to an EGM-4 instrument.

The soil volume water content near the chamber was also measured simultaneously to the measurement of CO_2 at a depth of 20 cm using the "TDR 300 Soil Moisture Meter" (Spectrum Technologies, Inc., Plainfield, IL - USA).

In both years, the output of the soil CO_2 flux was estimated as the amount of CO_2 accumulated (Reicosky, 1997; Curtin et al., 2000; Ding et al., 2007; Wilson and Al-Kaisi, 2008; Mancinelli et al., 2010; Mancinelli et al., 2013) throughout the period of study. The calculations were made by means of the linear interpolation of the two neighbouring measured fluxes and the numerical integration over time (trapezoid rule) as reported in the following equation:

$$\mathbf{CO_2 - C = \sum_{i}^{n} [(x_i + x_{i+1}) \times N \div 2] + ... + [(x_{n-1} + x_n) \times N \div 2]}$$

where: i = date of first measurement of CO_2 rate taken, n = date of last measurement of CO_2 rate taken; x = CO_2 rate (kg ha^{-1} day^{-1}), and N = number of days between the two consecutive CO_2 rate measurements.

2.4. Sampling and analysis of soil, cover crops and tomato

In each year of the experiment the sampling of soil, cover crops and tomato was performed.

Before starting the experiment, five soil cores (0–20cm depth) were taken from each plot and then pooled together for the physicochemical characterization analysis. Just before the transplanting and after the harvesting of the tomato crop, soil samples (0–20 cm depth) were collected from the central inter-row of each plot, after removing the litter layer. The soil samples were air-dried, sieved (<2 mm) and then kept at 4 °C in order to set up soil moisture and temperature at the most suitable level for potential microbial activity (Zornoza et al., 2009).

The samples of cover crop aboveground biomass were collected in a 0.5 m^{-2} central area of each plot just before mowing. The collected samples were dried at 70 °C until constant weight in order to determine their dry weight and the C and N content.

The yield and aboveground biomass of the tomato crop were sampled in 2 m^2 of a central area of each plot at crop physiological maturity. The samples of the yield and aboveground biomass of the tomato were dried at 70 °C until constant weight in order to determine their dry weight and C and N content.

Total organic carbon (TOC) and the total nitrogen (TN) contents of the soil, cover crops and tomato were determined using an elementary analyzer (Thermo Soil NC—Flash EA1112).

Since a cover crop - tomato sequence can be considered a cropping system which is part of a wider pluriannual crop rotation, belowground and aboveground biomass estimates of both cover crops and tomato were used to calculate soil C input of the system. Empirical equations were used for estimating the root residues derived from the C inputs of cover crops and tomato:

- Tomato roots (Mg dry wt. ha^{-1}) = 0.30 x aboveground biomass (Mg dry wt. ha^{-1}) [adapted from Kong et al., 2005];
- Vetch roots (Mg dry wt. ha^{-1}) = 0.7 x aboveground biomass (Mg dry wt. ha^{-1}) [adapted from Tian and Kang, 1998; Po et al., 2009];
- Phacelia roots (Mg dry wt. ha^{-1}) = 0.35 x aboveground biomass (Mg dry wt. ha^{-1}) [adapted from Talgre et al., 2011];
- White m. roots (Mg dry wt. ha^{-1}) = 0.39 x aboveground biomass (Mg dry wt. ha^{-1}) [adapted from Talgre et al., 2011].

2.5. Data analysis and statistics

The regression analyses of soil CO_2 evolution on soil temperature and volume water content was performed for each treatment separately. The data used for the regression analyses of the soil CO_2 emission evolution on soil temperature and soil volume water content represented the mean value of the two-year experiment. The effect of the soil temperature and the volume water content on the CO_2 emission was plotted as a quadratic polynomial function. The results of the polynomial regression analyses were useful for evaluating the influence of soil temperature and volume water content on CO_2emission.

For the data obtained in each experimental year, the differences in cover crop aboveground biomass and relative C and N content, TOC and TON before tomato transplanting were statistically analyzed by using the one-way ANOVA procedure. The data regarding the soil TOC and TON at tomato harvesting, C input, C output, C input/output ratio of both experimental years were analyzed as a split-split plot experimental design with the cover crop as main factor, the irrigation as the split factor and the N fertilization as the split-split factor. 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.

In the manuscript the data of the soil CO_2 emission, soil temperature, and soil volume water content are reported, which are presented as the means of the considered treatment ± standard error (SE).

Statistical analyses were performed using JMP statistical software package (SAS Institute, Cary, NC).

3. Results

3.1. Weather conditions

From the historical data of the temperatures and precipitations observed in the same site and recorded in the same agrometeorological station, the monthly average of the monthly minimum temperatures were 10, 13, 16 and 17 °C in May, June, July and August, respectively and the maximum temperatures were 23, 27, 31 and 31°C in May, June, July and August, respectively; while, the total rainfall was167 mm in the May-August period.

The minimum and maximum temperatures, and precipitation observed daily during the tomato cultivation in the two years of experimentation are shown in figure 1. The trends of both temperature and rainfall were considerably different in the 2012 and 2013 experimental years. The 2012 growing season of the tomato was generally hotter and drier compared to 2013. In fact, in the May-August period of 2012 the maximum and minimum daily temperatures were lower than the historical monthly values for 36 and 43 days, respectively; while in 2013 the maximum and minimum daily temperatures were lower than the historical monthly values for 72 and 53 days, respectively. In the two years experimental period, the amount of precipitation from May to August was 110 and 172 mm in 2012 and 2013, respectively.

3.2. Soil CO_2 emissions, temperature and volume water content

The trends concerning the dynamics of soil CO_2 emission were quite similar throughout the study period in both experimental years, with generally higher levels in July and lower levels at the end of August. On average the value of soil CO_2 emission in July tended to be higher in 2013 than 2012 and in the first half of August of the second year there was a second peak of CO_2in all treatments, corresponding to the increase in air temperature during the same period. However, in August the soil CO_2 emissions tended to be lower in all treatments, even if air temperatures remained rather high while rainfall was absent. Nevertheless, the various mulching treatments showed different trends of soil CO_2 emission in both experimental years (2012 and 2013) and the significant effects of mulching treatments on soil CO_2 emission were evident throughout the study period in both years (Fig. 2 and 3). In particular, the highest differences were observed among conventional, barley straw mulch and hairy vetch treatments in both years of experimentation. These treatments were influenced in different ways by the various climatic trends in the two-year experimentation. In fact, the soil CO_2 emissions during the study period tended to be higher in HV, intermediate in BS and lower in C in the first year, while, conversely, in the second year the soil CO_2 emissions tended to be higher in C and lower in HV. The soil temperature was strongly related to the climatic trend observed during the study period (Fig. 2 and 3). The highest significant differences between the mulching treatments were observed in 2012 compared to 2013. The highest soil temperature values (25.8 and 25.5 °C) were reached on August 20th, 2012 and July 10th, 2013 in the C and LP treatments, respectively, while the lowest values (21.5 and 19.0 °C) were observed on July 27th, 2012 and June 28th, 2013 in the C treatment. The volume water content of the soil was generally affected by the weather conditions (air temperatures and precipitation) during the measurement period of both years (Fig. 2 and 3). Nevertheless, significant differences among treatments were often observed, mainly in the first year of experimentation. From June onwards, the treatments with mulched soil generally showed higher volume water content than C which was consequently the most suitable management for determining lower levels of soil water retention, compared to the mulched treatments.

The soil CO_2 emissions depend on soil temperature and volume water content and can be described through the polynomial regression analysis for each of the mulching treatments (Fig. 4). With the exception of the LP treatment, the polynomial regression model proved to be highly significant in the relationship between soil temperature and soil CO_2 emissions in all other treatments (P= 0.0026, P=0.0008, P=0.0163 and P=0.0052 for BS, C, WM and HV, respectively). The polynomial regression curves show the highest level of soil CO_2 emission at 21.3, 21.6, 18.3 and 21.7 °C in BS, C, WM and HV, respectively, and the increase or decrease in soil temperature determines a higher reduction of soil CO_2 emissions in C compared to the other treatments (BS, WM and HV).There was significant variation in the soil CO_2 emission response of soil volume water content, as determined by the polynomial regression model for all mulching treatments (C: P<0.0001, BS: P=0.0003, LP: P<0.0001, WM: P<0.0001, HV: P<0.0001). The polynomial regression curves showed that all mulching treatments determine highest level of soil CO_2 values at different levels of soil volume water content (25, 29, 87, 55 and 30% in C, BS, LP, WM, HV, respectively).

Concerning the irrigation treatment, soil CO_2 emission showed a similar dynamic to the mulching treatment in the two-year study period. Throughout this period, significant differences were observed between the three irrigation levels, with the highest level of soil CO_2 emission mainly in the irr075 treatment, while the irr050 treatment often showed the lowest values (Fig. 5 and 6). In the three irrigation levels, the temperature of soil was averagely related to the climatic conditions and mainly to the daily maximum air temperature. Soil temperature generally increases according to the decrease of quantity of irrigation water distributed. In fact, during the study period the lowest soil temperature values were more often observed in the irr100, while the lowest values were observed in the irr050. The lowest soil temperature values in the irr100 treatment were 21.8 and 18.4 °C reached on July 27th, 2012 and June 28th, 2013, respectively.

Figure 1. Daily minimum [---] and maximum [—] temperatures (◦C), and rainfall [□] (mm) at the experimental site, throughout the periods of study in 2012 and 2013

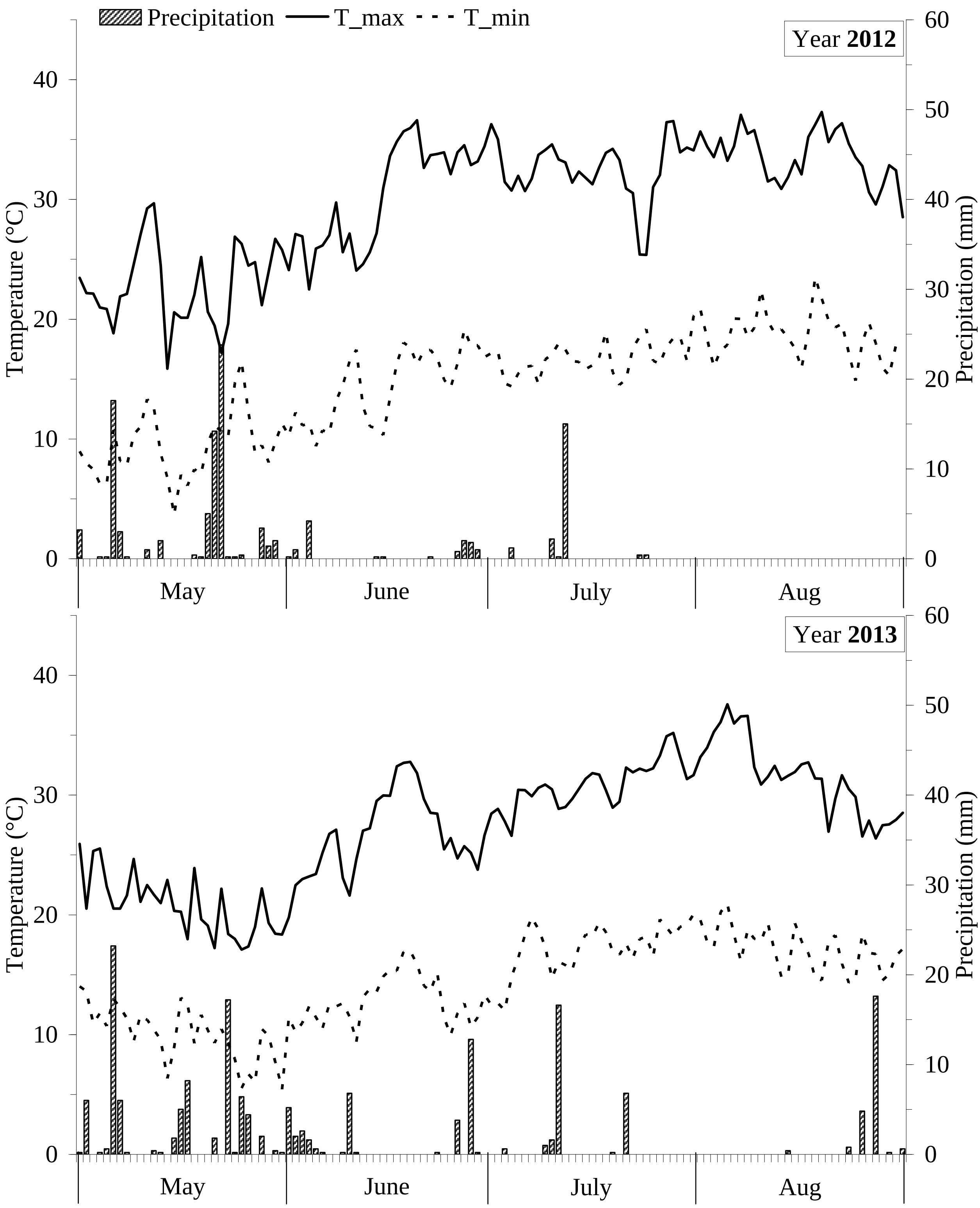


Figure 2 - Soil CO_2 flux, soil temperature and soil volume water content in the various mulching treatments, during the 2012tomato cycle. Bars are standard errors (n = 18).BS: barley straw; C: conventional; HV: hairy vetch; LF: lacy phacelia; WM: white mustard..

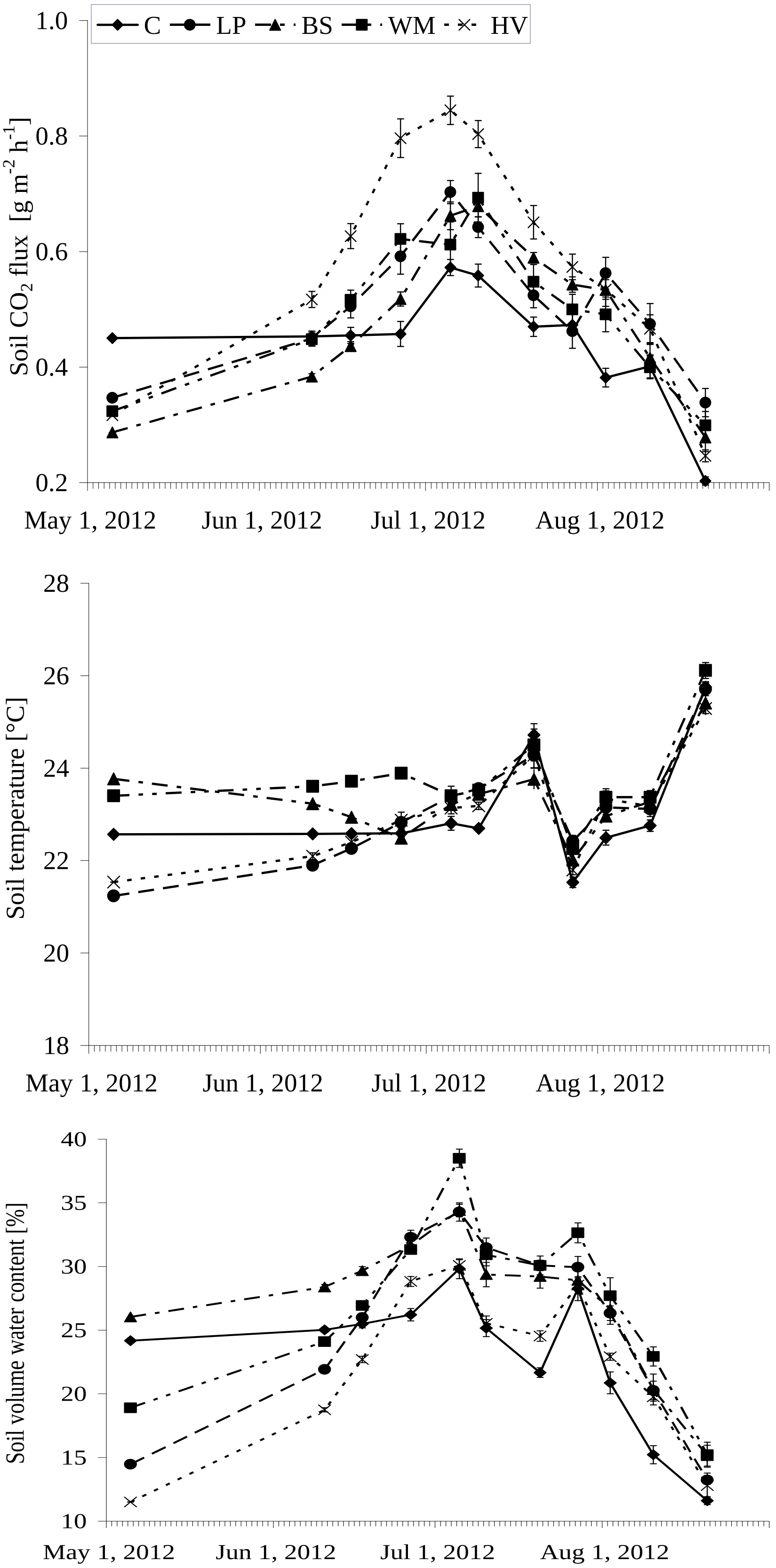
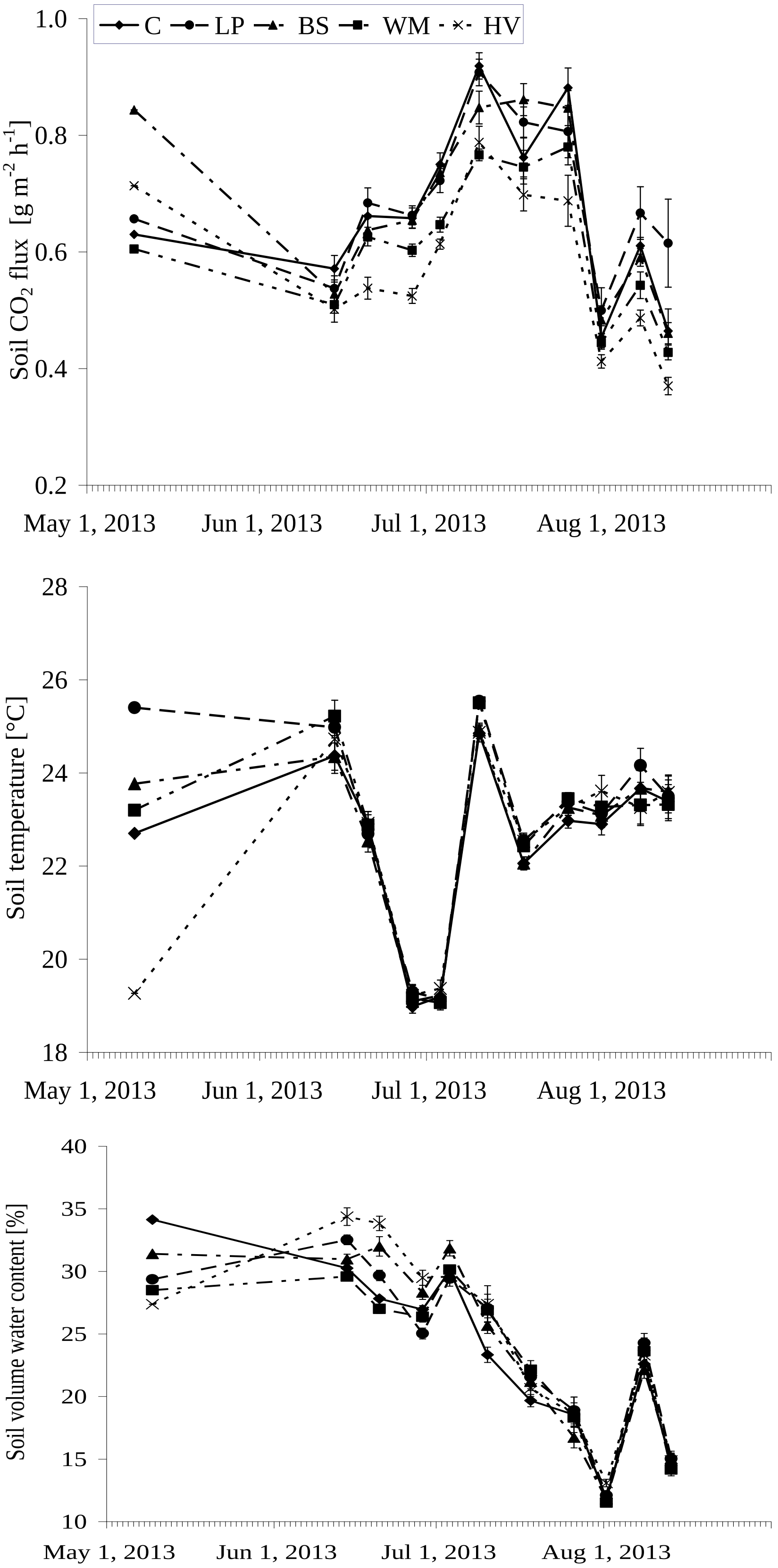


Figure 3 - Soil CO_2 flux, soil temperature and soil volume water content in the various mulching treatments, during the 2013 tomato cycle. Bars are standard errors (n = 18).BS: barley straw; C: conventional; HV: hairy vetch; LF: lacy phacelia; WM: white mustard.



As expected, the soil volume water content was averagely related to air temperature, while conversely the highest values were observed for the soil temperature in the irr100 treatment, moreover these values were lower in the treatments with reduced quantity of irrigation water distributed (Fig. 5 and 6). In the relationship between soil CO_2 emissions and soil temperature, the multiple regression model proved to be highly significant for the irrigation treatments irr050 and irr075 ($P<0.0001$), while the 100% irrigation (irr100) did not show a significant relationship (Fig. 7). The model showed the highest level of soil CO_2 emissions at soil temperature of 22 °C for both levels of irrigation irr050 and irr075 but with CO_2 flux higher in irr075 than irr050 and a difference of 0.08 g m^{-2} h^{-1} of CO_2. Soil CO_2 emissions plotted against soil volume water content were highly significant for all irrigation treatments ($P<0.0001$). The model showed that the reduction of the quantity of irrigation water (from 100% to 75% and 50%) causes a reduction of the soil water content required to determine the maximum level of CO_2 emissions (43, 32 and 23% in irr100, irr075 and irr050, respectively) and a decrease of CO_2 emitted (0.69, 0.65 and 0.58 g m^{-2} h^{-1} of CO_2 in the treatment irr100, irr075 and irr050, respectively).

In relation to the nitrogen fertilization effect on soil CO_2 emissions, the results showed a general trend similar to mulches and irrigation treatments throughout the measurement period. During the study period, the un-fertilized treatment (N0) often showed higher values than the treatment fertilized with 100 kg N ha^{-1} (N100), in both experimental years. The soil temperature did not show significant differences between N0 and N100, except at certain times of the second year. Soil volume water content proved to be significantly influenced by nitrogen fertilization with lower values in comparison to the un-fertilized treatment, mainly in July and August of both years (Fig. 8 and 9).

The polynomial regression model proved to be highly significant ($P<0.0001$) regarding soil CO_2 fluxes plotted against soil temperature and soil volume water content in both fertilized and unfertilized treatments (Fig. 10). The curves show the highest CO_2 emissions of soil temperature for fertilization treatments at 21 °C but with CO_2 flux lower in N100 compared to N0 (0.61 vs. 0.65 g m^{-2} h^{-1} of CO_2 in N100 and N0, respectively). The soil CO_2 emissions related to volume water content showed the highest values of CO_2 flux (0.62 vs. 0.64 g m^{-2} h^{-1} of CO_2 in N100 and N0, respectively) at 30 and 33% of soil volume water content.

Figure 4 – Soil CO_2 flux plotted against soil temperature and soil volume water content in the various mulching treatments. The data fit with second-order polynomial regression models.BS: barley straw; C: conventional; HV: hairy vetch; LF: lacy phacelia; WM: white mustard.

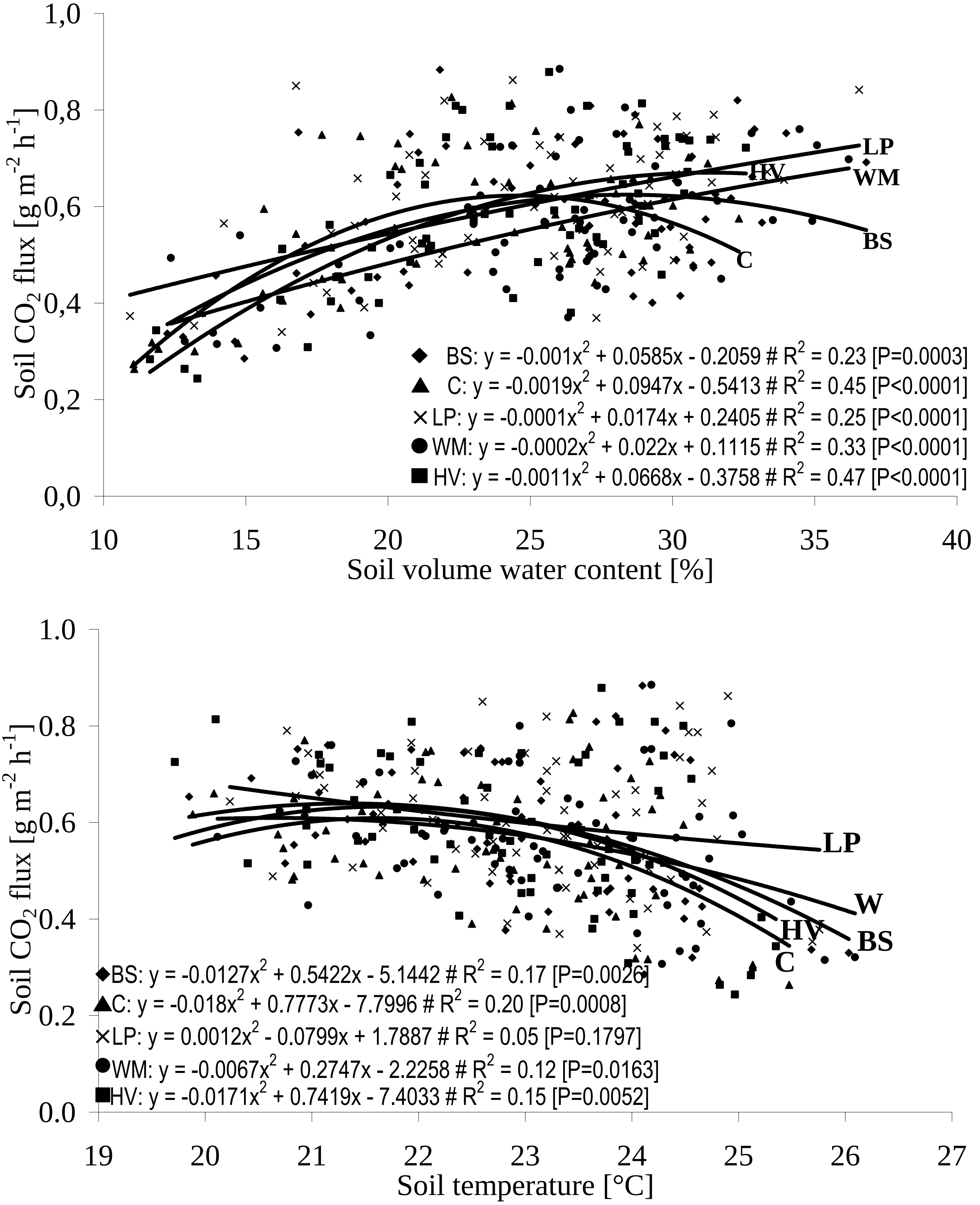


Figure 5 - Soil CO_2 flux, soil temperature and soil volume water content in the various irrigation levels, during the 2012tomato cycle. Bars are standard errors (n = 30). irr50: irrigation 50%; irr75: irrigation 75%;irr100: irrigation 100%..

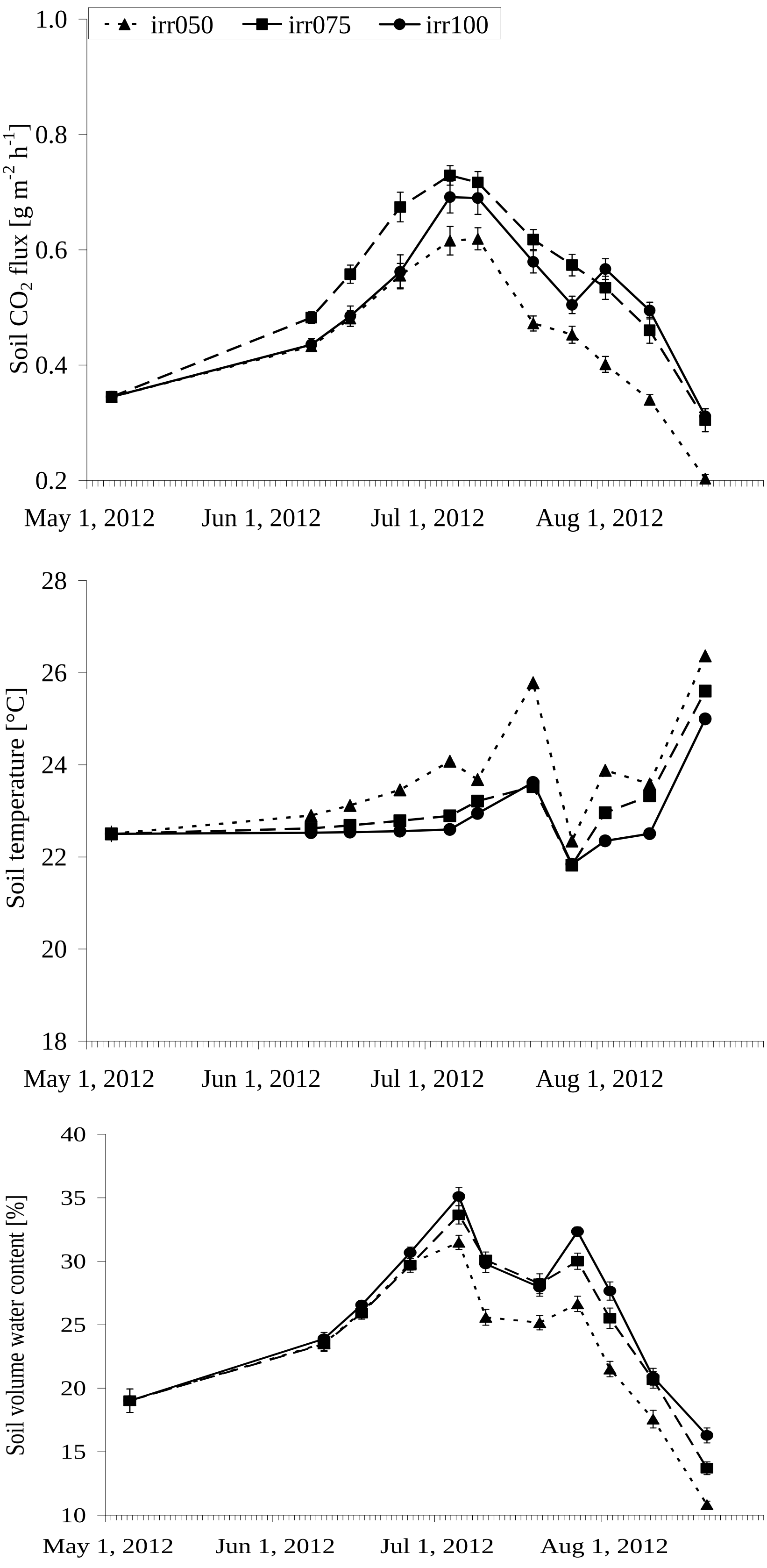


Figure 6 - Soil CO_2 flux, soil temperature and soil volume water content in the various irrigation levels, during the 2013 tomato cycle. Bars are standard errors (n = 30). irr50: irrigation 50%; irr75: irrigation 75%;irr100: irrigation 100%.

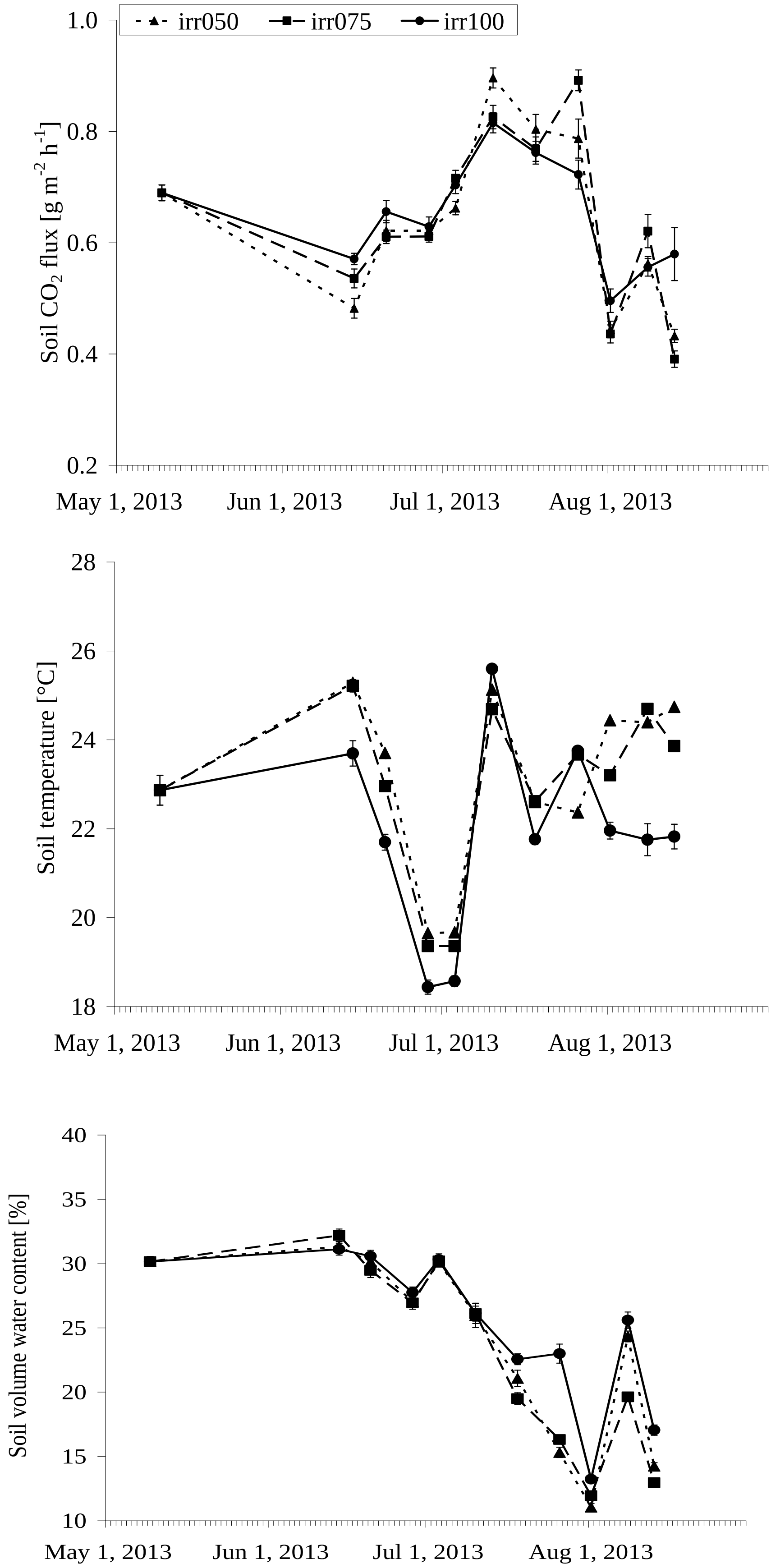
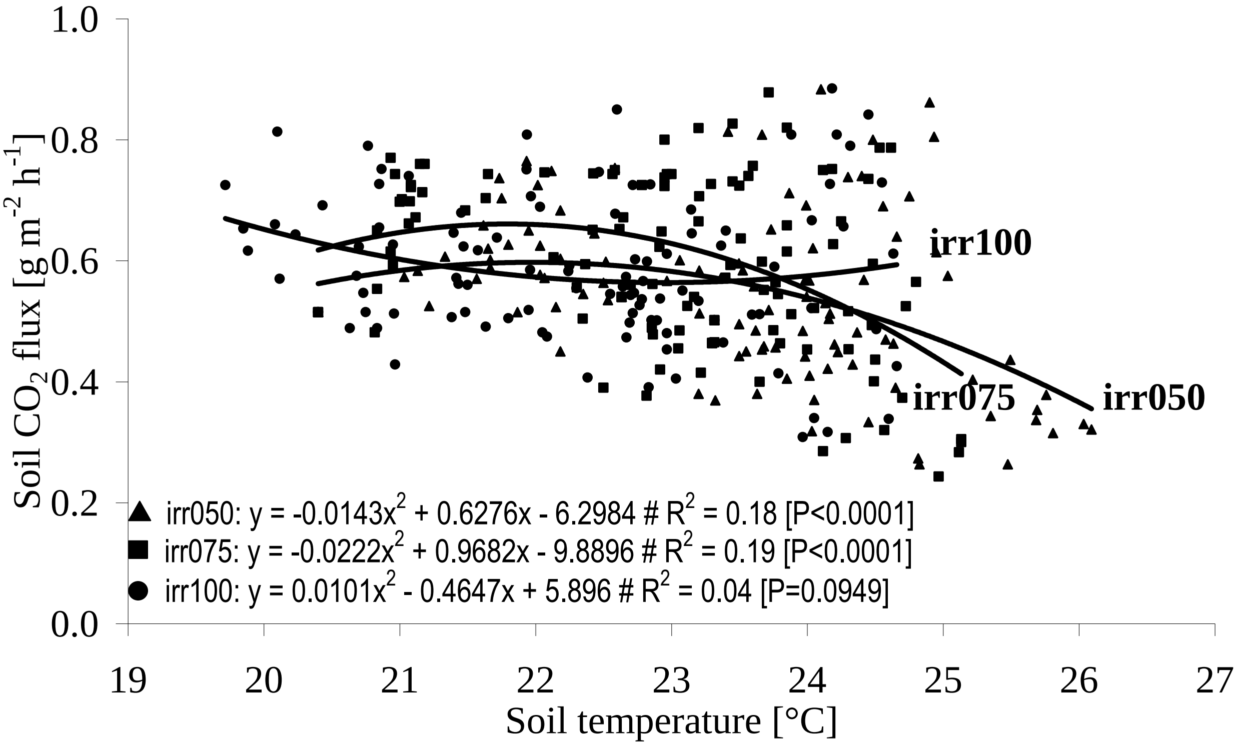


Figure 7 – Soil CO_2 flux plotted against soil temperature and soil volume water content at various irrigation levels. The data fit with second-order polynomial regression models. irr50: irrigation 50%; irr75: irrigation 75%;irr100: irrigation 100%.



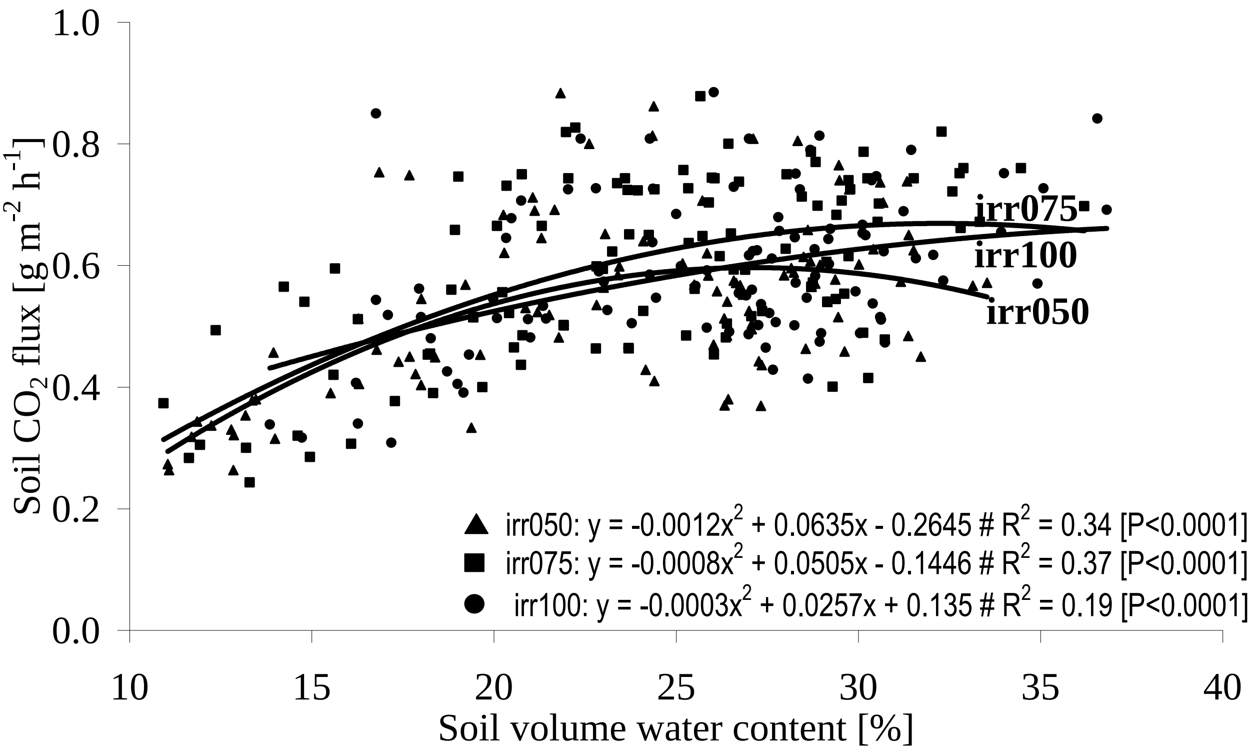


Figure 8 - Soil CO_2 flux, soil temperature and soil volume water content in the two fertilization levels, during the 2012tomato cycle. Bars are standard errors (n = 45). N0: unfertilized; N100: fertilized.

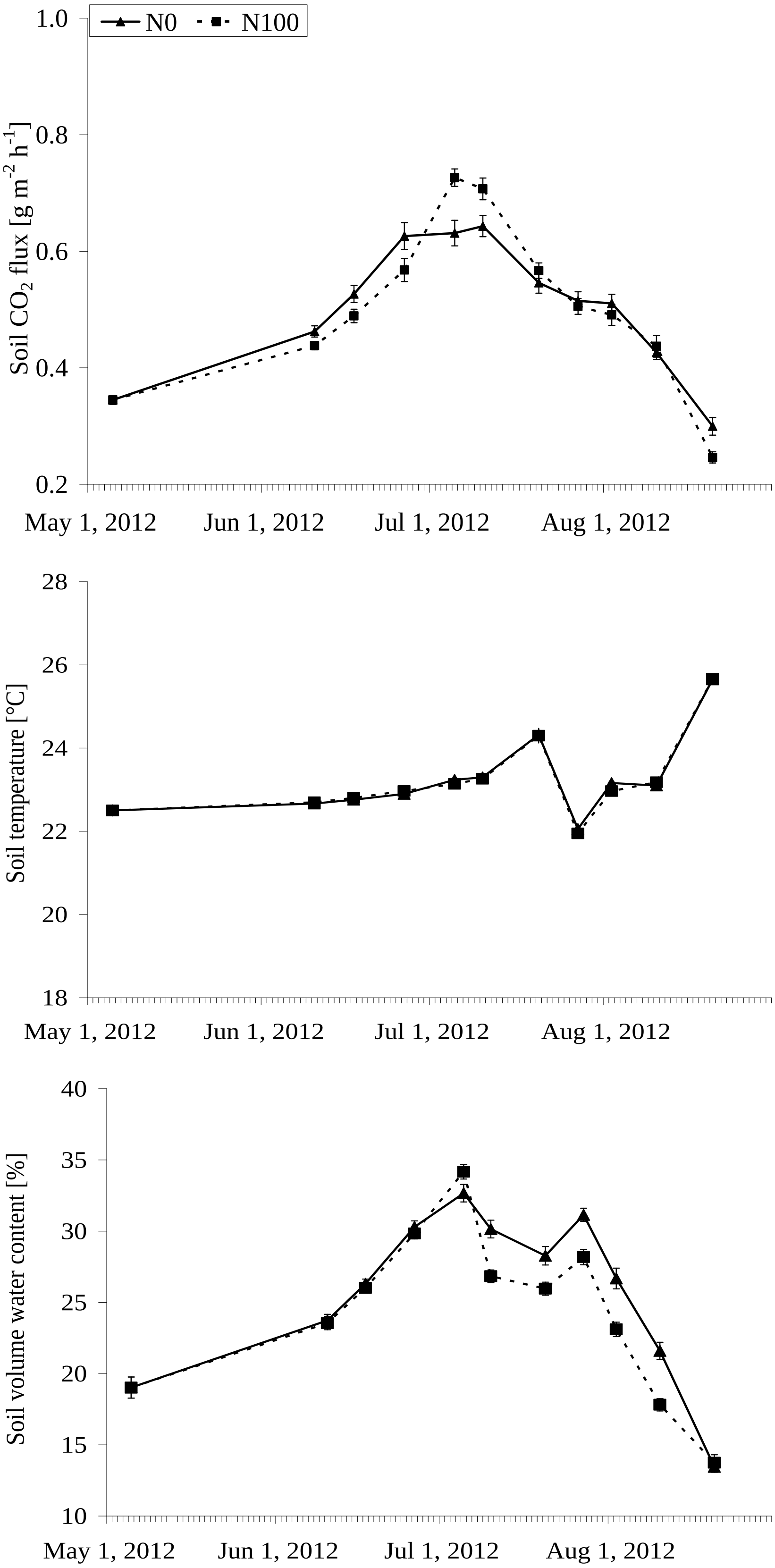


Figure 9 - Soil CO_2 flux, soil temperature and soil volume water content in the two fertilization levels, during the 2013 tomato cycle. Bars are standard errors (n = 45). N0: unfertilized; N100: fertilized.

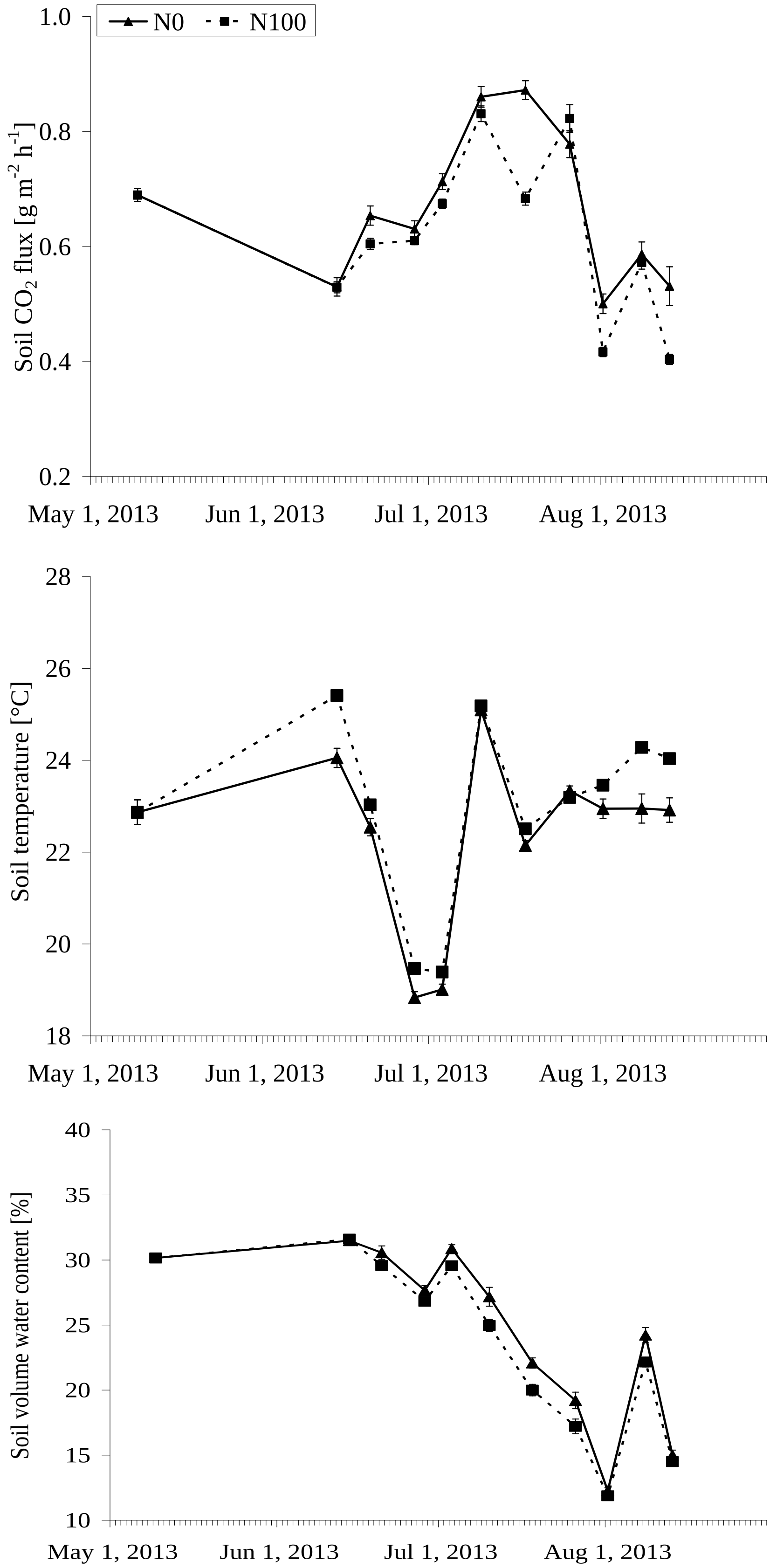
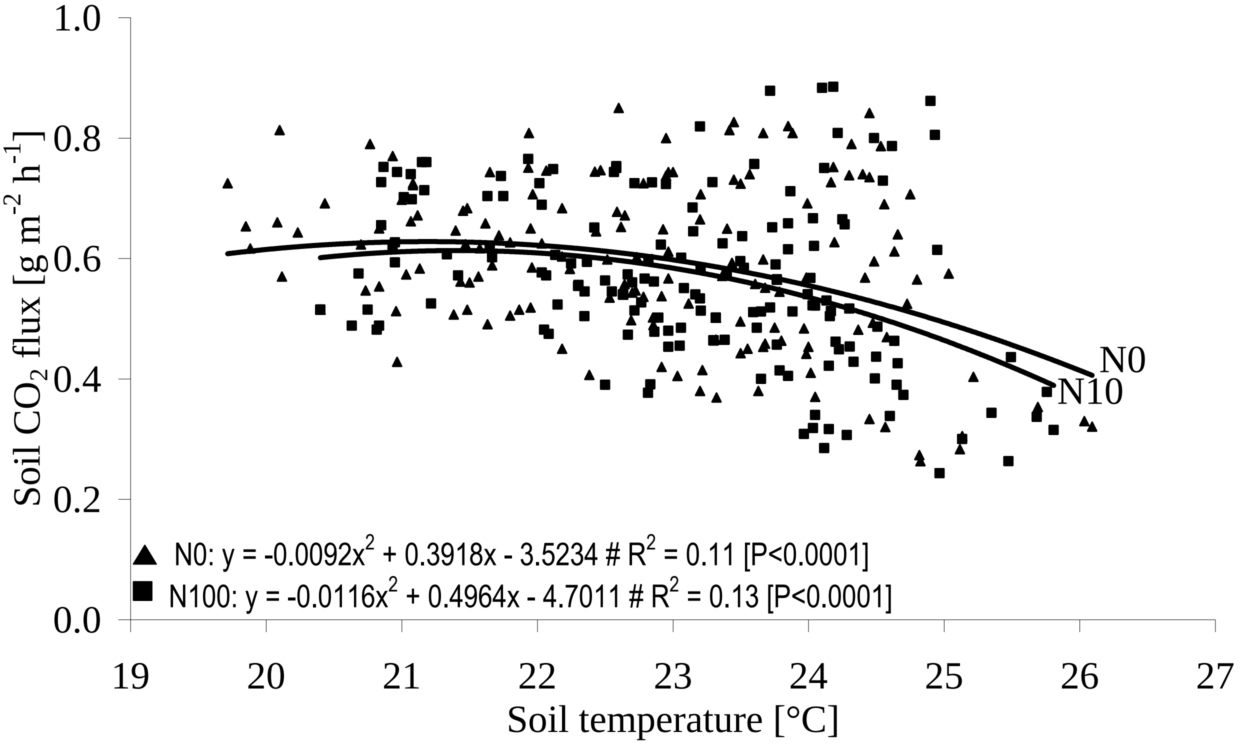
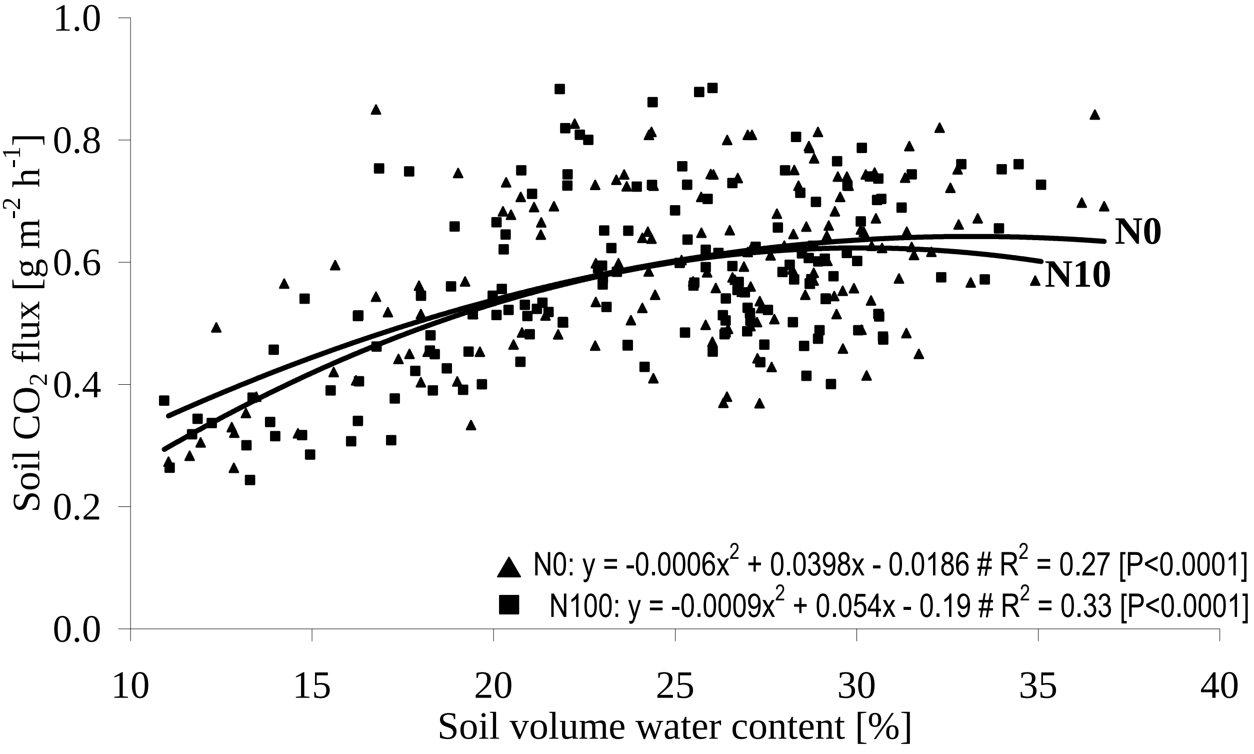


Figure 10 – Soil CO_2 flux plotted against soil temperature and soil volume water content in the two different fertilization levels. The data fit with second-order polynomial regression models. N0: unfertilized; N100: fertilized





3.3. Soil carbon and nitrogen content and crops productions

At tomato harvesting in 2012 the conventional management showed the lowest values in soil TOC and TON with 100% irrigation and in soil TOC with nitrogen fertilization, while in 2013 the conventional treatment showed the lowest values in all fertilization and irrigation treatments(Tab. 1).In 2012 as well in 2013, the treatments with mulching showed on average higher soil TOC and TON (1.44 and 0.14%, respectively) in comparison to the conventional treatment (1.33 and 0.13% of TOC and TON, respectively),which was probably due to the biomass input. The soil TOC tended to increase with irrigation at 100% (+0.01%) and nitrogen fertilization (+0.05%) in the treatments with mulching, while it tended to decrease in the conventional treatment (-0.06 and -0.09 with irrigation at 100% and nitrogen fertilization, respectively). Higher rainfall and lower air temperatures in 2013 compared to 2012 favored the carbon stock in the mulching treatment with WM without nitrogen fertilization and with irrigation reduced to 50%. The higher air temperatures which occurred in 2012 caused the highest levels of soil carbon accumulation in the HV mulching without nitrogen fertilization and in the LP mulching with nitrogen fertilization at all irrigation levels.

The value of HV aboveground biomass as mulching was higher in 2012 than 2013; conversely the LP aboveground biomass was lower in 2012 than 2013. Nevertheless, the percentages of carbon in the aboveground biomasses were similar in both years of experimentation. WM aboveground biomass showed values significantly lower compared to LP and HV in 2012 as in 2013(Tab. 2).In the two experimental years, the N percentage in the HV biomass was, as expected, significantly higher than WM and LP, determining a more favorable C/N rate for mineralization.

The higher amount of rainfall and lower air temperatures which occurred in 2013 generally tended to reduce the yield of the tomato crop (-1.13 kg m^{-2} of FM), which was significantly affected by all treatments applied (mulching, irrigation and nitrogen fertilization) (Tab. 2). Concerning the mulch effect, the management with HV mulch produced a significantly higher tomato yield compared to all other treatments in both experimental years (5.94 and 5.44 kg m^{-2} of FM). The WM mulch produced the worst tomato yield (3.81 and 2.44 kg m^{-2} of FM). By reducing the quantity of irrigation water from 100% to 75% and 50% there was a significant decrease in the tomato yield (-15% and -49% with irr75 and irr50, respectively in 2012 and -12% and -48% with irr75 and irr50, respectively in 2013). The nitrogen fertilization determined an increase in the yield of the tomato crop in both experimental years (+42% and +57% in 2012 and 2013, respectively).

Table 1 - The interaction effect of the mulching x fertilization and mulching x irrigation on soil TOC and TON at tomato harvesting in the two experimental years. Values belonging to the same parameter and year with different letters in rows for mulching effects (upper case letter), and in columns for fertilization and irrigation effect (lower case letter) are statistically different according to LSD (0.05).

	Mulching	Soil TOC (%)		Soil TON (%)	
		N-0	N-100	N-0	N-100
2012	conventional	1.122 Abc	1.070 Ae	0.111 Bab	0.140 Aa
	lacy phacelia	1.171 Bab	1.294 Aa	0.112 Bab	0.143 Aa
	barley straw	1.162 Aab	1.188 Abc	0.116 Bab	0.128 Ab
	white mustard	1.074 Bc	1.155 Acd	0.106 Bb	0.128 Ab
	hairy vetch	1.204 Aa	1.113 Bde	0.121 Aa	0.116 Ac
2013	conventional	1.588 Ab	1.531 Ab	0.143 Ae	0.139 Ab
	lacy phacelia	1.695 Aa	1.716 Aa	0.161 Abd	0.163 Aa
	barley straw	1.701 Aa	1.719 Aa	0.160 Acd	0.154 Aa
	white mustard	1.739 Aa	1.644 Ba	0.197 Aa	0.165 Ba
	hairy vetch	1.702 Aa	1.703 Aa	0.154 Bde	0.165 Aa
		irr50	irr100	irr50	irr100
2012	conventional	1.166 Aab	1.026 Be	0.146 Aa	0.106 Bd
	lacy phacelia	1.191 Ba	1.274 Aa	0.127 Abc	0.127 Aab
	barley straw	1.155 Aab	1.196 Abc	0.115 Be	0.128 Aab
	white mustard	1.117 Ab	1.112 Ad	0.116 Ade	0.118 Ac
	hairy vetch	1.151 Aab	1.166 Acd	0.119 Ace	0.119 Abc
2013	conventional	1.580 Ac	1.539 Ad	0.145 Ac	0.138 Ad
	lacy phacelia	1.711 Aa	1.699 Abc	0.164 Aab	0.159 Ac
	barley straw	1.640 Bac	1.780 Aa	0.152 Abc	0.161 Abc
	white mustard	1.713 Aa	1.670 Ac	0.168 Aa	0.194 Ba
	hairy vetch	1.622 Bbc	1.783 Aa	0.161 Aab	0.159 Ac

Table 2 - The cover crops aboveground biomass and relative C and N content and the effect of the mulching, irrigation, and fertilization on the tomato yield in the two experimental years. Values belonging to the same parameter and treatment with different letters in columns are statistically different according to LSD (0.05).

		Cover crops aboveground biomass (g m^{-2} of dm)	C in the aboveground biomass (%)	N in the aboveground biomass (%)	Tomato yield (kg m^{-2} of FM)
2012	conventional				5.23 b
	lacy phacelia	439 b	39.3 b	0.8 b	4.26 cd
	barley straw				4.02 de
	white mustard	365 b	43.9 a	1.3 b	3.81 e
	hairy vetch	785 a	42.3 a	2.7 a	5.94 a
	irr50				3.02 c
	irr75				5.01 b
	irr100				5.92 a
	N-0				3.85 b
	N-100				5.46 a
2013	conventional				3.56 b
	lacy phacelia	525 a	39.5 b	1.4 b	2.90 d
	barley straw				3.28 c
	white mustard	365 b	41.5 a	1.5 b	2.44 e
	hairy vetch	564 a	41.4 a	3.8 a	5.44 a
	irr50				2.31 c
	irr75				3.86 b
	irr100				4.41 a
	N-0				2.75 b
	N-100				4.31 a

3.4. Soil carbon balance

The cover crop - tomato sequence can be considered to be a cropping system which is part of the crop rotation of a farm. The higher carbon stock observed as average values in organic mulch treatments (1.44% of soil TOC) compared to the conventional treatments (1.33% of soil TOC) (Tab. 1) is probably due to the result of variations of the C inputs in the two types of management. Nevertheless, the amount of the soil CO_2 emissions was greater in the 2013 (4.05 Mg C ha^{-1}) growing season when the soil emitted on average a significantly greater amount of CO_2 than in 2012 (3.40 Mg C ha^{-1}). In fact, significant differences were observed in carbon inputs between the treatments of mulching, nitrogen fertilization and irrigation applied in both experimental years (Tab. 3). Conversely, the carbon outputs derived from CO_2 emissions showed no-significant differences in the various types of management. The highest C inputs were observed in the HV mulch management, while the conventional management showed the lowest carbon inputs in 2012 as in 2013. A net carbon depletion of the carbon balance was observed in the conventionally managed system (input/output ratio <1) while there was an increase in the systems managed with organic mulch from cover crops (1.4, 1.2, and 2.1 in LP, WM and HV respectively in 2012; 1.1, 1.1, 1.9 in LP, WM and HV respectively in 2013). The BS mulch showed a disadvantage concerning the input/output carbon rate in the second year. The nitrogen fertilization determined a significant increase of carbon inputs in the system and a positive balance of input/output which was higher in 2012 than 2013. Significant differences of the applied irrigation treatments were only observed in the second year concerning the input/output carbon rate for which the highest value was found in the irr100.

Table 3 - The effect of the mulching, irrigation, and fertilization on the C balance in the two experimental years. Values belonging to the same parameter and treatment with different letters in columns are statistically different according to LSD (0.05).

		C input by biomasses		C output by CO_2 fluxes		input/output rate	
		Mg C ha^{-1}					
2012	conventional	2.15	e	3.15	a	0.73	d
	lacy phacelia	4.37	bd	3.43	a	1.35	bd
	barley straw	3.69	d	3.19	a	1.21	cd
	white mustard	4.13	cd	3.34	a	1.46	ad
	hairy vetch	7.8	a	3.88	a	2.1	a
	irr50	4.13	b	3.09	b	1.43	a
	irr75	4.58	a	3.66	a	1.32	a
	irr100	4.59	a	3.45	ab	1.36	a
	N-0	4.11	b	3.41	a	1.28	b
	N-100	4.75	a	3.39	a	1.46	a
2013	conventional	1.76	e	4.17	a	0.43	e
	lacy phacelia	4.38	bc	4.18	a	1.09	bd
	barley straw	3.47	d	4.35	a	0.83	de
	white mustard	3.96	cd	3.81	a	1.05	cd
	hairy vetch	6.73	a	3.73	a	1.87	a
	irr50	3.78	b	3.99	a	0.99	b
	irr75	4.11	ab	4.08	a	1.05	ab
	irr100	4.3	a	4.08	a	1.12	a
	N-0	3.85	b	4.14	a	0.98	b
	N-100	4.27	a	3.95	a	1.13	a

4. Discussion

Many studies have been carried out and much is known regarding the beneficial effects on main crops, soil quality, weeds and pest control produced by introducing cover crops into agroecosystems. Although there are limited data and studies concerning the soil CO_2 emissions in vegetable crops grown on organic mulches derived from previously-grown cover crops in the Mediterranean environment.

The effect on soil CO_2 emissions was more pronounced in the tomato grown on organic mulches made from the previous cover crop compared to the conventional management, which was more significant in 2012 than 2013. Nevertheless, no significant statistical differences in cumulative CO_2 emissions were observed among the various agronomical techniques applied to the tomato (mulching, irrigation, nitrogen fertilization), in accordance with other studies carried out in agroecosystems (Wilson and Al-Kaisi, 2008; Mancinelli et al., 2010). A general effect was observed in temporal variations of soil CO_2 emissions throughout the study period, in all mulching treatments applied in 2012 as in 2013. Similarly, soil temperature and volume water content variation during the study period were evident for all three agronomic techniques adopted.

The difference in soil CO_2 emissions observed in the mulching treatments could be possibly due to the organic materials added in comparison to the conventional treatment, and also to the difference in amount and quality of these organic materials among the various type of mulches, which had different effects on the biological properties and organic carbon mineralization of the soil, in accordance with other studies carried out on organic C mineralization (Collins et al., 2000; Iqbal et al., 2009; Mancinelli et al., 2013). In fact, both cover crops and tomato produced a different quantity of biomass as well as different qualitative characteristics of cover crop biomass between one another and the two years of experimentation (Marinari et al., submitted paper). Several studies demonstrated that soil carbon dynamics are highly influenced by plant biomass production. Mancinelli et al (2010) found that the incorporation of cover crop biomass into the soil determined an increase of soil CO_2 emissions. In another study, the switchgrass cultivation showed greater soil CO_2 emissions than corn–soybean rotation, since twice the amount of crop biomass had been incorporated into the soil (Al-Kaisi and Grote, 2007).

In this study the influence of soil temperature and volume water content on soil CO_2 emissions were analyzed, since various studies have found that soil respiration may also depend on the temperature and water content of the soil (Gaumont-Guay et al., 2006). The optimal values of soil temperature and water content which cause the highest levels of activity of the biological processes for each mulching treatment were estimated with a polynomial regression model, which revealed the optimal conditions for soil respiration to be 21.5 °C as the average of the BS, C and

HV, and 18.3 °C of the WM. While the highest soil CO_2 emissions were estimated at a soil volume water content of 28% as the average of the BS, C and HV, at 55% of the WM, and 87% of the LP. The significantly higher levels of soil volume water content, generally observed in the mulching treatments rather than in conventional treatments, enable us to deduce that the mulches applied increased soil water availability which determined a more efficient use of the water, in agreement with Huang et al (2003) and Hatfield et al. (2001). Therefore, the results of this study suggest that in the Mediterranean environment agronomical management practices of soil tillage or no-tillage together with organic mulch could cause variations in soil temperature and water content with consequent beneficial effects on soil respiration.

The results of this study showed that soil CO_2 emission was positively affected by reducing the quantity of irrigation water distributed to reintegrate the evapotranspiration to 75%, while the soil CO_2 emission was negatively influenced when it was reduced to 50%. The increase of the quantity of irrigation water (50%, 75% and 100% of the evapotranspiration) negatively influenced soil temperature and positively influenced the soil volume water content as expected. However, temporal variations in soil CO_2 emission, temperature and volume water content were observed at all irrigation levels. In accordance with Sainju et al. (2006) and Jabro et al. (2008), by increasing the quantity of irrigation water there was an increase in soil water content and consequently in soil evaporation which probably reduced the soil temperature and therefore determined a higher soil respiration reaching optimal levels. In fact, the polynomial regression model showed that the soil CO_2 emission was higher in irr75 (0.67 g m^{-2} h^{-1}) than in irr50 (0.59 g m^{-2} h^{-1}) at the optimal soil temperature of 22 °C.

Throughout the study period in the two experimental years the nitrogen fertilization (100 kg N ha^{-1}) showed a reduction in soil CO_2 emissions except in July 2012; it is not surprising that soil respiration decreased with the application of N fertilizer as observed in other studies (Lee and Jose, 2003; Ding et al., 2007; Al-Kaisi et al., 2008; Wilson and Al-Kaisi, 2008; Peng et al., 2011). In fact, the polynomial model revealed a higher soil respiration in the fertilized treatment than in no-fertilized at same optimum soil temperature (0.65 and 0.61 g m^{-2} h^{-1} in N100 and N0, respectively)and similar values of soil volume water content (0.64 and 0.62 g m^{-2} h^{-1} in N100 and N0, respectively).

After harvesting tomato, the greater soil TOC in the mulching treatments than in the control treatments was probably due to the cover crop biomass and the greater growth of the total tomato biomass. Although C and N contents in cover crop roots and in tomato roots were not actually measured, some studies have shown that cover crop roots can provide up to 40% of the aboveground biomass, influencing soil TOC and TON (Kuo et al., 1997a, 1997b; Sainju et al.,

2003) and therefore determining a major growth of the tomato plants (Sainju et al., 2001) and increase of rhizodeposition of organic materials deriving from the tomato roots (Sainju et al., 2001) causing a further increase of TOC and TON in the soil following tomato harvesting. Although the total carbon was higher in the HV biomass than in LP and WM biomass in both years, soil TOC after tomato harvesting was not always significantly higher compared to other treatments, probably due to the its high N content and lower C/N rate, which facilitates the mineralization of biomass.

Teasdale et al. (2008) reported that hairy vetch biomass mineralizes rapidly after cutting consequently releasing nitrogen which favors subsequent vegetable crops. In this study, although different levels of production were observed in 2012 and 2013, the HV mulch determined the best marketable fruit yields compared to the conventional treatment and the other mulching treatments applied in both years of experimentation, in agreement with Campiglia et al. (2010, 2011). Conversely, although environmental benefits in terms of soil CO_2 emission and soil carbon stock were observed, the WM mulch provided the worst marketable fruit yield, as also reported also by Hartz et al. (2005). A significant reduction in tomato yields was also caused by the lack of N fertilization as well as the reduction of irrigation water. However, while the 50% reduction of irrigation water determined a decrease of almost 50% of the tomato yield, the 25% reduction of irrigation water showed a less than 15% decrease in yield.

In the concept of sustainable agriculture, agronomical techniques applied in the fields should be seen from a holistic point of view as a system of management practices aimed at optimizing the soil functions in agreement with Doran et al. (1994). Consequently, the cultivation of tomato on mulch following a cover crop sequence as proposed in this study can be considered to be a cropping system incorporated into a wider crop rotation. Therefore the soil carbon balance applied in this study enabled us to estimate the carbon contribution derived from the proposed cropping systems within a crop rotation. The soil carbon output intended as the cumulated CO_2 emissions over the tomato growing season was not affected by any of the experimental treatments applied (mulching, irrigation, N-fertilization). Conversely, the highest values were observed in the inputs as well as the input/output ratios in the HV mulching while the lowest values were observed in C (<1). However, the input/output ratios were always >1 in all mulch treatments, except the BS in 2013. The reduction of irrigation determined a decrease in the input/output ratio, which was always >1 by reducing the irrigation water to 75%. These results suggest that by applying organic mulches and/or slightly reducing irrigation water (-25%) could allow the soil to become a temporary carbon sink and therefore represent one of the possible solutions for reducing the soil CO_2 emissions in sustainable agroecosystems, in agreement with Paustian et al. (2000) who proposed the agronomical practices for increasing soil carbon inputs and reducing decomposition as the way for reducing the net CO_2 emissions in the soil. The carbon input/output ratio>1 in the first year and <1 in the second year generally caused by the lack of N-fertilization, made us assume that its rationalized distribution in function of climatic conditions is important for increasing the sustainability of agricultural systems.

5. Conclusions

This is one of the few experimental studies carried out in field conditions where the impact of organic mulching, N fertilization and various irrigation levels are evaluated on soil CO_2emissions, soil carbon content and processing tomato production in the Mediterranean environment. According to our results, the biomass quantity and qualitative characteristics of the cover crop appear to be the main factors influencing the net soil CO_2 emissions and yield in the processing tomato grown on organic mulches in no-tilled soil. There was a strong effect on soil CO_2 emissions in tomato grown on organic mulching derived from the previous cover crops compared to the conventional, and the highest influence was observed with the HV mulch, which determined higher positive values in carbon balance in terms of input/output ratio. The 50% reduction of irrigation water caused both a reduction in the soil CO_2 fluxes and in soil carbon inputs, therefore the 25% water reduction appeared to determine a positive soil carbon balance. The nitrogen fertilization seems to slightly reduce the CO_2 fluxes from soil but strongly increases the soil carbon inputs, therefore a rational use of N fertilization in accordance with climatic conditions is required in order to increase the sustainability of agricultural systems. However, the results highlighted that the fluctuations of soil CO_2 emissions were related to the soil temperature and water content, and the estimated optimal conditions for soil respiration differed according to the agronomical treatment applied.

In short it is possible to state that, although the cumulated CO_2 fluxes did not differ between the various treatments applied, it is possible to achieve environmental benefits in terms of net soil CO_2 emissions and agronomical benefits in terms of processing tomato yield. In the Mediterranean environment, environmental and agronomical benefits in agroecosystems could be obtained by increasing carbon inputs by choosing the most suitable agronomical practice such as the use of hairy vetch mulch on no-tilled soil, a slight reduction of irrigation water (-25%) and a rationalized use of N fertilizer which could potentially shift the C balance in favor of soil C accumulation.

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5.2 CHAPTER

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

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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 LPand5.2 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: Lacyphacelia; white mustard, hairy vetch; mulching; microbial biomass, microbial functional diversity; Mediterranean climate.

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1.Introduction

In the last century, humans have 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):

$$AI = Pi/(Ti + 10) \quad (1)$$

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).

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

2.2. 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. Enzyme 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 byEq. (2) (Bending et al., 2002)

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

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.

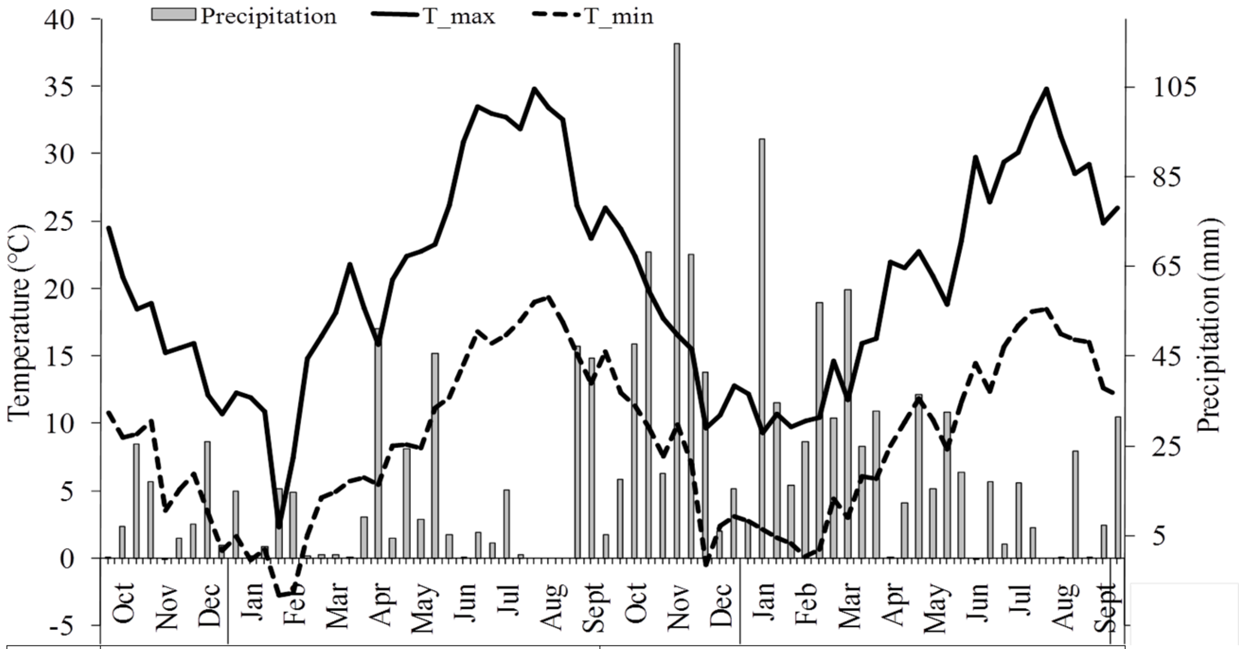
The principal component analysis (PCA) was performed with the JMP 9.0 statistical software package. The soil treatments were grouped using soil chemical and biochemical properties as variables.

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).

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



	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											

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).

Figure 2: Yield of tomato in the two years of mulching experiments (2012 and 2013). Values without common letters are statistically different according to LSD (P < 0.05) (C= Control, LP= LasyPhacelia, WM= White Mustard, HV= Hairy Vetch).

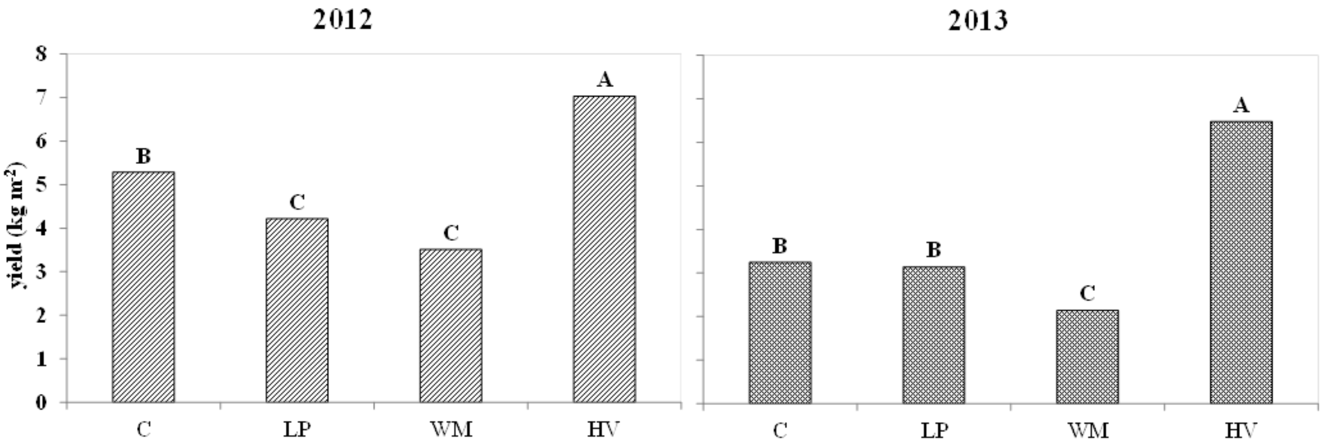


Table2: Soil input from mulching after tomato transplanting(initial effect) after tomato harvest (residual effect), and variation during Tomato growth season (**Δ%**).Dry matter production, input of carbon and nitrogen, C/N ratio of aboveground biomass. In rows values belonging to the same years without 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).LP= LasyPhacelia, 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

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.

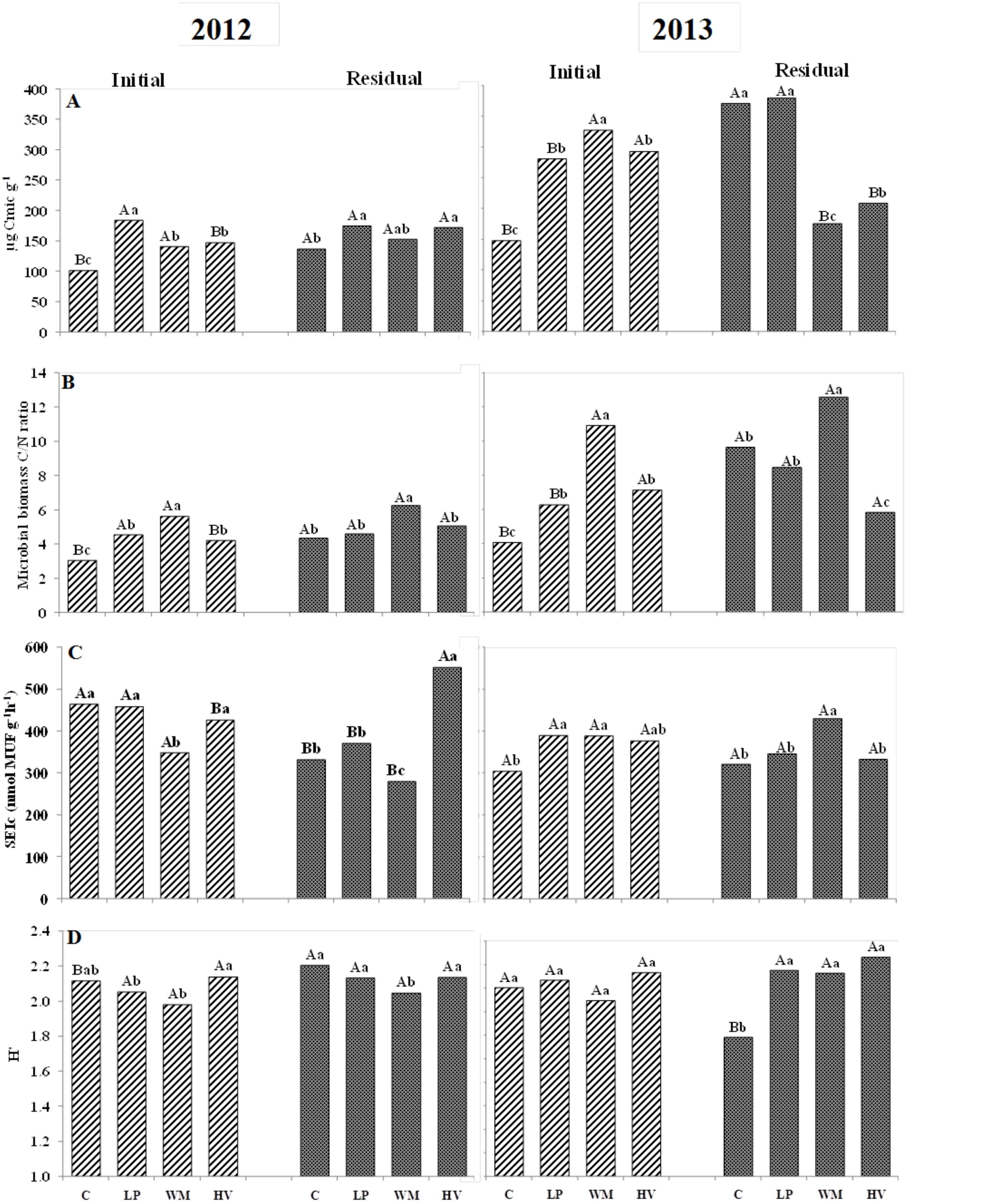
3.4. Soil microbial biomass, its activity and functional diversity

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).

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

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= LasyPhacelia, WM= White Mustard, HV= Hairy Vetch).



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.

Table 4: Person 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 4: Soil extractable carbon 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)

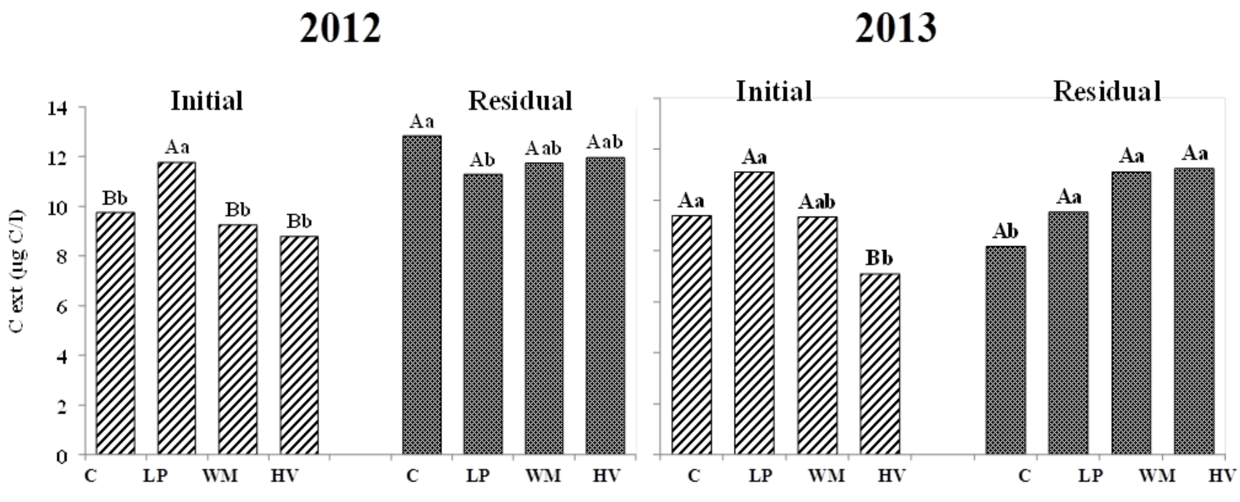
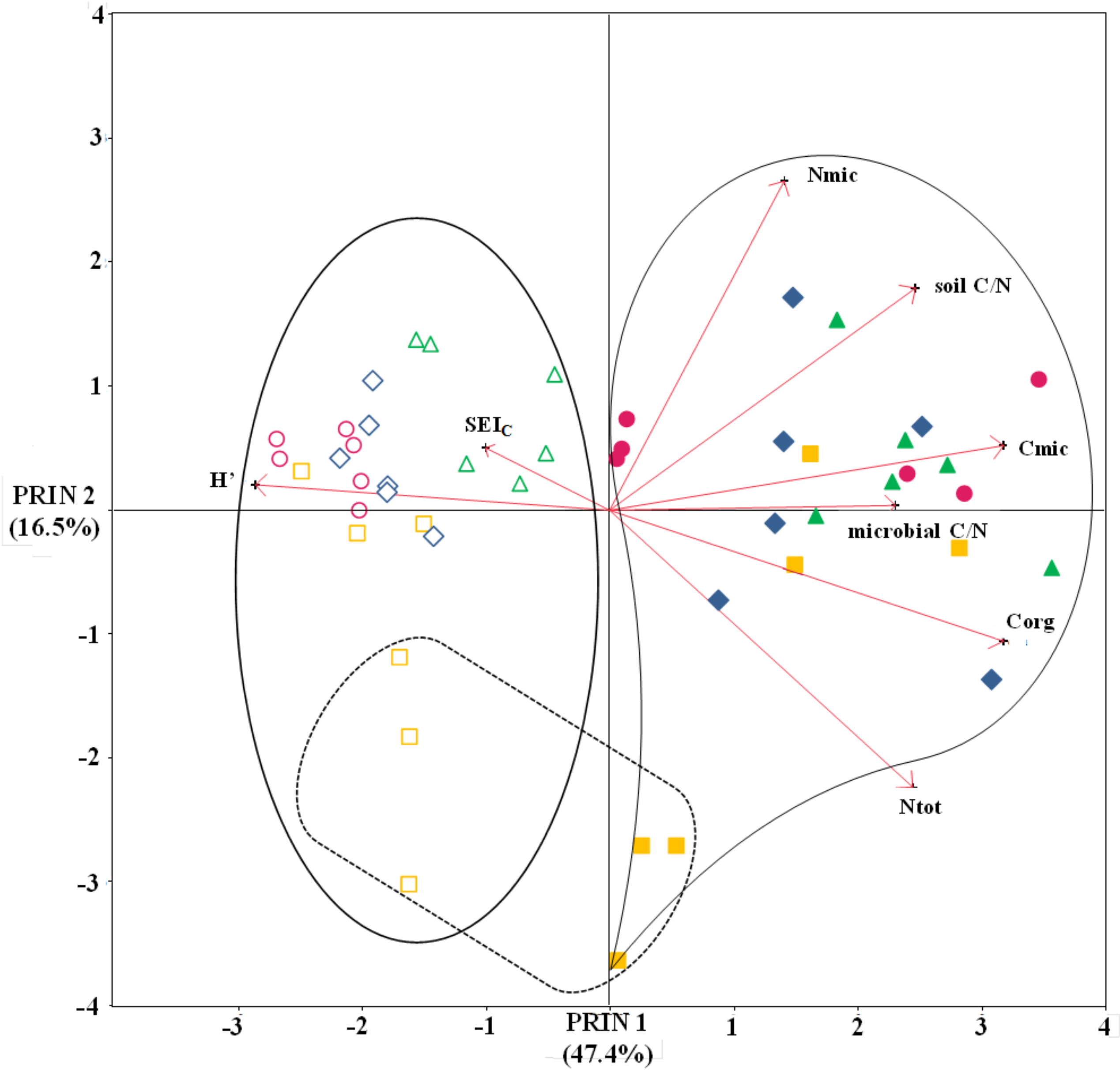


Figure 5: Principal Component Analysis. Symbols are: Circle = C; Triangle = LP; Square = WM; Rhombus = HW. Empty = 2012; Full = 2013. Solid line to distinguish two years groups; dashed line to distinguish soil properties under WM mulching in August.



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.

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5.3 CHAPTER

Do cover crop species and residue management play a leading role in pepper productivity?

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ABSTRACT

There is a growing interest in improving sustainability in Mediterranean vegetable cropping systems by using winter cover crops. A 2-year field experiment was carried out with the aim of assessing the effect of cover crops and their residue managements on the following pepper (*Capsicum annuum* L.) crop productivity. Treatments consisted of factorial combinations of cover crops [hairy vetch (*Vicia villosa* Roth.), oat (*Avenasativa* L.) and no cover], residue management systems [tilled (as green manure, GM), or mowed and placed in strips in the crop row (as dead mulch, M)]. Cover crops were sown in early September and mechanically suppressed in May about one week before pepper transplanting. The pepper was transplanted in paired rows which were placed in the middle of the mulch strips in M treatments, the same geometry was maintained in the other treatments. The pepper crop was not fertilized and mechanically weeded twice only in the inter-row space between the paired rows in order to minimize agronomical inputs. At cover crop suppression, hairy vetch showed the highest aboveground biomass and total nitrogen content (712 g m^{-2} of DM and 197 kg of N ha^{-1}, respectively), while oat exerted the strongest weed reduction. The marketable pepper yield was higher in hairy vetch than oat and no cover regardless of residue management (on average 25.4, 9.9 and 12.0 t ha^{-1}, respectively) probably due to an abundant availability of soil nitrate throughout the pepper growing season. This was confirmed by high and constant values of SPAD readings of pepper plants grown in hairy vetch. Cover crop residues placed in strips suppressed weeds more effectively than incorporated residues. A better nitrogen nutrition and weed control led to an increase in pepper productivity cultivated in vetch mulch strips. Therefore combining legume cover crops and a strip mulching technique to manage cover crop residues, could contribute to effectively increasing the crop productivity and consequently the yield of the following pepper crop. This management package could be considered an important option for organic and conventional growers seeking a way to reduce the agronomical inputs in a winter cover crop – pepper sequence.

KEY WORDS: Green manure; Mulch; Hairy vetch; Oat; Pepper; Weeds; Nitrogen.

1. INTRODUCTION

Winter annual legume and non-legume species have been used for centuries as cover crops in many agricultural systems, but their use over the past decades has been minimal (Hartwig and Ammon, 2002). In fact, modern external inputs such as synthetic fertilizers and pesticides have replaced some cover crop functions (Liebman and Davis, 2000), although the use of chemicals is often associated with environmental and human health risks (Kropff and Walter, 2000; Schroeder et al., 1993). More sustainable agricultural practices are required in order to reduce the occurrence of these problems (Uchino et al., 2011). Therefore, in recent years cover crops have gained popularity as part of modern sustainable agricultural systems (Picard et al., 2010). Although they can provide several benefits (Sarrantonio and Gallandt, 2003; Hartwig and Ammon, 2002), cover crops are often used primarily for providing nutrients and supporting weed control in the subsequent cash crop (Kruidhof et al., 2008). In fact, nitrogen fertilization and weed control are crucial issues in agricultural production systems as a good availability of nutrients and low weed competition enhance the crop biomass and productivity (Blackshaw et al., 2004). However, the quality and quantity of cover crop residues and their management affect nutrient dynamics (Ruffo and Bollero, 2003) and weed establishment in different ways (Kruidhof et al., 2009). Grass cover crop residues show a strong weed suppressive ability partly due to allelophatic potential (Hooker et al., 2008; Weston, 1990), nevertheless they are the most resistant to decomposition mainly due to the high C:N ratio which causes nitrogen immobilization (Ranells and Wagger, 1996) and competition with the following cash crop for the available nitrogen in the soil (Döring et al., 2005). The use of legume cover crops represents a potentially valuable source of nitrogen for replenishing soil nitrogen pools (People and Craswell, 1992), and their residues mineralize rapidly releasing nutrients for the following main crop (Rosecrance et al., 2000). However, the release of nutrients by cover crop residues may favor both crops and weeds depending on their relative growth rate and earliness of competitors (Teasdale and Pillai, 2005). In order to maximize the beneficial effects obtained by the potential release of nutrients, it is important to seek cover crop residue management practices which favor crop competitiveness and reduce weed aggressiveness, resulting in increased productivity of the crop.

When cover crops are incorporated into the soil as green manure (hereafter called GM), their residues mineralize rapidly and release nutrients and allelochemicals (Kuo and Jellum, 2002; Putnam et al., 1983). When cover crops are used in no-tillage systems and their residues are left on the soil surface, as dead mulches (hereafter called M), the residues mineralize more slowly but they make a physical barrier which negatively affects the emergence of weeds (Teasdale and Mohler, 2000).

In the Mediterranean environment, cover crops are usually grown during the winter season and killed in spring prior to planting the summer crop (Teasdale, 1996). The most widely used winter cover crops are grasses which are considered the most suitable catch crops, and legumes are appreciated for their nitrogen supply to the cropping system. Due to the cold growing period, the winter cover crop residues are usually not abundant and they cannot effectively suppress the weeds when left uniformly on the soil surface (Teasdale and Mohler, 2000). Due to the hot growing period of the subsequent cash crop, cover crop residues can mineralize too fast when incorporated into the soil (Ruffo and Bollero, 2003). Therefore, in order to maximize the positive effects associated with their use, it is important to improve cover crop residue management. The objectives of this study were: i) to identify the best cover crop residue management for improving weed control and nitrogen availability in the subsequent crop; 2) to quantify the influence of cover crop species and their residue management on pepper productivity; 3) to find a management package to minimize the agronomical inputs in a winter cover crop – pepper sequence.

2. MATERIALS AND METHODS

2.1. Experimental site and design

The study was carried out at the Experimental farm of the University of Tuscia in Viterbo (upper Latium, 85 km NW of Rome, lat. 42°26’ N, long. 12°40’ E, alt. 310 m a.s.l.). The region has an attenuate thermo-Mediterranean climate (UNESCO-FAO classification) with a mean annual precipitation of 780 mm, mostly concentrated during the autumn and spring seasons, minimum temperatures a little below 0°C in the winter and about 36°C as maximum temperatures in the summer. A winter cover crop-pepper sequence was carried out for two growing seasons (2010/2011 and 2011/2012) in two nearby and homogeneous fields previously cropped with durum wheat (*Triticum durum* Desf.). Average soil characteristics at cover crop sowing (0-30 cm layer) were: 76.3 % sand, 13.3 % silt, and 10.4 % clay; pH 6.9 (water, 1:2.5); 1.32 % organic matter (Lotti) and 0.094 % total nitrogen (Kjeldahl). Each year, a split-split plot experimental design with three replications in randomized blocks was used, the experimental factors were: (i) two cover crop species [hairy vetch (*Vicia villosa* Roth., var. Capello) and oat (*Avena sativa* L., var Donata)] and a no covered soil – (hereafter called no cover); (ii) two different cover crop residue managements [residues mowed and left in strips on the soil surface as organic dead mulch in no-tillage (M treatment) and residues finely chopped and incorporated into the soil in a layer 0-30 cm deep in conventional tillage (GM treatment)]; (iii) two different levels of weed management applied to pepper crop [weed free (hereafter called WF) and weedy (hereafter called W)]. The experimental main plot size was 128 m^2 (16 m x 8 m), the sub-plot size was 64 m^2 (8 m x 8 m) and the sub-sub-plot size was 32 m^2 (8 m x 4 m).

2.2. Cover crop and pepper establishment

In early September of each year (2010 and 2011), the soil was plowed to a depth of 30 cm and fertilized with 100 kg ha^{-1} of P_2O_5 as triple super phosphate. The soil was disked twice (about 15 cm depth) for seed bed preparation. Cover crop seeds were broadcast manually and lightly buried by gentle harrowing on 13 September 2010 and 26 September 2011. The seed rates were the same in both years (60 and 100 kg ha^{-1} for hairy vetch and oat, respectively). After seed bed preparation, the soil in the no cover plots was kept bare by chemical means (glyphosate) until cover crop suppression. On 4 May 2011 and 8 May 2012, hairy vetch and oat cover crops were suppressed as follows: i) the aboveground biomass was mowed at about 4-5 cm above the soil surface, and arranged in strips by a hay-conditioner farm machine with a cut front of 200 cm (M treatment); ii) the aboveground biomass was chopped with a straw chopper and incorporated into the soil using a mould-board plough to a depth of 30 cm and then the soil was disked twice for pepper seedling bed preparation (GM treatment). In M treatment, the mulch strips were 80 cm wide, about 10 cm high, and placed 2 m from center to center of each strip, covering 40 % of the total ground area (Campiglia et al., 2012). At the same time in no cover treatment the soil was: i) left untilled; ii) mould-board ploughed and disked.

On 12 May 2011 and 15 May 2012 one month old pepper seedlings (*Capsicum annum* L.) of the Cleor variety were transplanted by hand. The pepper seedlings were arranged in paired rows at a distance of 40 cm between them and distance of 160 cm between the paired rows. The distance between the pepper plants in the rows was 33 cm, and pepper density was 3 plants m^{-2}. In M treatment the pepper paired rows were placed in the middle of the mulch strips so that the pepper plants were surrounded by a minimum of 20 cm of mulch (Campiglia et al., 2012). The pepper seedlings were over irrigated immediately after transplanting in order to avoid moisture stress. Irrigation water was supplied by drip irrigation tape with 30 cm spaced emitters laid over the mulch layer (in M plots) and the soil surface (in GM and no cover plots) in the middle of paired rows parallel to crop rows. The water input was calculated by evapotranspiration estimated by class A pan evaporimeter and converted by crop coefficients during the pepper growing cycle (Allen et al., 1998), returning 100 % of the evapotranspirated water. Irrigation was stopped one week before the final pepper harvesting. All plots were maintained weed free by mechanical means (rotary hoe) applied twice at 25 and 50 days after pepper transplanting (here after called DAT) between the paired rows All rotary-hoeing operations were carried out in the same orientation with the same driving speed and setting along the pepper rows. Inside the paired pepper rows, the weeds were removed manually whenever necessary (weed free) or left to grow undisturbed throughout the pepper cropping season (weedy). In order to control pepper diseases, repeated copper treatments were applied during the pepper growing cycle. In both years the pepper was harvested twice, on 25 August and 13 September, 2011, and 5 and 20 September, 2012.

2.3. Sampling and Measurements

Cover crop and weed aboveground biomass was separately collected before cover crop suppression. The plants were hand-clipped at the soil surface and sampled using a 50 x 50 cm quadrat (0.25 m^2) randomly placed four times in the middle of each plot. The samples were oven dried (70 °C until constant weight) weighed and their nitrogen (Kjeldahl method) and carbon (Walkley-Black method) contents were measured. One day before pepper transplanting between 11.00 and 13.00 h, the photo-synthetically active radiation flux (PAR, µmol m^{-2} s^{-1}) was measured under the mulch layer at ground level with a linear ceptometer (SS1-UM-2.0, DELTA-T Devices LDT, Cambridge, England) placed ten times in the central mulch strip of each plot. The same measurements of PAR were taken in no mulched plots. Five soil samples per plot were collected at cover crop sowing, at pepper transplanting, at 50 DAT, and at final pepper harvesting in the 0 – 30 cm layer of the weed free plots and mixed together, in order to obtain a uniform sample for soil NO_3-N determination by means of colorimetric methods (Cataldo et al., 1975). In the presence of the pepper crop, soil samples were taken in the middle area of the paired pepper rows. Pepper-N status was evaluated using a chlorophyll meter (SPAD-502, Konica Monolta Holding, Inc.).

SPAD readings were taken on the uppermost fully expanded leaf in 10 plants per plot and averaged (Minotti et al., 1994) every 10 days in the weed free plots. In order to evaluate the effect of the cover crops and their residue management on weed suppression, total weed density and total weed aboveground biomass (over dried at 70 °C until constant weight) were sampled at 50 DAT and at final pepper harvesting using a 50 x 50 cm quadrat randomly placed four times within the paired pepper rows.

The pepper fruits of 12 plants, placed in the middle paired rows of each sub-sub-plot, were harvested twice in order to determine the fresh marketable pepper fruits and their number. At final pepper harvesting, the same plants were cut manually at soil surface level and dried at 70 °C together with their fruits until constant weight in order to determine the total aboveground biomass (fruits + straw).

The relative response index (RRI) was adopted in order to assess the effects of cover crops and their residue management on pepper (RRIp) or weed (RRIw) fitness (Williams II et al.,1998). The RRI is calculated as:

RRI = (Pnc – Pc)/(Pnc + Pc)

where Pnc represents pepper or weed aboveground biomass in the no cover treatments, and Pc represents pepper or weed aboveground biomass in the cover crop treatments. The RRI index expresses plant response to cover crop residue in relation to the bare soil control, RRI higher, equal to or lower than 0 indicates that cover crop residue management decreased, had no effect, or increased plant fitness compared to no cover treatments (Grace, 1995). The response comparison index (RCI), at pepper harvesting, was used to quantify whether the addition of the cover crop has a more positive influence on peppers or on pepper weeds. The RCI index is calculated as:

RCI = RRIw – RRIp

RCI higher, equal to or lower than 0 means that the positive influence of the addition of cover crop in pepper is greater, equal to or lower than the positive influence on the pepper weeds.

2.5. Statistical analyses

The analysis of variance (ANOVA) was carried out for all of the data of the 2-year period using the JMP statistical software package 4.0 (SAS Institute, Cary, NC) considering the year as a random effect. A split plot experimental design was adopted for cover crop characteristics and soil nitrate at cover crop sowing where the year was treated as the main factor and the cover crop as the split factor. The data regarding the weed density and aboveground biomass, the RRI of weeds, the RCI, and the soil nitrate concentration measured throughout the pepper cultivation were analyzed as a split-split plot experimental design, where the year was treated as the main factor, the cover crop as the split factor and the cover crop residue management as the split-split factor. The pepper characteristics were analyzed as a split-split-split plot experimental design, where the year was treated as the main factor, the cover crop as the split factor, the cover crop residue management as the split-split factor, and the weed management as the split-split-split factor. Before analysis, the weed density data were transformed as square root (x + 0.05) and percentages as angular transformation, in order to homogenize the variance (Bartlett's test; Gomez and Gomez, 1984). The data reported in the tables and in the figures were back transformed. 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. Linear regressions were performed for selected parameters.

3. RESULTS

3.1. Cover crop characteristics

Hairy vetch and oat cover crops emerged about 2 weeks after sowing and grew regularly from September to May in both years. At cover crop suppression, hairy vetch was at the late flowering stage, while oat was at the beginning of milk stage. The total aboveground biomass (cover crops + weeds) was higher in 2010/2011 than 2011/2012 (654 vs. 568 g m^{-2} of DM, respectively), and in hairy vetch (713 g m^{-2} of DM) than oat (509 g m^{-2} of DM). Hairy vetch showed a higher weed content than oat (11 vs. 2 % of the total aboveground biomass). As expected the nitrogen content in the cover crop aboveground biomass was much lower in oat compared to hairy vetch (58 vs 197 kg N ha^{-1}), while the C/N ratio was higher in oat than hairy vetch (Table 1).

Table 1. The main effect cover crop and year on total cover crop aboveground biomass and its weed content, total N content, N accumulation in the cover crop aboveground biomass, and C/N ratio at cover crop suppression. Values belonging to the same variable followed by the same letter are not significantly different according to LSD (0.05).

	Total aboveground biomass (g m^{-2} of DM)		Weed content (%)		Total N (%)		Total N (kg ha^{-1})		C/N ratio	
2010/2011	654.20	a	5.82	b	2.04	a	133.46	a	18.0	b
2011/2012	567.50	b	9.16	a	1.85	b	104.99	b	20.8	a
Oat	509.14	b	3.86	b	1.12	b	58.25	b	27.1	a
Hairy vetch	712.56	a	11.12	a	2.76	a	196.64	a	11.7	b

3.2. Pepper characteristics

There were significant interactions among year x cover crop and cover crop x residue management x weed management on pepper characteristics ($P \leq 0.05$, Table 2). All pepper values were similar between the years in no cover treatment, higher values were observed in hairy vetch in 2011 compared to 2012, while an opposite trend was noticed in oat (Table 2). Biomass, marketable yield, and the number of marketable fruits of the pepper were higher in weed free than weedy treatments and in hairy vetch compared to oat and no cover, while they differed in response to the residue management treatments. In weed free conditions, the pepper yield varied from 32.9 to 7.4 t ha^{-1} of FM in hairy vetch and oat green manured (GM) respectively. It was similar in hairy vetch between mulch (M) and GM, in oat it was higher in M than in GM, while in no cover it was higher in tilled (GM) than no-tilled (M) soil. In weedy conditions, the pepper yield was highest in M hairy vetch and lowest in GM oat (25.3 and 4.4 t ha^{-1} of FM, respectively), and it was always higher in hairy vetch than oat and no cover (Table 2). The number of marketable pepper fruits was less variable among the treatments than pepper yield (from 9 to 38 fruits m^{-2}), however it showed a similar trend. A similar tendency was also observed in the total pepper biomass which ranged from 5.3 to 1.0 t ha^{-1} of DM in GM weed-free hairy vetch and GM weedy oat, respectively.

Table 2. The effect of interaction of year x cover crop and cover crop x residue management x weed management on the total pepper aboveground biomass (yield + straw), marketable pepper yield, and number of marketable fruits at final pepper harvesting. Values belonging to the same characteristic without common letters are statistically different according to LSD (0.05) in columns for each cover crops (lower case letter), and in rows for each residue management (upper case letter) of each weed management. M = mulch; GM = green manure

	2011	2012	Weed-Free M	Weed-Free GM	Weedy M	Weedy GM
	Total pepper biomass (t ha^{-1} of DM)					
Oat	1.54 cB	2.08 bA	2.79 bA	1.50 cB	1.98 bA	0.98 cB
Hairy vetch	4.73 aA	3.83 aB	4.96 aB	5.30 aA	4.08 aA	2.79 aB
No cover	2.05 bA	2.47 bA	2.95 bB	3.44 bA	1.17 cB	1.49 bA
	Marketable pepper yield (t ha^{-1} of FM)					
Oat	8.97 cB	10.75 bA	16.99 bA	7.36 cB	10.70 bA	4.39 cB
Hairy vetch	26.48 aA	24.26 aB	31.90 aA	32.86 aA	25.30 aA	11.43 aB
No cover	12.74 bA	11.78 bA	15.73 bB	19.63 bA	5.40 cA	7.07 bA
	Marketable pepper fruits (n. m^{-2})					
Oat	12.4 cB	16.2 bA	27.2 bA	15.2 bB	15.3 bA	9.1 cB
Hairy vetch	31.1 aA	26.5 aB	38.2 aA	35.3 aA	27.4 aA	17.2 aB
No cover	19.6 bA	16.2 bA	28.5 bB	33.3 aA	15.8 bA	15.4 bA

3.3. Soil NO3-N and pepper leaf SPAD values

The soil nitrate concentration measured at different dates in the cover crop – pepper sequence is reported in Table 3. It tended to increase over time, from cover crop sowing to pepper cultivation although there were significant differences among treatments. In general it was higher in hairy vetch compared to oat and no cover regardless the residue management treatments, while it was similar between mulched (M) and soil incorporated residues (GM) except in hairy vetch. In fact, at 50 days after transplanting (DAT) the soil nitrate concentration was higher in vetch GM than vetch M (33 and 25 mg NO3-N kg-1 dry soil, respectively) while at final pepper harvesting the soil nitrate concentration showed greater values in vetch M compared to vetch GM (28 and 24 mg NO3-N kg-1 dry soil, respectively).

The chlorophyll content, which is strongly related to nitrogen availability, was characterized on the basis of SPAD readings. As expected high soil nitrate concentrations corresponded to high values of SPAD readings, in fact peppers grown in hairy vetch residues showed higher values of SPAD compared to peppers cultivated in oat residues and no cover regardless the residue management treatments. In general the pepper grown in oat had similar SPAD values to no cover when the residues were mulched, while the SPAD values were lower when the residues were soil incorporated (data not shown). In weedy conditions at 50 DAT, the SPAD readings of the pepper decreased when the cover crop residues were incorporated into the soil compared to those mulched , a similar trend was observed at pepper harvesting regardless weed management treatments (Table 4). At pepper harvesting a reduction of SPAD readings was also observed in hairy vetch and no cover in the presence of weeds (Table 4).

Table 3. Mean effects of cover crop at cover crop sowing and pepper transplanting, and interaction effect of cover crop x residue management at 50 days after pepper transplanting (DAT) and final pepper harvest on soil nitrate (mg NO_3-N kg^{-1} dry soil) on 0 – 30 cm layer. Values belonging to the same characteristic and treatment without common letters are statistically different according to LSD (0.05), in columns per cover crop (lower case letter) and in rows per residue management (upper case letter). ns = not significant. M = mulch; GM = green manure

	Soil nitrate content											
	At cover crop sowing		At pepper transplanting		50 DAT				At pepper harvesting			
					M		GM		M		GM	
	mg NO_3-N kg^{-1} dry soil											
Oat	16.7	ns	15.0	c	19.3	bA	17.8	cA	20.2	bA	18.1	bA
Hairy vetch	16.2	ns	21.4	a	24.8	aB	33.3	aA	27.8	aA	23.6	aB
No cover	17.5	ns	17.5	b	20.8	bA	23.2	bA	20.0	bA	19.3	bA

Table 4. The effect of interaction of the cover crop x residue management x weed management on the pepper leaf chlorophyll content (SPAD readings) measured at 50 days after pepper transplanting (DAT) and the effect of interactions of the cover crop x residue management and the cover crop x weed management on the pepper leaf chlorophyll content (SPAD readings) measured at pepper harvesting. Values belonging to the same characteristic without common letters are statistically different according to LSD (0.05) in columns for each cover crops (lower case letter), and at 50 DAT in rows for each cover crop residue management (upper case letter) of each weed management and at pepper harvesting in rows for cover crop residue management or weed management. WF = weed free; W = weedy; M = mulch; GM = green manure

	Leaf SPAD value at 50 DAT							
	WF				W			
	M		GM		M		GM	
Oat	54.2	bA	49.2	cB	49.4	bA	42.7	bB
Hairy vetch	57.8	aA	55.5	aA	56.9	aA	50.5	aB
No cover	49.9	cA	52.5	bA	45.6	cA	44.2	bA
	Leaf SPAD values at pepper harvesting							
	M		GM		WF		W	
Oat	45.0	bA	41.8	bB	44.8	bA	42.1	bA
Hairy vetch	52.6	aA	47.9	aB	54.3	aA	46.2	aB
No cover	43.7	bA	42.3	bA	46.7	bA	39.3	cB

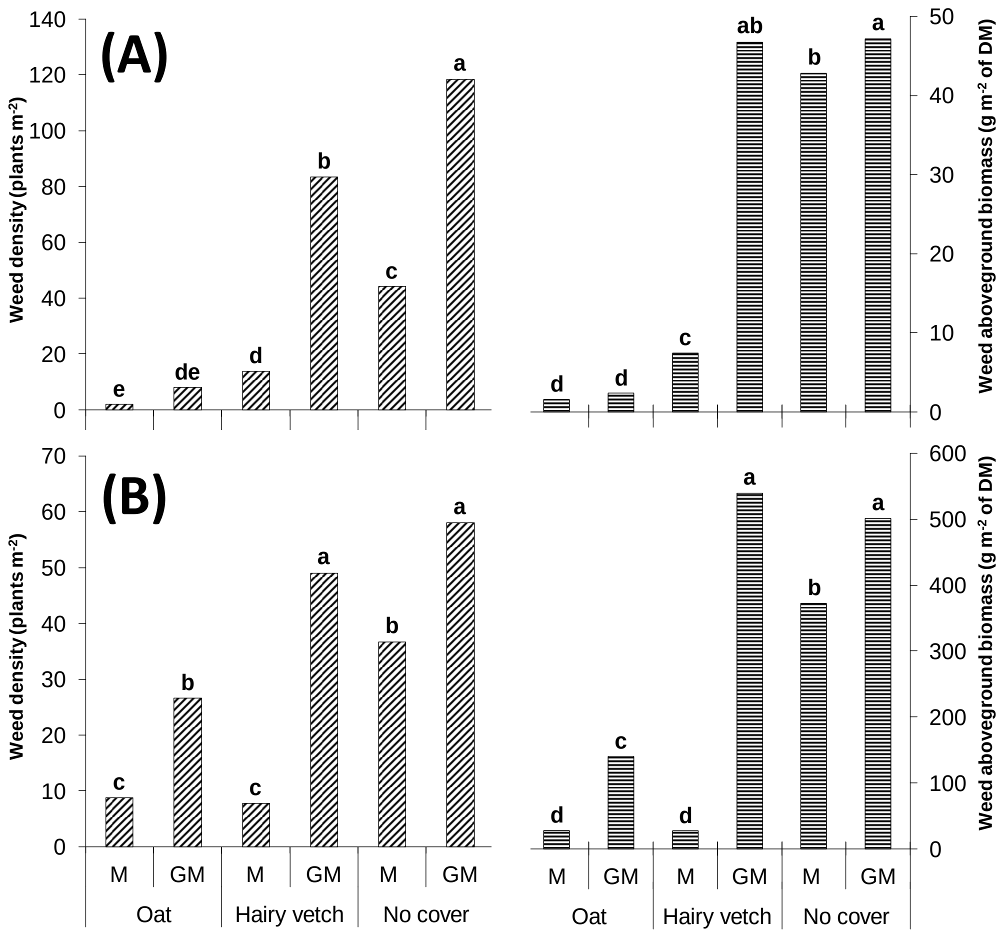
3.4. Weeds in pepper

The analysis of variance showed that both cover crop species and management systems affected the weed density and weed aboveground biomass at 50 DAT and at pepper harvesting ($P \leq 0.001$), moreover there were significant interactions year x cover crop ($P \leq 0.05$, Table 5) and cover crop x residue management ($P \leq 0.05$, Fig. 1). At 50 DAT, both weed density and weed aboveground biomass were higher in 2011 than 2012 (on average 55.5 plants m^{-2} and 30.1 g m^{-2} of DM vs. 33.8 plants m^{-2} and 19.2 g m^{-2} of DM, respectively), except in oat where weed density and weed aboveground biomass showed similar values between the years (on average 4.5 plants m^{-2} and 17.9 g m^{-2} of DM, respectively). Furthermore, weed density and weed aboveground biomass tended to be higher in GM than M treatments (on average 69.9 plants m^{-2} and 32.1 g m^{-2} of DM vs. 19.6 plants m^{-2} and 17.2 g m^{-2} of DM, respectively). In general oat residues led to the lowest level of weed infestation regardless the residue management, while vetch residues led to low weed infestation only when they were mulched (Fig. 1). At pepper harvesting, weed density and weed aboveground biomass followed a similar trend of those observed at 50 DAT (Table 5 and Fig. 1).

Table 5. The effect of interaction of year x cover crop on weed density and weed aboveground biomass at 50 days after pepper transplanting (50 DAT) and at pepper harvesting. Values belonging to the same characteristic without common letters are statistically different according to LSD (0.05) in columns for each cover crop (lower case letter), and in rows for each year (upper case letter).

	50 DAT				Pepper harvesting			
	2011		2012		2011		2012	
	Weed density (n. plants m^{-2})							
Oat	5.1	cA	3.9	cA	2.3	cA	1.5	cA
Hairy vetch	56.3	bA	40.8	bB	31.0	bA	23.1	bB
No cover	105.9	aA	56.6	aB	57.0	aA	32.9	aB
	Weed aboveground biomass (g m^{-2} of DM)							
Oat	19.4	cA	15.9	bA	119.6	cA	47.1	bA
Hairy vetch	24.8	bA	32.0	aA	264.9	bA	301.2	aA
No cover	58.1	aA	36.7	aB	525.3	aA	347.7	aB

Figure 1. The effect of interaction cover crop x residue management on the weed density and the weed aboveground biomass at 50 days after pepper transplanting (A)) and at pepper harvesting (B). Values belonging to the same characteristic and treatment without common letters are statistically different according to LSD (0.05). M = mulch; GM = green manure



While weed density tended to be similar and even less than that observed at 50 DAT, weed aboveground biomass was about 10 times higher, especially where the cover crop residues were soil incorporated and in no cover treatments.

Linear positive correlations were found between the PAR flux measured under the mulch strips at pepper transplanting and the weed density in pepper observed at 50 DAT ($R^2 = 0.91$, $P \leq 0.001$) and at pepper harvesting ($R^2 = 0.87$, $P \leq 0.001$).

3.5. Pepper and weed biomass

The fitness of the pepper and the weeds was affected by all treatments as main effects ($P < 0.001$), furthermore there was an interaction cover crop x residue management x weed management on the relative response index of pepper (RRIp), (Table 6). RRIp ranged from – 0.460 and 0.409 in weed-free M hairy vetch and weed-free GM oat, respectively. In general it was lower in hairy vetch than oat (on average -0.291 vs. 0.104, respectively) and in M than GM treatments (on average -0.194 vs. 0.006, respectively) except in weed-free hairy vetch where it showed similar values regardless the residue management. There were linear negative correlations between the number of marketable pepper fruits and the RRIp in both weed free

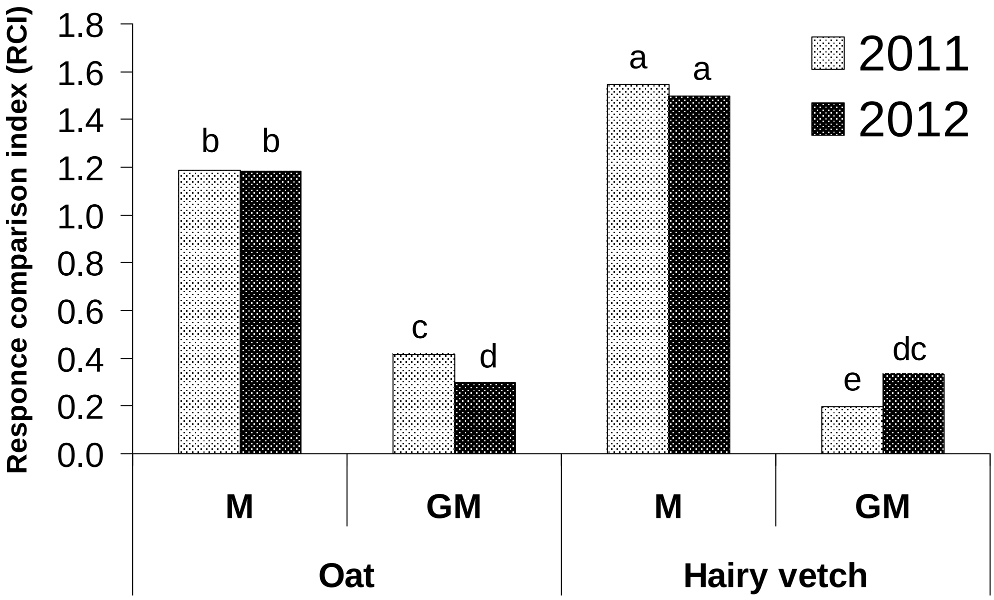
(R^2= 0.87, P $\leq$ 0.001) and weedy (R^2 = 0.85, P $\leq$ 0.001) conditions. The relative response index of weeds (RRIw), which indicated the weed biomass, was significantly affected by the interaction of cover crop x residue management (Table 6). RRIw was always higher in M than GM treatments (on average 0.865 vs. 0.276, respectively), while it was higher in oat compared to hairy vetch only in GM (0.590 vs. -0.039, respectively).

The difference in plant biomass between peppers and weeds, expressed as response comparison index (RCI), was affected by an interaction year x cover crop x residue (Fig. 2). The RCI was constant over the years and always higher in M compared to GM (on average x and y, respectively) and in hairy vetch compared to oat (on average x and y, respectively). In GM conditions the RCI varied between the years and it was lower in 2011 than 2012 in hairy vetch, while an opposite trend was observed in oat (Fig. 2).

Table 6. The effect of interaction of the cover crop x residue management x weed management on the relative response index (RRI) of pepper at pepper harvesting, and the effect of interaction of the cover crop x residue management on the relative response index (RRI) of weeds at pepper harvesting. Values belonging to the same characteristic without common letters are statistically different according to LSD (0.05) in columns for each cover crops (lower case letter) and in rows for each residue management (upper case letter) of each weed management. M = mulch; GM = green manure

	Relative response index (RRI)					
	Pepper (RRIp)				Weeds (RRIw)	
	Weed-Free		Weedy			
	M	GM	M	GM	M	GM
Oat	0.107 aB	0.409 aA	-0.135 aB	0.033 aA	0.874 aA	0.590 aB
Hairy vetch	-0.279 bA	-0.213 bA	-0.460 bB	-0.213 bA	0.856 aA	-0.039 bB

Figure 2. The effect of interaction of the year x cover crop x residue management on the response comparison index (RCI) at pepper harvesting. Values without common letters are statistically different according to LSD (0.05).



4. DISCUSSION

The aboveground biomass and the nitrogen accumulated by cover crops at cover crop suppression were similar to those observed in previous experiments in the Mediterranean environment of Central Italy (Campiglia et al., 2011). Although the cover crop species emerged uniformly in both years, total cover crop aboveground biomass and total nitrogen content were higher in 2011 than subsequent years, probably due to more suitable air temperatures and rainfall which occurred throughout the cover crop growing period (data not shown). At cover crop suppression, oat was more weed suppressive than hairy vetch, probably due to its great ability to act as smother crop (Zerner et al., 2008) capable of releasing allelochemicals and reducing the germination and growth of weed seeds (Putman et al., 1993). Hairy vetch produced a higher aboveground biomass and accumulated more nitrogen than oat, thus showing its great potential as a supplier of nitrogen to the system when used as cover crop. A high nitrogen content in the vetch residues at pepper transplanting led to a high nitrate content both in mulched and green-manured soils. It is interesting to note that the increase of soil nitrate due to vetch residues occurred immediately after cover crop suppression and was higher at 50 days after pepper transplanting in soil incorporated residues than mulched soil. Kuo et al. (1997) reported that hairy vetch residues release about half of their nitrogen content within 2-4 weeks after their incorporation into the soil during the summer period due to the higher nitrogen content and lower C:N ratio than oat (Sainju and Singh, 2001). However, the rapid release of nitrogen from cover crop residues is not always a desirable process due to the difficulty in synchronizing the nitrogen uptake of the crop (Crew and Peoples, 2004), especially in pepper which showed a slow growth in the first period after transplanting. An excess of soil nitrogen availability can determine nitrogen loss by leaching (Sainju et al., 2009). Although Sainju et al. (2006) observed a slight effect of tillage conditions on hairy vetch residue mineralization, in this study the highest values of soil nitrate were found in the green-manured soil compared to mulched soil at 50 days after pepper transplanting. The vetch residues incorporated into the soil were probably subjected to more suitable conditions of moisture and temperature for mineralization than the residues left on the soil surface (Sainju et al., 2006). Therefore, if there is a risk of losing nitrogen via leaching due to a fast mineralization of the cover crop residues incorporated into the soil, leaving the residues on the soil surface as mulch can be a viable management practice for slowing down the mineralization process. In contrast, oat residues did not provide much nitrogen, indeed they may have promoted the sequestration of the available soil nitrogen (Dabney et al., 2001).

Consequently a greater soil nitrogen availability increased the amount of marketable fruits, biomass yields and relative fitness of pepper (RRIp) in the soil previously cover cropped with vetch regardless the residue management treatment. The SPAD values clearly showed that pepper plants cultivated after vetch cover crop had higher chlorophyll content than pepper plants grown after oat or without a cover crop as observed by Abdul-Baki et al. (1996). For this reason legume cover crops seem to be advantageous compared to other cover crop species for improving soil nitrogen fertility and subsequent no-legume crop fitness by reducing or cutting out nitrogen fertilizer inputs.

Although vetch cover crop always led to an increase of the pepper biomass (RRIp < 0), it also caused an increase of the weed biomass. In fact, when the vetch residues were incorporated into the soil, they provided a relatively weaker suppressive effect on weed emergence and growth and the weeds took advantage of the presence of vetch residues (RRIw < 0). On the contrary, the cover crop residues placed in mulch strips always led to a reduction of the weed biomass. The compact and thicker mulch layer left on the soil surface probably inhibited the germination of many annual weed species through a decrease in soil temperature fluctuation and a reduction in light penetration. The proposed mulch strip system whereby cover crop residues are concentrated within the crop row and tillage performed only between rows, in our case between the paired rows, enabled us to increase residue levels within rows by using the natural quantities of residue which are often insufficient in themselves for providing adequate weed suppression if left uniformly distributed across the field. These higher levels of biomass residues are more likely to provide weed suppression in an area where it is more difficult to control weeds mechanically, while this system leaves an un-mulched area between the rows where it is easy to control weeds mechanically. As proof of this, wherever the cover crop residues were soil incorporated and the weeds in pepper were controlled only by two passages of a rotary hoe in the space between the paired rows, the weed flora was abundant and mainly dominated by annual photoblastic weeds such as *Amaranthus retroflexus* L., *Chenopodium album* L., *Portulaca oleracea* L., *Solanum nigrum* L. (data not shown). Only a few weeds escaped the mulch control in the hairy vetch mulch strips and although they individually accumulated a consistent quantity of aboveground biomass, the total weed biomass was always lower than the pepper biomass. Consequently the response comparison index (RCI) at pepper harvesting indicated that the relative fitness of pepper was much greater than the fitness of the weeds when the pepper was cultivated in mulched conditions. Considering those crop biomass and crop yields were closely and positively related, an increase of RRI of pepper corresponded to an increase in pepper yield.

It is important to note that replacing the traditional incorporation of cover crop residues into the soil with the conversion of cover crop residues in dead mulch left on the soil surface, would lead to a reduction of agronomical inputs. In fact, the cover crop aboveground biomass was arranged in mulch strips with only one farm operation, while for preparing the pepper seedling beds it was necessary to chop the cover crop biomass, incorporate it into the soil and then disk the soil twice. Moreover the excellent weed suppression provided by the thick mulch layer on the pepper row reduced the area where the weeds were to be controlled by mechanical means.

5. CONCLUSIONS

The results of this experiment show that legume cover crops, such as hairy vetch, should be considered preferable to other no-legume cover crops in order to enhance soil nitrogen availability in the system. The placement of cover crop residues in strips, where subsequent vegetable seedlings are transplanted, seems to be a suitable approach for controlling the weeds in the intra-row space, while the weeds can easily be suppressed by a mechanical means in the inter-row space. Therefore, combining legume cover crops with a strip mulching technique to manage cover crop residues could contribute to effectively increasing biomass and the yield of the following vegetable crop. This management package could be considered to be a profitable option for organic and conventional growers seeking a way to reduce the agronomical inputs in a winter cover crop – pepper sequence.

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6. PERSONAL REFLECTIONS

The deep structural changes knowing at the global level (population growth, economic growth accelerated in some emerging countries, increased consumption of energy on a global scale, etc...) impose a more careful evaluation of the long-term sustainability of current dynamics of socio-economic development. The pressure on natural resources in different country of the world, in fact, is very strong and the attention on efficient use of natural resources and on mitigation of economic development effects is growing. Specifically, the greater concern is the negative impact that human activity has on the Earth's climate. The Climate changes in fact is imposed in the last decade to the attention of the governments of major industrialized countries, becoming one of the most important issues of the international political agenda. These changes are causing an increasing loss of biodiversity and an increasing pressure on terrestrial and marine ecosystems. For the important role of CO_2 cycle, soils and plant biomass are crucial in mitigating climate change because they are able to store carbon captured by plants through photosynthesis. The soil is the largest sink of organic carbon between all terrestrial ecosystems and can acts both as a source of emission and as a sink of carbon sequestration depending on local and management conditions. Several authors, analyzing the potential for GHG mitigation by farm management practices, have concluded that about 90%comes from the sequestration of carbon in soils, in particular by sustainable agriculture that generally reduces vulnerability to climate change (IPCC, 2007).

In this dissertation an ecological approach was applied at different crop production (tomato and pepper) to investigated three main aspects related to crop management system: soil C cycle, soil quality and microbial biomass, and weed management.

This study resulted to be very interesting on the aspect concerning CO_2 emissions from agricultural soil in Mediterranean environment, because is one of the few experimental researches where organic mulching, N fertilization and different irrigation levels were studied all together in field conditions. The net soil CO_2 emissions and tomato yield result to be influenced, overall, by the quality and quality of the cover crop biomass. Among the different treatments applied, Hairy Vetch showed the bigger positive values in net soil carbon balance in terms of input/output ratio. Among three irrigation levels, the irr75 was the one that determined a positive soil carbon balance. It necessary, instead, to use appropriate N fertilization applications in function of the climatic condition thus they can strongly increases soil carbon inputs and slightly reduce the CO_2 fluxes from soil. Then, the agronomic choices result to be very important to maximize yield and can be used as a means to CO_2 reduction and soil C storage increase.

During the period of study, the different climatic conditions affect soil microbial biomass, its activity and other analysis regarding the functional activity of the soil. In particular, precipitation and temperature regulated the decomposition rate of mulch and soil carbon availability, as K_2SO_4extractable-C, which promoted microbial functional diversity. Among all the cover crops selected, Lacy Phacelia and White Mustard are those that more are sensitive to climatic condition: Lacy Phacelia mulching in terms of aboveground decomposition rate and tomato yield production, White Mustard mulching in terms of soil carbon and nitrogen storage. This information are important to evaluate which plant have the best performances on crop yield and soil quality.

How concerns the weed issue, the management package illustrated in the experiment had have positive effects on weed control, biomass produced and on nitrogen availability. In fact the placement of cover crop residues in strips seems to be a suitable approach for controlling weeds in the intra-row space. Moreover the use of legume cover crops, such as hairy vetch, should be considered preferable to other no-legume cover crops in order to enhance soil nitrogen availability in the system. Then combining legume cover crops with a strip mulching technique to manage cover crop residues could be a profitable option for organic and conventional growers seeking a way to reduce the agronomical inputs in a winter cover crop–pepper sequence.

The results of the experiments carried out for this dissertation have shown that ecological knowledge on agroecosystem could be important to resolve, at list partly, the anthropogenic footprint on environment. A right soil management, that it results from less soil disturbance, can improve soil fertility and decrease man-made inputs due to machinery, fertilizers and pesticides use.From the point of view of environmental policy, the ecological approach positively affects on CO_2 emission decreasing from soil to atmosphere.

However, the results observed in the experiments suggest that by integrating ecological approaches with farming practices it is possible to improve the effectiveness of agronomic management. Above all, further research is required in order to develop the use of cover crop residues as organic dead mulch, overall in Mediterranean environment. For this purpose new technologies and studies are required in order to improve knowledge about which plant species is better to use as dead mulch and in which vegetable crop system they can use. Since the soil could be a potential means to CO2 emissions reduction, more soil analysis are necessary to better understand what are the agronomic practices able to increase soil C storage and improve soil quality and health.

7. CURRICULUM VITAE

Paola Brunetti was born on the 20th of February, 1979 in Rome, Italy. In 1998 she graduated from secondary education at high school science in Montefiascone (VT). She began Forestry and Environmental sciences course at University of Tuscia in 1998 and ended her MSc course in February of 2005 with final dissertation on the “Analysis of Red Spruce growth in a chronosequence of Tharandt forest (Germany)” graduating *cum laude*. After her graduation, she started agronomic consultant as freelance after accrediting professional Forestry and Environmental since 2006 by Ordinedei Dottori Agronomi e Forestali della Provincia di Viterbo. In November 2010 she achieved a one-year Master’s degree in "New Jobs: The environment as an opportunity"- specific address - "carbon credits market: recognition and certification”. During the course specific subjects have been addressed like climate change and pollutions, national and European environmental policy, agro-forest carbon credit measurement and certification, the Kyoto protocol and its mechanisms: Clean Development Mechanisms (CDM), Joint implementation (JI) and ETS (emission trading systems).

In February 2011, she started the Ph.D. course in Environmental Sciences at Tuscia University, Viterbo (VT), under supervision of dr. Roberto Mancinelli, Ph.D. The Ph.D. project focus its attention on the following themes: effect of cropping system management(conventional and organic) on CO_2 fluxes from the soil to the atmosphere, soil carbon cycle, soil fertility and weeds control.