Tesis doctoral

**Valorización Agronómica de Restos de Pimiento:
Influencia en las Propiedades del Suelo y
Producción de Brócoli.**

**Agronomic Recycling of Pepper Crop Residues:
Their Influence on Soil Properties and Broccoli
Crop.**

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producción de brócoli”

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RESUMEN

La producción agrícola en la Región de Murcia ocupa un lugar muy importante en la economía regional y especialmente en la zona del *Campo de Cartagena*. Esta intensísima agricultura conlleva la generación de un problema de contaminación difusa debido a un excesivo uso de fertilizantes químicos. Además, esta actividad también genera una gran cantidad de residuos post-cosecha resultantes de los ciclos productivos, especialmente de cultivos bajo invernadero. En esta tesis se propone la valorización de los restos de cosecha como enmienda orgánica del suelo para intentar mejorar sus propiedades y reducir el uso de fertilizantes químicos.

Los residuos de cosecha poseen un gran potencial como enmienda en suelos dedicados a producción agrícola ya que representan una fuente de materia orgánica y de nutrientes, lo que justifica el estudio de sus efectos en el suelo una vez incorporados. Por ello se ha evaluado, bajo condiciones controladas, la influencia a medio plazo de diferentes dosis de residuos de pimiento (muy comunes en el *Campo de Cartagena*) sobre la dinámica del carbono y del nitrógeno. Los resultados resaltan la importancia de la dosis cuando se aplican restos vegetales, ya que la adición de los mismos a dosis bajas (2 g kg^{-1}) causó una inmovilización de N mientras que a dosis más altas ($3 \text{ and } 5 \text{ g kg}^{-1}$) se produjo una mineralización de N neta de 13.7 y 56.7 mg kg^{-1} .

También se ha estudiado el efecto de la aplicación de residuos frescos de pimiento sobre un cultivo de brócoli, en comparación con el uso exclusivo de fertilizantes químicos. Los objetivos fueron evaluar el efecto de dichos residuos sobre las propiedades físico-químicas y bioquímicas del suelo relacionadas con el ciclo del carbono y fertilidad del suelo, así como sobre la calidad, nutrientes y producción en un cultivo de brócoli. Los resultados mostraron que la combinación de altas tasas de aplicación de restos vegetales con una mínima fertilización química mejora las propiedades edáficas incrementando entre otros la biomasa microbiana y algunas actividades enzimáticas si se compara con el control. Además, existen efectos positivos sobre la calidad del brócoli sin que exista un impacto negativo sobre su producción.

Finalmente se ha estudiado el *turnover* de carbono mediado por las poblaciones microbianas utilizando técnicas de marcaje isotópico (^{13}C) para conocer la cantidad de carbono que se incorpora a las diferentes fracciones del suelo, considerando la influencia de tres restos vegetales y dos formas de manejo, orgánica y convencional. Los resultados indicaron que el ^{13}C , incorporado como glucosa fue asimilado en mayor medida por la

biomasa microbiana en los suelos enmendados con restos vegetales. Sin embargo, la adición de restos también causó una mayor mineralización del ^{13}C orgánico comparado con los controles, por lo que su incorporación en el suelo, tanto en la fracción lábil como recalcitrante, fue menor en dichos suelos enmendados con restos vegetales.

ABSTRACT

The agricultural production in the Murcia Region occupies a very important place in the regional economy and especially in the agricultural land of *Campo de Cartagena*. The intensive agricultural production has resulted in a diffuse pollution problem due to an excessive use of chemical fertilizers and a large amount generation of post-harvest crop residues resulting after the growing season, especially from greenhouse crops. In this thesis the recycling of crop residues applied as soil organic amendment is proposed to improve soil quality and reduce the use of chemical fertilizer.

The crop residues have a great potential as organic amendment in agricultural lands representing a source of both organic matter and nutrients; however it is necessary to study their effect when they are applied in soil. We have evaluated, under laboratory conditions, the medium-term influence of pepper residues (very common in *Campo de Cartagena*) applied at different rates on carbon and nitrogen dynamics in an agricultural soil. The results highlight the importance of dose when applying crop residues. The addition of pepper residues at low doses (2 g kg^{-1}) produce a net N immobilization in soil, while higher doses (3 and 5 g kg^{-1}) show a net N mineralization of 13.7 and 56.7 mg kg^{-1} .

Was also studied the effect of pepper crop residues application on broccoli production compared with the exclusive use of chemical fertilizers. We focused on the effect of crop residues on soil physico-chemical and biochemical properties related to the carbon cycle, soil fertility and broccoli nutrient, yield and quality. The results showed that large application rates of crop residues with a minimum chemical fertilization improved chemical and biochemical soil properties increasing, among others, microbial biomass and some enzymatic activities compared with control. Likewise, positive effects on broccoli quality were found with no negative impact on broccoli yield.

Finally, the carbon turnover mediated by soil microbial populations was assessed using isotopic labeling techniques to know the amount of labeled C (^{13}C) incorporated into different organic pools considering the influence of three plant materials and under two management systems. Results indicated that the ^{13}C -glucose was much more assimilated into microbial biomass C in plants-amended samples. However, plants addition also caused a positive priming effect and enhanced mineralization of organic ^{13}C , thus C from glucose was incorporated in a lower proportion into soil recalcitrant and labile carbon in plants-amended soils.

SCIENTIFIC PAPERS

On the basis of the present thesis the following manuscripts have been prepared:

1. Moreno-Cornejo, J; Zornoza, R; Faz, A and Rosales, RM (2013)
Effects of pepper crop residues and inorganic fertilizers on soil properties relevant to carbon cycling and broccoli production
Use and Soil Management, doi: 10.1111/sum.12062
2. Moreno-Cornejo, J; Zornoza, R and Faz, A
Pepper crop residues and chemical fertilizers effect on soil fertility and biochemical properties for a crop of *Brassica oleracea*
Spanish Journal of agricultural research (under review)
3. Moreno-Cornejo, J; Zornoza, R and Faz, A
Carbon and nitrogen mineralization during decomposition of crop residues in a calcareous soil
Geoderma (under review)
4. Moreno-Cornejo, J; Doane, T; Horwath, WR; Zornoza, R and Faz, A
Use of ¹³C-glucose to study turnover of C under the influence of three plant materials and two soil managements
Biology and fertility of soils (under review)

Additionally, a book chapter has been prepared:

Moreno, J and Faz, A (2009)

Dinámica nutricional en Agrosistemas del campo de Cartagena. Estudios comparativos entre valorización de restos vegetales y fertilización mineral para la protección del Mar Menor. In: El Mar Menor, Estado actual del conocimiento científico. 2009. Pag: 285-320. Instituto Euromediterráneo del Agua (ed). Murcia 2009, España. ISBN: 978-84-966326-8-7.

Dedico esta tesis a mis padres, Rafaela y Juan Antonio quienes
me inculcaron el amor por el trabajo bien hecho

I dedicate this thesis to my parents, Rafaela and Juan Antonio, who
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CHAPTER 1

RELEVANCE AND

OBJECTIVES

1.1 RELEVANCE

In the Murcia Region, as a result of agricultural activity, a large amount of plant residues are produced. In the agricultural area of *Campo de Cartagena* this is a particular problem, especially with crop residues from greenhouses, most of them dedicated to pepper cultivation, which causes a very punctual-high volume of residues at the end of the growing cycle. Sometimes residues are incorporated into the soil as an amendment but in most cases the common practice is burning. This involves the loss of the possibility to be recycled as a source of nutrients and organic matter in soil. Another known effect of modern agricultural systems is the leaching of nutrients due to excessive use of chemical fertilizers leading to water pollution.

One of the main problems in the Mediterranean agroecosystems is the loss of soil quality owing to drastic decreases in organic matter content. We can take advantage of using crop residues as organic amendments to improve soil quality and like a source of nutrients to reduce the use of chemical fertilizers.

Therefore, it is necessary to carry out experimental studies to assess the beneficial or harmful effects of the use of plant residues as amendments on physical, chemical and biochemical soil properties, quality and turnover of organic matter, and quality and yield of the crop.

1.2 OBJECTIVES

The main purpose of this thesis was to study the carbon and nitrogen turnover of fresh crop residues once they are used in soil as organic amendments, and assess their effects on soil quality, soil fertility, crop quality and nutritional status and yield. To achieve this general objective, three different experimental surveys were carried out.

The first experiment was aimed at evaluating, under laboratory conditions, the medium-term influence of pepper residues applied at different rates on carbon and nitrogen cycles in an agricultural soil, to assess the degree of decomposition, release of nutrients, and incorporation of organic carbon into the soil.

A second comparative field study was carried out on the basis of the development of a broccoli crop using several application rates of chemical fertilizers and pepper crop residues during two crop cycles. The hypothesis was that the use of pepper crop residues as organic amendment may lead to improvements in soil quality and fertility, and

reductions in the need of N fertilization, preserving broccoli yield quantity and quality. Several aims were formulated for this study:

- To determine the effect of pepper crop residue on soil properties related to the carbon cycle
- To assess the effect of pepper crop residues on soil fertility
- To analyze the effect of using pepper crop residues on broccoli yield, quality and nutritional status
- To assess if there are relationships between soil properties and broccoli yield and quality
- To establish the most suitable application rates of pepper residues to increase soil quality and fertility by maintaining high yield and crop quality.

The third experiment was aimed at assessing, under laboratory conditions, the soil carbon turnover using ^{13}C -glucose as tracker, and the effect of application of different crop residues on this turnover.

1.3 STRUCTURE OF THE THESIS

The background and state of art are explained in Chapter 2 including: an introduction of agriculture in Murcia Region and Campo de Cartagena, main environmental problems derived from agriculture, the advantages of using crop residues as amendment in agricultural lands and a brief introduction about isotope techniques used in soil C studies.

The experimental results obtained during the development of the study are organized in Chapters 3, 4, 5, and 6 of this document. Each chapter is written in the form of a scientific paper and consists of the following sections: abstract, introduction, materials and methods, results, discussion and conclusions.

Finally, chapter 7 includes the main scientific conclusions of the work and Chapter 8 the literature cited in this thesis.

CHAPTER 2

BACKGROUND AND

STATE OF THE ART

2.1 AGRICULTURE IN THE MURCIA REGION

In the Murcia Region of Spain, where there is a dichotomy between high agricultural production and scarce water resources, agriculture occupies a very important place in the regional economy and especially in the agricultural land of *Campo de Cartagena* in which our study is framed.

The evolution of agricultural land in Murcia Region, demonstrated in table 2.1, has been enormous. In the early 1970's, food production was based on non-irrigated agriculture. After the increase in the use of groundwater and the construction of the Tajo – Segura aqueduct, opened in 1980, the land use changed, and new and modern irrigation systems were established (Álvarez-Rogel, 2006). Thus, between 1976 and 1996 the surface increased about 35,000 ha of irrigated land (vegetable and woody crops), which directly related to water availability, showing great potential for growth. Surface increases were much more pronounced in the period 1976-1986. A great expansion of vegetables production was recorded, being about 8500 ha in the first period and 2000 ha in the second.

Table 2.1 Historical evolution of agricultural surface in Murcia Region. Data in ha.

Crop	1976		1986		1996	
	Irrigated	Dry	Irrigated	Dry	Irrigated	Dry
Grains	11,134	98,239	19,539	104,083	8,412	87,449
Legumes	620	292	420	72	252	73
Potatos	5161	55	5094	0	2345	0
Industrial	6042	18	11,140	685	4515	108
Fodder	11,394	160	6610	16	2572	286
Vegetables	21,477	1,409	29,680	280	31,983	5
Citrus	16,039	0	26,877	0	31,893	0
Orchards	22,963	1,749	23,367	690	30,971	540
Dry fruit	5456	40,882	9496	53,998	12,918	59,634
Vineyard	4434	56,998	6584	69,889	7415	34,633
Olive	13,294	13,294	9,858	9,858	14,117	14,117
TOTAL	118,014	213,096	148,665	239,571	147,393	196,845

Modified from Avellá et al. (2012).

Since 1996 the total cultivated surface in Murcia Region has plateaued around 190,000 ha (being 187,064 ha in 2011). However, vegetables surface (figure 2.1) continued increasing from 31,938 in 1996 to 48,837 ha in 2011 (Econet, 2013).

2.2 VEGETABLE PRODUCTION IN THE CAMPO DE CARTAGENA

Campo de Cartagena is a semiarid Mediterranean agricultural area that extends 1200 km². Today, the *Campo de Cartagena* is one of the main horticulture growing areas in Europe (Álvarez-Rogel et al., 2006). The main crops in this irrigated area are: vegetables (lettuce, broccoli, melon and artichoke), citrus (lemon and orange trees), and greenhouse crops (pepper and flowers) with an occupied surface that change every year (figure 2.2).

Figure 2.1 Vegetable distribution in Murcia Region. From Robledo et al. (2006). Protected crops are crops grown in greenhouses and plastic tunnels.

Figure 2.2 Surface distribution in *Campo de Cartagena*. From Carreño et al. (2004).

Increments in agricultural areas has stagnated in recent years, due to the lack of water resources and increased production costs. Despite this stalemate, in the *Campo de Cartagena*, as figure 2.3a shows, vegetable crops continue to rank first in terms of cultivated surface for irrigated, plastic tunnels and also in greenhouses, occupying 95, 93 and almost 97% of those surfaces, respectively, in 2009, according to Econet (2013) (figure 2.3a).

2.2.1 BROCCOLI CROP

Through research within the last few years, broccoli (*Brassica oleracea* L. var. *italica*) has been considered a health-promoting food. The healthy food attributes assigned to broccoli are a consequence of its high contents of bioactive phytochemicals such as nitrogen-sulfur compounds (glucosinolates and isothiocyanates), phenolic compounds (flavonoids) and nutrients (vitamin A, B1, B2, B5, B6, C, E, K and essential nutrients: N, K, Ca, Mg, Cu, Zn, Fe and Se) (Dominguez-Perles, 2010). Its consumption is beneficial for the prevention of chronic disorders, such as carcinogenic and cardiovascular pathologies (Premier, 2002; Jeffery et al., 2003).

Broccoli is cultivated in many European and American countries. The broccoli trade represents a major agro-economic activity in Murcia Region, where the volume of land dedicated to growing broccoli has increased twelve-fold for 33 years when was first introduced. In 1980, the area used for growing this crop was 878 ha, and now in 2013 it reaches 10,781 ha. The acreage given to broccoli is the second largest in the Region (after lettuce) and it is the third largest agricultural activity, after lettuce and lemons, with a higher value in exports (Murciatoday, 2013).

In *Campo de Cartagena*, almost 90% of broccoli is planted under drip irrigation and the rest in plastic tunnels. Nothing is cropped in greenhouses (Econet, 2013). Broccoli crop requires high supply of N for optimum yields (Karitonas, 2003). We used the local law regulation Order of 26 November 2007 “Producción integrada de brócoli” lately actualized (BORM 2012, Nr 118), the recommendations of the Sistema de información Agraria de Murcia (SIAM) and Order of 3 December 2003 “Código de Buenas Prácticas Agrícolas” (BORM 2003, Nr 286) to establish the fertilization doses for our experiments (table 2.2).

Table 2.2 Sources for establishing the optimal dose for growing broccoli.

Source	Nutrients (kg ha ⁻¹)				
	N (%)	P ₂ O ₅ (%)	K ₂ O (%)	Ca (%)	Mg (%)
Order 26 November 2007 ^a	245	100	300	35	15
Order 3 December 2003 ^b	225-275	-	-	-	-
SIAM	285	110	437.5	183	35

^a For a yield of 20 t ha⁻¹. ^b Applied as fertigation.

2.2.2 PEPPER CROP

Pepper (*Capsicum annum* L.) is an important horticultural crop in the Mediterranean area and is considered an excellent source of bioactive nutrients. Ascorbic acid (vitamin C), carotenoids and phenolic compounds are its main antioxidant constituents (Flores et al., 2009). Carotenoids help to prevent a broad range of cancers and cardiovascular diseases (Tavani and La Vecchia, 1999).

It is in the Southeast of the Iberian Peninsula (Murcia and Almería) where more than 76% of national pepper was produced in 2005. Murcia, the second province after Almería, contains the largest area of pepper in greenhouses (around 12%).

Almost 100% of regional pepper is produced in *Campo de Cartagena* area (figure 2.3a). This crop occupied in *Campo de Cartagena* 1527 ha in 2009, most of it mainly developed in greenhouses (95 %). Vegetable production in greenhouses has expanded tremendously in recent decades. Among all vegetable crops, pepper (as monocrop) is the most widespread. Thus, the greenhouse pepper surface in this area reached 1447 ha in 2009, representing the 90% of total vegetable surface (figure 2.3b). Hydroponics procedures have also been substantially extended in the Region (*Campo de Cartagena*, Águilas and Mazarrón) with more than 500 ha (Econet, 2013).

Figure 2.3 Crops distribution in greenhouses in *Campo de Cartagena*, 2009 (Econet).

The planting schedule is from November to December and the production cycle from March to September. The main varieties grown are California (Requena, Orlando, Quito, Vélez) and Lamuyo (Herminio, Almuden) with a planting density of 25,000 plants ha⁻¹ (FECOAM, 2006).

2.3 ENVIRONMENTAL PROBLEMS IN AGRICULTURE

2.3.1 CROP RESIDUES PRODUCTION

As a result of the crop production, a large amount of wastes are produced in agricultural lands such as fertilizers, pesticides packaging, plastics, metals, or crop residues. Crop residues are the plant remains after harvesting the usable portion (Cooperband, 2002) such as leaves, stems, branches, roots or non-commercial fruits.

The crop residues are listed in the European Waste Catalogue and in the Hazardous List as plant-tissues wastes (02 01 03). The management of these crop residues involves problems mainly because of the difficulty of handling during loading and transport due to their large volumes. According to waste hierarchy of the Spanish State law “Ley 22/2011 de residuos y suelos contaminados”, the worst management option is to eliminate the residue (producing greenhouse gases from dumping or incineration). The energy recovery would be the following possibility while the best management option would consist of recycling or reusing them to minimize losses of greenhouse gases.

In the case of crops in greenhouses, the crop residues are produced when the plant remains have to be removed from the soil or substrate (soilless) after harvest to continue the productive activity. In the first case, these residues cannot be incorporated in the same soil because residues can present phytosanitary disrepair as a result of diseases (being a source of them for the next season). Regarding substrate, there is no soil in which to incorporate the residues. Crop residues are dried inside the greenhouses after harvest (taking advantage of the high temperatures at the end of the growing season, August-September) to reduce their volume and they are piled outside the greenhouses. However, nowadays the problem about an adequate management solution has not been solved. Sometimes residues are incorporated into the soil as an amendment but in most cases a common practice is burning (FECOAM, 2007). This results in loss of the C and N sequestered in the biomass, increasing atmospheric CO₂ contents (Anderson, 1992).

If we considered the 1447 ha of greenhouse pepper surface with a normal planting of 20,000 plants per ha and each plant weighing 2 kg (with a humidity of 85%), then, almost 9000 t of plant residues were produced in 2009 (own data).

The results obtained from crop-residue application studies are of importance for residue-management practices. There is an increasing need for such information because in many countries new legislation has been introduced to ban the on-site burning of crop residues, for environmental protection (IAEA Division, 2003).

2.3.2 DIFUSE POLLUTION

Another problem associated with the agriculture in this region is the increase of using chemical fertilizers. The European Union, aware of the problem, approved the Directive 91/676/EEC concerning the protection of waters against pollution caused by nitrates from agricultural sources. It states the need to reduce pollution by nitrates from agricultural diffuse source and establish preventive measures. It was incorporated into the Spanish regulation through R.D. 261/96. The *Campo de Cartagena* was declared a vulnerable zone in December 2001 (BORM 2001, Nr 301). In Murcia Region, the Directive is developed by Order of 3 December 2003 “Código de Buenas Prácticas Agrícolas” (BORM 2003, Nr 286) and by Order of 12 December 2003 “Programa de Actuación en Zona Vulnerable” (BORM 2003, Nr 301) which establishes the Action Program in the Vulnerable Zone of *Campo de Cartagena*. In this last regulation, it was established that in vulnerable zones maximum doses defined depending on crop and irrigation type had to be respected.

As a result of agricultural activities there is an excess of nutrients, mainly N and P. The most important environmental impact on the N cycle is the accumulation of nitrates in the soil, due to the low absorption capacity of soil to retain negatively charged particles, having a high solubility (Mellgren, 2008). Ammonium in the other hand is less mobile in soils because it attaches to soil particles. Pare et al. (2006) showed that nitrate accounted for >99% of the N leaching in an experiment using ammonium nitrate as fertilizer. Ammonium loss was negligible. They are exported to surface and ground waters by runoff and leaching (Velasco et al., 2006). Furthermore, there are some researches showing that the main source of pollution in the *Mar Menor* (the largest lagoon of the Spanish Mediterranean Basin adjacent to the cultivated area) is the nutrient flux from agricultural lands through the saturated subsurface flow system (Pérez-Ruzafa et al., 2002; Martínez and Esteve, 2005). Thus, diffuse pollution has become one of the major environmental problems in southeast Spain. It is estimated that in recent decades annual averages have reached values around 60 t of P and 2000 t of N, which are important values considering the water volume of the lagoon (Martínez and Esteve, 2005).

Since broccoli crop requires high supply of N (Cutcliffe et al., 1968) for optimum yields, chemical fertilizers are often used. They are easier to apply and the farmer knows the amount of nitrogen applied. Excessive or insufficient N applications may cause a reduction in the crop production (Everaarts, 1994) and some physiological disorders such as hollow stem were found when high levels of N were applied (Tremblay, 1989; Belec et al., 2001). Therefore, it is important to increase the efficiency of nitrogenous fertilizers. Fertilizers losses are mainly due to losses by volatilization, leaching and denitrification processes (Janssen, 1998). When crop has a high N requirements, fertigation technology entails these losses significantly as a consequence of the high frequency of N application, which prevents their prolonged stay in the soil (Torres, 1999). In drip irrigation systems nutrients are applied through emitters directly into the zone of maximum root activity and consequently fertilizer-use efficiency can be improved over conventional method of fertilizer application (Abouel-Magd, 2009). Not only the way of application is important, but also the amount of water applied to the crop. Drip irrigation may contribute substantially to increase water use efficiency for vegetable crops (Thompson et al., 2002). Irrigation to replenish water losses through evapotranspiration provided higher yields and greater N-use efficiency compared with excessive irrigation (Guteiz, 2004).

2.4 USE OF CROP RESIDUES AS ORGANIC AMENDMENT IN AGRICULTURE

Crop residues (CR) application as an organic amendment into the soil is of great interest for both agricultural and environmental aspect. Incorporating CR can improve soil properties while they help to reduce the use of chemical fertilizers as a source of nutrients (figure 2.4). Many long-term studies have shown that combinations of both organic and inorganic nutrient sources lead to enhance nutrient availability (Mugwe et al., 2009). There is a revival of the centuries-long tradition of recycling organic residues, but now combined with mineral fertilizers (Wang et al., 2007). Therefore, in Murcia region it is necessary to improve nutrient management practices, including also the recycling of crop residues.

The beneficial effects of crop residues (CR) on the physical, chemical, and biological properties of soil are well documented. Returning CR into the soil improves soil physical properties mainly because they protect and stabilize the soil particles into aggregates which provide the structural stability against external agents such as wind and water. Thus, crop residues addition decrease soil erosion (Doran et al., 1984; Perret et al., 1999; Cooperband, 2002), increase water conservation reducing runoff and soil evaporation

(Lal, 2007), increase porosity and gases exchange and reduce soil compaction (Moyin-Jesu, 2007).

Figure 2.4 Strategies for decreasing chemical fertilizer requirements. From United Nation University, 2007.

2.4.1 POSITIVE EFFECTS OF USING CROP RESIDUES AS ORGANIC AMENDMENT

Mediterranean agro-ecosystems are characterized by comparatively low annual, erratic rainfall and high evapotranspiration. These climatic conditions may limit accumulation of soil organic matter (SOM) in upper soil layer (López-Garrido et al., 2011) and sometimes is also due to excessive soil aeration as a consequence of tillage, which oxidizes organic matter. Likewise, in agricultural systems, because plants are harvested, only 20% of production will on average, be accumulated into the soil organic fraction (Batjes and Sombroek, 1997). In parts of Spain, as Murcia Region, the presence of SOM is often poor, below 2%. Decline in SOM content in intensive cropping systems is considered to be the major problem in maintaining agricultural productivity. A solution to this problem is the utilization of crop residues to increase organic material levels in the soil (Doran et al., 1984). Long-term field experiments have shown that there is a direct linear relationship between the quantity of carbon added to soil as CR and the amount of carbon accumulated in the soil, with other factors remaining constant (Duiker and Lal, 1999). Only between 1% (Schlesinger, 1990) and 15% (Lal, 1997) of crop residues returned to

the soil can be expected to be converted to passive soil organic matter. According to Farage et al. (2003), the most efficient method of accumulating carbon in soils must be by direct decomposition of plant material. If carbon passes through the heterotrophe chain some will be lost directly to the atmosphere as CO₂ as a consequence of respiratory activity and through digestive and assimilatory inefficiencies.

Regarding chemical properties, applying organic amendments to soil, like crop residues, increases the organic carbon content and plays an important role in the recycling of nutrients (IAEA, 2003). The addition of CR into the soils is an alternative to replace some of the nutrient losses caused by crop removal, leaching or erosion (España et al., 2001) such as N and other nutrients for plants (Porta et al., 1999; Shafi et al., 2007). Thereby, Moyin-Jesu (2007) found a general improvement in the soil N, P, K, Ca and SOM by application of 6 t ha⁻¹ of different plant residues. Their addition also improved cation exchange capacity, buffer effect of soil against temperature changes (Hadas et al., 2004), and helps in carbon sequestration (Post et al., 2004).

Among the positive effects on biological soil properties we can highlight the provision of a carbon, nutrients and energy source reactivating macro and microfauna and increasing microbial proliferation and activity (Smith et al., 1993; Boyer et al., 1996; Tejada et al., 2006). Roldán et al. (2003) found an increase of microbial activity and soil enzyme activities applying crop residues and planting leguminous species. The microorganisms present in soil play an important role in many elements cycles such as C, N and P, because their availability is strongly influenced by mineralization and immobilization of the organic matter as a result of microorganisms activity (García et al., 2000).

Crop residues not only improve soil properties, since also present several beneficial agricultural effects such as increasing of crops yield or reduction of chemical fertilizers use (Lal, 1980; Aggarwal et al., 1997; Bakht et al., 2009).

2.4.2 CROP RESIDUES MOST COMMONLY USED IN AGRICULTURE

Cereal plants residues have been extensively studied, specially maize and wheat all over the world (Smith and Sharply, 1990; España et al., 2001; Roldán et al., 2003; Shafi et al., 2007; Wang et al., 2007; Cayuela et al., 2009) with positive effects on soil properties and crop yields. Other studied crop residues were tobacco, corn, cocoa husk, rice hulls, cotton, alfalfa, sorghum or soybean, among others. In table 2.3 we can find a list with the most common crop residues used by different authors. Perucci et al. (1984) studied, under

laboratory conditions the effect of pepper residues on soil enzymes and detected that they increased all the studied enzyme activities except for phosphomonoesterase.

Table 2.3 Common crop residues used as organic amendment in agricultural lands.

Authors	Year	Crop residue	Country
Aggarwal et al.	1997	Clusterbean, mung bean, pearl milled	India
Chaves et al.	2004	Several Brassicaceas	Belgium
De Neve et al.	1998	Several Brassicaceas and lettuce	Belgium
España et al.	2001	Maize	Venezuela
Ghosh et al.	2011	Cotton gin	Australia
Hadas et al.	2004	Tobacco, rice hulls, wheat straw, corn	Israel
Hopkins et al.	2001	Tobacco	UK
Jensen et al.	2005	37 species (cereals, pasture, grasses, legumes, vegetables, fibre, energy and catch crops)	Nordic countries
Kasongo et al.	2011	Coffee wastes	Congo
Lopez-Piñeiro et al.	2008	Olive mill waste	Portugal
Moyin-Jesu	2007	Wood ash, sawdust, cocoa husk, rice bran	Nigeria
Mugwe et al.	2009	<i>Calliandra</i> , <i>Leucaena</i> , <i>Tithonia</i> and legumes	Kenya
Perucci et al.	1984	Wheat straw, tobacco, maize, sunflower, sorghum, tomato and pepper	Italy
Roldan et al.	2003	Maize	Mexico
Saviozzi et al.	1995	Rape, sunflower, soyabean	Italy
Schroeder	1998	Cowpea crop	USA
Shafi et al.	2007	Wheat, Maize, Mung bean	Pakistan
Smith et al.	1990	Alfalfa, corn, oat shorgum, soybean, wheat	USA
Wang et al.	2007	Maize	China
In Spain			
Acea & Carballas	1996	Wheat Straw	Galicia
Bustamante et al.	2007	Winery organic wastes	Alicante
Navarro et al.	1996	Almond nut skin	Alicante
Ordoñez et al.	2001	Olive tree pruning residues	Córdoba
Piedra buena et al.	2006	Pepper crop residues	Madrid

In Spain, we can find a huge amount of researches regarding the traditional olive oil industry. Ordoñez et al. (2001) used olive tree pruning residues during 6 years to increase organic matter content and improve soil fertility (P and K). Several authors studied the olive mill solid waste from olive oil extraction process, like Lopez-Piñeiro et al. (2008) who found a significantly increased in aggregate stability and organic matter content, total N, and available P and K. The repeated application of these residues to an olive grove soil caused neither N nor P deficiencies, and had a positive effect on olive yield. Other organic residues used were some derived from winery. Bustamante et al. (2007) studied grape

stalks and grape marc, among others, finding that they had low N fertilizing capacity and increased soil salinity. Almond residue, used by Navarro et al. (1996), increased organic matter and K content in soil. Piedrabuena et al. (2001) studied the pepper crop residues under laboratory conditions to know their biofumigant effect; nonetheless, literature about use of pepper crop residues as a soil amendment is very limited.

2.4.3 CROP RESIDUES CHARACTERISTICS

The effects of applied organic inputs vary with cropping systems, soil types, environmental factors, management and organic material characteristics. Chemical composition of the decomposing materials have long been considered a critical factor in determining the extent and rate of decay according to Prasad and Power (1991) and Chaves et al. (2004), especially N content, C/N ratio, soluble compounds, hemicellulose, cellulose, lignin and polyphenols content.

On average, crop residues contain about 40-50% of C but N is much variable component (Farage et al., 2003). The C/N ratio of plant residues is most often used as an index to assess whether the residues will release or immobilize inorganic N. This ratio is important because intense competition among microorganisms for available soil N occurs when the ratio is high. Higher rates of organic matter decomposition by soil microorganisms can be expected in cropping systems with more available N (Kong et al., 2011). Vigil and Kissel (1991) integrated N immobilization data from several medium to long-term experiments with residues having a wide range of C/N ratios, and showed that the break-even point between net N immobilization and mineralization of residues was at a C/N ratio of 41. Stevenson and Cole (1999) pointed out values under 20 for mineralization and above 40 for immobilization.

For modeling the crop residues transformation in soil, it is essential to know the composition of input materials (Kögel-Knabner, 2002). The higher content of residues in cellulose and hemicellulose, which are more easily degradable, the more susceptibility to mineralization (Cook and Allen, 1992); when plant residues have high lignin content, decomposition will be slower due to it is very resistant to microbial decomposition (Melillo et al., 1982; España et al., 2001).

Residues location is relevant for decomposition rates, since surface residues decompose more slowly than soil-incorporated (Schomberg et al., 1994), together with soil type (Smith and Sharpley, 1990) and the amount and size of residues that determine

accessibility by microbial biomass (Nelson and Oades, 1998; Trinsoutrot et al., 2000; Barzegar et al., 2002).

When N supply is done by means of N mineralization of crop residues, it must be synchronized with nutrient demand by the growing crops (Cobo et al., 2002). Knowledge of crop residue decomposition is important for assessing the long-term prediction of their potential fertilizing power in soil (Saviozzi et al., 1995). Hadas et al. (2004), demonstrated the need to determine the decomposability of C and N rather than total contents of residues, because decomposability has direct implications on the appropriate timing and quality of fertilizer-N application when residues are incorporated, either to avoid N deficiency when immobilization is expected or to avoid excessive available N when the residue releases inorganic N.

Thereby, numerous researchers have investigated the mineralization rate of fresh different crop residues. Schroeder et al. (1998) incorporated cowpea crop residues before broccoli crop, aiming to reduce the amount of N fertilizer applied as side dressing. These authors found that the N derived from residues (103 kg ha^{-1}) was not sufficient to result in higher broccoli yield, but reduces from 168 kg ha^{-1} to 84 kg ha^{-1} the need of N fertilizer supply. Chaves et al. (2004) studied the N mineralization of root residues in different species of brassicaceas and found a net N release after a period of 4 months. Aggarwal et al. (1997) studied residues of cluster bean, mung bean and pearl millet to grow pearl millet and observed that crop residues plus $20 \text{ kg fertilizer N ha}^{-1}$ provided pearl millet grain yield equivalent to that for $40 \text{ kg fertilizer N ha}^{-1}$ with no residues.

2.5 SOIL PROPERTIES INDICATIVE OF SOIL QUALITY

In operation of the soil ecosystem its physical, chemical and biological properties are implicated. Overall physical and chemical parameters are not very useful for assessing soil quality in a short term, since they are altered only when changes are drastic (Filip, 2002), although in many cases have been used to assess soil quality (Arshad and Coen, 1992).

The physical properties that can be used as indicators of soil quality are those which reflect the manner in which this resource accepts retains and transmit water to the plants as well as the limitations that roots can found to grow, seedling emergence, infiltration or water movement within the profile. We studied changes in water holding capacity and texture (Bautista et al., 2004).

Chemical indicators refer to conditions affecting the soil-plant water quality, the buffering capacity of the soil, water and nutrient availability to plants and microorganisms. Some chemical indicators measured are the availability of nutrients, total organic carbon, pH, electrical conductivity, cation exchange capacity, changes in total nitrogen and available N, P and K mineral contents.

However, changes in physical and chemical properties are difficult to measure in the short or medium term as Power et al. (1998) and Saffigna et al. (1989) suggested. Therefore, biochemical parameters, considered as bioindicators of soil microbial activity, biomass and composition, are used because of their highest sensitivity to changes (Dick et al., 1996). The biological component of the soil is essential for numerous processes and functions that are carried out by soils (Dick et al., 1996), such as decomposition of organic waste, the nutrients cycle or synthesis of humic substances.

Due to the complex dynamic of the soil system, it is difficult to choose only one parameter to accurately reflect its microbiological status, and it is advisable to use several parameters and to jointly analyze the information they provide (García et al., 2000). Soil microbial biomass and enzymes are very common indicators and they are crucial in the degradation of soil organic matter and cycling of nutrients. Therefore, they can be used as sensitive indicators to reflect the effects of land management practices on soil quality (Bandick and Dick, 1999; Masciandaro et al., 2004). Gianfreda and Bollag (1996) observed that stimulation of microbial biomass and enzyme activities in soil is usually greater in organically than in inorganically fertilized soils. Likewise, they have been also used as indicators of productivity, soil quality or potential rate of soil to decompose organic wastes (Paolini, 2002).

Microbial biomass carbon is a biochemical parameter that reflects the size of the soil microbial community (Nannipieri et al., 1990). The microbial biomass carbon can be used more effectively than the organic matter content as an indicator of variations in soil quality since it responds more rapidly and with a greater degree of sensitivity to such changes; it means that short term measurements of biomass can reflect the long term tendency of the organic matter (Powlson et al., 1987). A positive effect of organic amendments with readily available C is observed on soil microbial biomass (Tejada et al., 2006; Trinsoutrot et al., 2000).

Another important biochemical parameter is basal soil respiration. It is used related to soil quality and as indicator of soil fertility (Staben et al., 1997). Respiration is a widely used parameter to estimate the soil microbial activity. The activity of all heterotrophic

microorganisms leads to the degradation of organic matter and such decomposition has frequently been used to indicate the biological state of soils (Nannipieri et al., 1990). Soil CO₂ release by microbial respiration has been widely used for many years to quantify the impact of various treatment and management inputs on soil microbial community (Haney et al., 2008). The basal respiration rate of natural soils is usually higher than that observed in the degraded soils. Soil respiration is usually positively correlated with organic matter content and often with the microbial biomass and microbial activity (Alef, 1995).

The metabolic quotient (qCO₂) was first described by Anderson and Domsch in 1985 and is related with the hypothesis of energy optimization in developing ecosystems. It is the equivalent of respiration per unit of biomass. Metabolic quotient has often been used as an indicator of efficiency in carbon mineralization (Insam and Haselwandter, 1989). The qCO₂ could be indicative of stress or disturbance when comparing the same soil (Zornoza et al., 2009a). A decreasing in qCO₂ is related to lower soil disturbance and stress and increase in microbial efficiency (Leita et al., 1999).

Soil enzymes have been used as soil quality indicators because they respond immediately to changes in soil fertility status due to their strong relationship with quantity and quality of SOM (Pascual et al., 1998; Garcia et al., 2000; Gil-Sotres et al., 2005). Soil enzyme assays measure the potential activities of persistent and accumulative enzymes in soils via monitoring the formation of products or disappearance of substrates under optimal conditions (Iyyemperumal and Shi, 2008). The nutrient cycling in soils is mediated by enzymes which catalyze biochemical reactions and thereby mediate the transformation of organically bound nutrients into inorganic plant available forms. The soil enzymes mainly are of microbial origin although they can also be derived from plants and animals residues. In the soil, they can exist in several stages, ranging from those associated with living organisms (endoenzymes) to those immobilized in clays minerals and humic complexes (extracellular enzymes or abiotic) (Paolini, 2002). The simultaneous measurements of several enzymes may be useful as a marker of bioactivity and can be used as an index of soil biochemical fertility (Stefanic, 1984).

According to Pascual et al. (1998) hydrolase activities involved in C cycle are positively influenced by the greater quantity of carbonated sources because organic amendments increase the concentration of labile substrates in the soil. Measurement of β -glucosidase activity is very usual. This enzyme is produced by a variety of organisms (plant, animals, fungi and bacteria) (Esen, 1993). It catalyzes the final step in the biodegradation of cellulose compound and the subsequent release of glucose to

microorganisms. β -glucosidase has been found to be sensitive to soil management within relatively short periods (Dick et al., 1996; Leirós et al., 1999) and it is relatively stable with seasons (Ndiaye et al., 2000). Another activities involves in C cycle are arylesterase which catalyzes the hydrolysis of phenolic esters as phenyl acetate to phenol and acetate; it occurs in microorganisms, plants, and other living organisms, and β -galactosidase which catalyzes the hydrolysis of the disaccharides β -D-galactopyranosides. Most of the N input into soil is in the form of polymers, which first have to be broken down into smaller units by extracellular enzymes (Schimel and Bennett, 2004). The mineralization of organic nitrogen refers to the degradation of proteins, amino acids and nucleic acids to NH_4^+ (Paul and Clark, 1989). The small organic molecules released by the enzymes can then be taken up directly or degraded further and the N taken up as NH_4^+ . This process is the product of reactions catalyzed by specific enzymes such as protease and urease. Protease-BAA is involved in the hydrolysis of N compounds to NH_4^+ , using low-molecular-weight protein substrates. More specifically, this enzyme acts on dipeptides, which are the shortest fragments resulting from the hydrolysis of long proteins chain. Urease can be produced by bacteria, yeasts, filamentous fungi, and algae as well as plants (Follmer, 2008). It is responsible for breaking down urea into CO_2 and NH_3 . Ammonium is considered the preferred source of N for bacteria and fungi (Merrick and Edwards, 1995; Marzluf, 1997). Some studies correlated urease activity with the carbon content or nitrogen in the microbial biomass (Klose and Tabatabai, 1999). Phosphatase is a general name for a broad group of enzymes that catalyze the hydrolysis of both esters and anhydrides of phosphoric acid. Among the most used phosphatases we can find acid phosphatase and alkaline phosphatase. These enzymes catalyze the conversion of organic P into inorganic P, making it available for plants and microorganisms.

2.6 ISOTOPICS TECHNIQUES

Isotopes are forms of the same element that differ in the number of neutrons in the nucleus. There are two kinds of isotopes, stables or radioactive isotopes. Stable isotopes are safe isotopes that do not decay and unlike the radioactive isotopes, are not at all hazardous to human health. In fact, stable isotopes are quite abundant and natural parts of each one of us. The extra neutron/s makes the nucleus more massive or “heavier” than the “light” isotope. In cases of C, N, O, S or H the lightest stable isotope accounts for more than 95% of all the isotopes (Fry, 2006).

2.6.1 SOLID-STATE NUCLEAR MAGNETIC RESONANCE (NMR)

The nuclear magnetic resonance (NMR) spectroscopy relies on the interaction of the nuclei of certain atomic isotopes with a static magnetic field. Common NMR active nuclei are ^1H , ^{13}C , ^{17}O , ^{31}P , ^{15}N , ^{29}Si , and many more. Nearly every element has at least one isotope that is NMR active. The ^{13}C -NMR has been the most frequently used and the most widely accepted. Natural abundance for C isotopes is 98.9% and 1.1% for ^{12}C and ^{13}C , respectively. The ^{13}C nuclei can behave as a little magnet (nuclear spin = $1/2$), while ^{12}C do not have this property (nuclear spin = 0). Thus, ^{13}C nucleus can be aligned with an external magnetic field. This magnetic field makes the possible spin-states of the nucleus differ in energy, and using NMR techniques the spins can be made to create observable transitions between the spin states. This technique is currently very widely used for soil organic matter characterization (Stevenson, 1994). Two general approaches have been used: liquid-state and solid-state ^{13}C -NMR spectroscopy both with some advantages and limitations. The main attraction of solid-state NMR is its non-destructive nature. Other advantages of ^{13}C NMR are: (1) the need to extract the organic carbon from the mineral fraction with various reactive reagents is eliminated, (2) the whole of the organic fraction is analyzed rather than that portion soluble in a specific extractant, and (3) the analysis is nondestructive and allows additional measurements to be made on the sample utilized for the ^{13}C NMR analysis (Baldock et al., 1989). The use of solid-state ^{13}C -NMR spectroscopy in combination with other techniques has led improve spectral quality. Cross-polarization (CP) and Magic-angle spinning (MAS) techniques reduces line broadening and overcomes the problem of dipolar line broadening enhancing resolution. The combination of both result in the CP/MAS designation. Through the analysis of ^{13}C cross-polarization/magic angle spinning nuclear magnetic resonance (CP/MAS ^{13}C -NMR) spectroscopy we can elucidate the atomic and molecular structure of soil SOM or plant residues. This technique has been used for about 25 years to study SOM quality (Preston, 1996; Mathers and Xu, 2003; Kavdir et al., 2005).

This technique is also valid even to study organic residues composition as crop residues (Kögel-Knabner, 2002; Lorenz et al., 2006; Carvalho et al., 2009). Plant litter materials provide the primary resources for organic matter formation in soil and it is important to know their composition. Although specific compounds are hardly identified, NMR spectroscopy can give good results concerning the gross chemical composition of plant residues (Kögel-Knabner, 2002).

The spectrum obtained shows a horizontal scale (measured in parts per million – ppm) that can be divided in different chemical shift regions in which the chemistry of the C atoms within each region is similar. Figure 2.5 provides a tentative position of functional groups of the ^{13}C NMR spectra.

Figure 2.5 ^{13}C NMR absorptions of major functional groups (modified from Bruno and Svoronos, 2011).

2.6.2 LABELLING METHODOLOGIES

Owing to the concern of its radioactivity by human being, radioactive ^{14}C labelling technique used to study the turnover of organic materials added to soils has gradually been replaced by stable isotope ^{13}C labelling technique since 1980's (Quiron et al., 2000). Stable isotopes are a powerful tool for tracing elements (^{13}C , ^{18}O or ^{15}N). By using isotope composition we can trace elements movement thorough biogeochemical cycles at the plant-soil-microbial interface. Stable carbon isotopes are a viable alternative to ^{14}C -labelling, as recent developments in their use with either natural abundance or with labelled substrate in soil ecology have shown (Staddon, 2004).

Labelling methodologies enable the introduction of carbon compounds either enriched or depleted in one or more of the rarer carbon isotopes. In principle, any carbon

pool can be studied by using a ^{13}C -labelling approach so long as there is a mean of labelling the pool of interest. The most common technique is to add a ^{13}C -labelled substrate to the medium and then the fate of the carbon originating from the introduced compound can then be tracked. Following ^{13}C mineralization through measuring ^{13}C values in the respired CO_2 , the dynamics of the decomposition processes could be analyzed. The conventional method to measure the relative abundance of stable isotope is by isotope ratio mass spectrometry (IRMS). Prior analysis by IRMS, samples are converted to simple gases such as CO_2

To study C turnover, labelled glucose added into the soil is the substrate more widely used (Baldock et al., 1989; Aoyama et al., 2000; Hoyle et al., 2008; Dungait et al., 2011), although labelled substrates can vary in complexity from CO_2 or CH_4 to different fresh organic matter including amino acids, or plant materials. De nobili et al. (2001) studied the addition of ^{13}C -glucose, amino acids and root exudates. Fontaine et al. (2004) used ^{13}C -labelled cellulose to investigate the effect of fresh organic carbon on soil organic respiration rate finding that the addition of cellulose at a rate of 200 mg C kg^{-1} , without any supplement of nutrient, increased the soil organic carbon mineralization or priming effect of the control by 12–16%. Guenet et al. (2010) also found a positive priming effect after adding ^{13}C -labelled straw at different rates. Thereby, many field and controlled observations have demonstrated that despite organic inputs are incorporated into the soil in large quantities, soil total organic C content does not necessarily increase.

Therefore, it is essential to know how much of the applied amendment is mineralized and which part becomes as stable organic matter to establish the best organic amendment management when they are applied into the soil. Changes in soil quality and quantity of fresh organic matte inputs may disturb the proportion of C submitted to priming effect and modify the C balance (Guenet et al., 2012).

CHAPTER 3

CARBON AND NITROGEN

MINERALIZATION DURING

DECOMPOSITION OF PEPPER

CROP RESIDUES

CHAPTER 3

Carbon and Nitrogen mineralization during decomposition of pepper crop residues

Abstract

The present essay was aimed at evaluating, under laboratory conditions, the medium-term influence of pepper residues applied at different rates (2, 3 and 5 g kg⁻¹) on carbon and nitrogen cycles in an agricultural soil. The cumulative quantities of CO₂-C evolved from the pepper residues mineralization were fitted to a first-order kinetic model. These values are equivalent to ~30% of the organic carbon added for all doses, with similar mineralization constant rate (~0.09 d⁻¹). Net ammonification followed the same pattern in all doses indicating a net loss of NH₄⁺-N by nitrification or immobilization. Net nitrification followed a different pattern depending on the dose. Final positive values were observed for the two highest doses, but when 2 g kg⁻¹ was applied, net nitrification was always negative, suggesting net immobilization of N. The net N mineralization was -3.1, 13.7 and 56.7 mg kg⁻¹ for doses 2, 3 and 5 g kg⁻¹, respectively. Thus, the addition of pepper residues at low doses produced a net N immobilization in soil. This trend may indicate that N is likely a limiting factor for microbial growth. The activities of soil enzymes involved in C and N cycles responded differently according to the dose, indicating that only the highest dose would increase the capacity of soil to perform useful ecosystem functions. These results highlight the importance of dose when applying pepper residues, since doses <5 g kg⁻¹ do not easily release available N for plant growth in the loamy calcareous soil used for this experiment.

3.1 INTRODUCTION

The decline in soil organic matter (SOM) in many Mediterranean semiarid agricultural zones is associated with a decrease in soil fertility. This SOM depletion associated with the intensification of agriculture is considered one of the foremost environmental threats in the EU (Marmo, 2008). This intensification has also led to over-application of inorganic fertilizers turning this practice into an important focus of contamination (Conway and Pretty, 1991; Bumb and Baanante, 1996). Thus, it is essential to find appropriate crop management to reduce the inorganic fertilization and increase soil quality. The use of organic residues as soil fertilizers has been proposed as a sustainable strategy to achieve this aims (Reeves, 1997; Eghball, 2001). Nonetheless, better knowledge about carbon and nitrogen mineralization dynamics of residues is essential to quantify the potential benefits of residue management on soil quality and fertility.

Organic residues often exhibit different physicochemical properties and therefore they affect the soil ecosystem in different ways. Thus, the study of their impact on soil is essential to benefit from their potential as amendments and to avoid adverse environmental effects (Cayuela et al., 2009). Organic matter mineralization processes are of great importance in maintaining soil quality and fertility, and hence agricultural sustainability. An extensive amount of research has been done on the investigation of crop residues as an organic amendment such as wheat, mungbean, maize, rape, olive tree pruning, almond nut skin, straw, among others (Trinsoutrot et al., 2000; Barzegar et al., 2002; Cayuela et al., 2009); however little information about the use of greenhouse crop residues as organic amendments is available. Studies demonstrated that biochemical composition (C:N ratio, hemicellulose, lignin...), rates, and the size of crop residues affect C and N decomposition rates (Barzegar, 2002; Raiesi, 2006). Biochemical composition, and particularly the soluble C content, determines the initial rate of residue decomposition (Hadas et al., 2004), whereas the lignin content controls the medium to long-term fate of added C (Trinsoutrot et al., 2000).

Previous studies have indicated that organic residues management affected microbial population and activity (Raiesi et al., 2006; Guerrero et al., 2007; Cayuela et al., 2009). Soil microbial populations and enzymes are crucial in the degradation of SOM and cycling of nutrients and, therefore, can be used as sensitive indicators to reflect the effects of land management practices on soil quality (Bandick and Dick, 1999). The enzyme activities are directly involved in the transformation of complex carbon forms of organic matter to readily available nutrients for the plants, determining the pattern of most of

chemical transformations in soil (Stryer, 1995). Changes in microbiological indicators may decrease or increase C and N dynamics and have an influence on nutrient uptake by plants. In fact, the synchronization of N supply with plant demand is important for both environmental and agronomic reasons.

The present work was aimed at evaluating, under laboratory conditions, the medium-term influence of pepper residues applied at different rates on carbon and nitrogen cycles in an agricultural soil. This type of research is needed to understand and explore soil organic matter enrichment and nutrients availability under controlled conditions and provide scientifically based information to support decision regarding crop residues management and its agronomic importance.

3.2 MATERIALS AND METHODS

3.2.1 SOIL AND CROP RESIDUE

The soil used was collected from the top 30-cm Ap horizon from an agricultural land called Campo de Cartagena (UTM: 4191030N, 68625016E) (Murcia Region, SE Spain). The climate of the area is semiarid Mediterranean, with a mean annual temperature of 17°C and a mean annual rainfall of 300 mm. The soil is a Typic Petrocalcic (Soil Survey Staff, 2010) with loam texture. Characteristics of the soil are shown in table 3.1. The soil was air dried and sieved <2 mm.

Table 3.1 Soil characteristics of the study site. Values are mean $\pm$ standard deviation (n=24).

Soil properties	0 - 30 cm
Sand (g kg ⁻¹)	407 $\pm$ 25
Silt (g kg ⁻¹)	356 $\pm$ 24
Clay (g kg ⁻¹)	237 $\pm$ 27
Textural Class	Loam
Water Holding Capacity (%)	53.8 $\pm$ 2.27
pH	7.81 $\pm$ 0.15
Electrical Conductivity (μ S cm ⁻¹)	921 $\pm$ 171
CaCO ₃ (g kg ⁻¹)	584 $\pm$ 32
Soil Organic Carbon (g kg ⁻¹)	19.3 $\pm$ 2.4
Total Nitrogen (g kg ⁻¹)	1.52 $\pm$ 0.13
Cation Exchange Capacity (cmol ⁺ kg ⁻¹)	7.95 $\pm$ 0.72

The crop residue consisted of a dehydrated and crushed mix of stems, leaves and peppers fruits which do not meet the standards for commercial use from pepper plants (*Capsicum annuum*, L.). Pepper plants were collected after harvesting from a greenhouse. The residue was dried at 55 °C until constant weight for the determination of dry matter; then it was ground to homogenize the particle size before application.

3.2.2 SOIL INCUBATION

Potential C and N mineralization was studied in a laboratory incubation experiment in dark, at constant humidity (55% water holding capacity) and temperature (25°C), under aerobic conditions for 155 days. The residues were thoroughly mixed at three different application rates: 2 g kg⁻¹ soil (D1), 3 g kg⁻¹ soil (D2) and 5 g kg⁻¹ soil (D3) dry weigh basis, with soil samples. Untreated soil was also included as control (CT). For C mineralization, 10 g of amended soil was placed in 0.5 L polyethylene jars containing alkali traps (0.2N NaOH) to determine CO₂ emissions at 1, 6, 10, 15, 23, 34, 45, 59, 76, 94, 114, 133 and 155 days of incubation. Three jars without soil or plant residue were considered as blanks. For N mineralization, 150 g of amended soil was placed in 0.5 L polyethylene jars with thin plastic film perforated to ensure gas exchange. Soils were sampled to monitor the evolution of NH₄⁺-N and NO₃⁻-N at 0, 4, 7, 15, 24, 30, 42, 63, 83, 126 and 155 days of incubation. Each treatment was replicated three times, and moisture levels were gravimetrically maintained every 3 days using deionised water.

Total organic C, total N, soluble C, microbial biomass C and the enzyme activities β-galactosidase, β-glucosidase, arylesterase, urease and protease were determined at the beginning and at the end of the incubation.

3.2.3 ANALYTICAL METHODS

Characteristics of pepper residues are shown in table 3.2. The pepper residues were dried, ground and analyzed for total nitrogen (TN) according to the Kjeldahl method (Hoeger, 1998) and total organic carbon (TOC) (Golueke, 1977). P, Ca, Mg, Na, K, Fe, Mn, Cu and Zn were analysed after incineration at 500°C and redilution using 6N HNO₃, by ICP-MS (7500 CE, Agilent). Cellulose was determined by Brendel et al. (2000). Lignin was determined after a two stage sulphuric acid hydrolysis (Hatfield et al., 1994). Soluble organic carbon was extracted in water (1:500 w/v) and determined UV-persulfate digestion.

Soil pH and electrical conductivity (EC) were measured in deionised water (1:1 and 1:5 w/v, respectively). Particle size distribution was determined using the Robinson pipette method. Soil organic carbon (SOC) was analyzed with dichromate oxidation technique, while total nitrogen (TN) was determined using the Kjeldahl digestion method (Hoeger, 1998). For equivalent calcium carbonate the volumetric method (Bernard calcimeter) was used and cation exchange capacity (CEC) by Chapman (1965). The concentrations of $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ were determined by UV-visible spectrophotometry in a 2M KCl extract (1:10 w/v) (Kandeler et al., 1999). The total inorganic-N (IN) is the sum of $\text{NO}_3^-\text{-N}$ and $\text{NH}_4^+\text{-N}$. CO_2 emissions were measured by titration of the unreacted NaOH with standardized 0.1N HCl to phenolphthalein end point (Anderson, 1982). The microbial biomass carbon (MBC) was determined using the chloroform fumigation-extraction method proposed by Vance et al. (1987). The non-fumigated fraction was considered as soluble carbon (SC). The arylesterase activity was quantified following the method of Zornoza et al. (2009b). β -Glucosidase and β -Galactosidase activities were measured according to Tabatabai (1982) and Eivazi and Tabatabai (1988), respectively. Urease activity was determined according to Tabatabai and Bremner (1972) modified by Nannipieri et al. (1978). Protease activity was analysed following the method of Ladd and Butler (1972), modified by Bonmatí et al. (1988).

Table 3.2 Main chemical properties of pepper residues used for incubation with soil (n=5).

Properties	Value
Total Nitrogen (g kg^{-1})	22.9 $\pm$ 2.9
Total Organic Carbon (g kg^{-1})	438 $\pm$ 13
C:N	19 $\pm$ 2
P (g kg^{-1})	6.02 $\pm$ 0.37
Ca (g kg^{-1})	23.9 $\pm$ 6.2
Mg (g kg^{-1})	7.23 $\pm$ 1.33
Na (g kg^{-1})	1.89 $\pm$ 0.22
K (g kg^{-1})	40.7 $\pm$ 3.2
Cu (mg kg^{-1})	12.5 $\pm$ 1.0
Zn (mg kg^{-1})	77.6 $\pm$ 11.8
Fe (mg kg^{-1})	184 $\pm$ 58
Mn (mg kg^{-1})	87.4 $\pm$ 18.8
Cellulose (g kg^{-1})	200 $\pm$ 1
Lignin (g kg^{-1})	179 $\pm$ 5
Soluble Organic Carbon (g kg^{-1})	34 $\pm$ 3

3.2.4 DATA ANALYSIS AND STATISTICAL PROCEDURES

Normal distribution of data was checked using the Kolmogorov–Smirnov test. The average value of CO₂-C from the control soil was subtracted from each application rate to obtain the increment data (Δ data). Equally, the average value of NH₄⁺-N and NO₃⁻-N measured at each sampling day from the control was subtracted from each application rate to obtain the Δ data, expressed as mg inorganic-N per kg of dry soil. This approach supposes that the possible priming effect due to the enhanced decomposition of native SOM will not occur or they are small compared to the mineralization of the organic amendment. Net mineralized inorganic-N was calculated by subtracting the soil inorganic N content at day 0 from the amount present in soil after *t* days of incubation, expressed as mg inorganic-N kg⁻¹ dry soil. Cumulative net nitrification and ammonification were calculated analogously using NO₃⁻-N and NH₄⁺-N results. Then, for each application rate, the Δ data were averaged and the cumulative values were fitted to different kinetics functions. Values of net C and N mineralization for the different application rates were fitted versus time to several kinetics models, and the coefficients of regression (R²) and assumption of normal distribution of residuals were used as a criterion to decide the best fit. To assess the effect of incubation (initial and final sampling days) on soil properties, data were analysed by paired-samples t-test, separately for each application rate. A one-way ANOVA was carried out to assess differences among application rates separately for each sampling day. The comparison of means was made according to Tukey's Honestly significant difference test (*P*<0.05). The fits and kinetic parameters were carried out using the SigmaPlot 10.0 software. ANOVA and t-test analysis were performed with the software IBM SPSS for Windows, Version 19.

3.3 RESULTS AND DISCUSSION

3.3.1 RESPIRATION RATES AND CARBON MINERALIZATION KINETICS

The values of soil respiration rates in CT (unamended soil) ranged from 2.18 to 0.23 mg CO₂-C kg⁻¹ h⁻¹, following a decreasing trend during the incubation, also reported in other studies (Van Gestel et al., 1991; Guerrero et al., 2007). Higher rates of basal respiration at first stages of incubations are typical due to the sieve disturbance, the rewetting and the presence of easy decomposable substrates (Denef et al., 2001; Fierer and Schimel, 2003; Guerrero et al., 2007). Nutrients depletion has been also described as a cause for this behaviour (Dilly, 1999). The addition of crop residues caused a significant increase in respiration rates during the first day of incubation, with values of 3.77, 4.68

and $6.00 \text{ mg CO}_2\text{-C kg}^{-1} \text{ h}^{-1}$ for D1, D2 and D3, respectively. Respiration rates in amended soil followed a trend to decrease reaching values similar to CT from day 76 up to the end of the experiment. These values and patterns were similar to those observed in soils amended with plant residues (Trinsoutrot, 2000; Jin et al., 2008; Cayuela et al., 2009), but lower than those observed in soils amended with animal manures (Bernal et al., 1998; Guerrero et al., 2007).

The cumulative quantities of $\text{CO}_2\text{-C}$ evolved from the pepper residues mineralization (figure 3.1) were fitted to a first-order kinetic model $C_{\min}=C_0 (1-e^{-K t})$, being $C_{\min}$ the carbon mineralized from soil once the control is subtracted (as $\text{CO}_2\text{-C}$) in a given time, C_0 the potentially mineralizable pool of organic C, K the mineralization constant rate, and t the time.

Figure 3.1 Cumulative values of $\text{CO}_2\text{-C}$ mineralised from pepper residues where control values have been subtracted (symbols are experimental data and lines represent the fitting of the first order kinetic model). Error bars denote standard deviation.

These models explained more than 94% of the experimental data for all application rates (table 3.3). These data are approximately equivalent to 33, 28 and 32% of the organic carbon added for D1, D2 and D3, respectively, and ~4x higher than the soluble organic carbon added for all application rates. The mineralization constant rate (K) and the index $C_0 \cdot K / \text{added C}$ were similar for all application rates. The index $C_0 \cdot K / \text{added C}$ is an indicator of degradability for organic materials because it positively correlates with organic N and negatively with lignin-humus contents (Saviozzi et al., 1993). The data pointed out the same trend and rate of C mineralization irrespective of the application rate applied to soil. The C mineralized at the end of incubation was positively correlated with the initial concentrations in SC ($r=0.70$; $P<0.01$). Thus, it seems that organic carbon content, and mainly the soluble fraction, directly controls the carbon mineralization rates. The mineralization rate in this experiment was lower than that found in alfalfa, wheat or rape residues (Trinsoutrot et al., 2000; Raiesi, 2006; Geisseler et al., 2009), but similar to application of peanut straw (Jin et al., 2008).

Table 3.3 Parameters of first order kinetic model ($C_{\min} = C_0 [1 - e^{-Kt}]$) fitted to the cumulative values of CO₂-C mineralised from pepper residues.

	Application rate 1	Application rate 2	Application rate 3
Added C (mg kg ⁻¹)	876	1314	2190
C_0 (mg C kg ⁻¹)	296.91	353.83	692.56
K (d ⁻¹)	0.0836	0.1178	0.091
$C_0 \cdot K / \text{added C}$	0.0283	0.0317	0.0288
R ² adj	0.966	0.946	0.986
F-value	347 ($P<0.001$)	213 ($P<0.001$)	856 ($P<0.001$)

3.3.2 NITROGEN DYNAMICS AND KINETICS

Net ammonification followed the same pattern in all application rates, indicating a loss of NH₄⁺-N in the amended soil (figure 3.2A). Concretely, we detected two periods of nitrification or immobilization ($t=0-15$ d, and $t=63-83$ d), and two periods of ammonification ($t=15-63$ d, and $t>83$ d). NH₄⁺-N losses were significantly lower ($P<0.05$) in D1 at the end of the incubation, with similar values for D2 and D3 (figure 3.2A). At the end of the experimental period, the NH₄⁺-N values were 5.12, 5.71 and 5.13 mg NH₄⁺-N kg⁻¹ in D1, D2 and D3, respectively; the net ammonification (values of day 0 subtracted from final values) was -3.02, -4.24 and -5.58 mg NO₃⁻-N kg⁻¹ for D1, D2 and D3, respectively.

Net nitrification followed a different pattern depending on the concrete application rate of crop residue applied to the soil (figure 3.2B). A first phase of decrements in NO_3^- -N was observed for D1 and D2 up to day 7, probably due to N immobilization by microbial growth. From this time, an increasing trend was observed up to the end of incubation, indicating predominance of nitrification, although a slight decrease was detected between days 30-63 in D1. In D1, values were always negative, indicating net immobilization of NO_3^- -N mineralized from crop residues. Application rate 2 values turned positive from day 42, indicating available free NO_3^- -N in soil at the end of the incubation period. NO_3^- -N values in D3 were always positive, indicating the predominance of nitrification and release of available NO_3^- -N in soil. Even though nitrification was observed most of the time in D3, a decreasing trend was observed between 42-83 d, suggesting an immobilization period, like observed in D1, and in all application rates for NH_4^+ -N mineralization. At the end of the experimental period, the NO_3^- -N values were 173, 194 and 248 mg NO_3^- -N kg^{-1} in D1, D2 and D3, respectively; the net nitrification (values of day 0 subtracted from final values) was -0.08, 17.96 and 62.26 mg NO_3^- -N kg^{-1} for D1, D2 and D3, respectively.

The net N mineralization (IN) followed the same trend than that observed for NO_3^- -N mineralization (figure 3.2C), since the inorganic N content in soil was principally as NO_3^- -N (~97%). Thus, the net inorganic N mineralization was negative for D1, indicating a complete immobilization of N forms mineralized; positive from day ~50 in D2, and always positive in D3. At the end of the experimental period, the inorganic N values were 178, 200 and 253 mg IN kg^{-1} in D1, D2 and D3, respectively; the net mineralization (values of day 0 subtracted from final values) was -3.1, 13.7 and 56.7 mg IN kg^{-1} for D1, D2 and D3, respectively. The net increment in total inorganic N at the end of the incubation period, expressed as percentage of the added N, was 20% and 50% for D2 and D3, respectively. These results are similar to those reported by Chaves et al. (2004) for fine plant root residues and green manures, but lower than tobacco and rape residues (Hadas et al., 2004).

Figure 3.2 Variation in $\text{NH}_4^+\text{-N}$ (A), $\text{NO}_3^-\text{-N}$ (B), and net N mineralization ($\text{NH}_4^+\text{-N} + \text{NO}_3^-\text{-N}$) (C) from pepper residues where control and day 0 of incubation values have been subtracted. Error bars denote standard deviation.

The observed pattern in figure 3.2 represents N mineralization from the crop residues. In this experiment, the addition of crop residues at low application rates produced a rapid N immobilization in soil with a noticeable decrease in $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$, also reported in literature (Chaves et al., 2004; Hadas et al., 2004; Jin et al., 2008; Cayuela et al., 2009). It has also been reported that ~15-20% of the C added with the residues is present in the microbial biomass shortly after the start of decomposition (Trinsoutrot et al., 2000). Thus, the application of organic residues triggers the immobilization of inorganic N by microbial communities, indicating stimulation in microbial activity. Some of the $\text{NH}_4^+\text{-N}$ losses may be due to NH_3 volatilization. Alkalinity and low organic matter content can move the equilibrium from NH_4^+ to NH_3 , highly volatile. Nonetheless, we estimate that this process is not significant in this study, pretending to the chemical composition of pepper residues (table 3.2); in addition, decreases in $\text{NH}_4^+\text{-N}$ are accompanied by decreases in $\text{NO}_3^-\text{-N}$ only in D1 and D2, indicating immobilization and in some extent, denitrification induced by intensive respiration (Hadas et al., 2004). Nonetheless, denitrification is normally very low in aerated ambient to totally explain the losses in inorganic nitrogen, and it is usually well correlated with C mineralization (Huang et al., 2004). Therefore, lower denitrification should be expected in D1, where higher decreases in inorganic nitrogen were detected. Soil heterotrophs normally prefer NH_4^+ for their growth, so microbial consumption of NO_3^- is not generally an important process. Nonetheless, it has been reported when the concentration of NH_4^+ is low and there is a high amount of available C (Shi and Norton, 2000).

No differences with application rate were detected with C mineralization, with mineralization rates proportional to application rate. However, this trend is not valid for N. Thus, this could indicate that N is likely a limiting factor for microbial growth when crop residues are added. Cayuela et al. (2009) also observed this trend in soils amended with wheat straw and cotton cardings. There were two clear cycles observed in N mineralization: 1) from 0 d to 40-60 d, with initial immobilization and following ammonification/nitrification; and 2) from 40-60 d up to the end of the incubation period, with a second cycle of immobilization and then ammonification/nitrification bound to microbial death (figure 3.2). This microbial death is inferred by the decrease in MBC at the end of the incubation period (table 3.4). We hypothesized that these cycles are indicative of shifts in microbial community structure. The first cycle may represent the initial response of fast growing strategists to rapid mineralization of the more labile plant fraction, followed by enzyme induction before mineralization of more resistant fractions by a parallel growth of K-strategists (Fontaine et al., 2003; Cayuela et al., 2009).

These results highlight the importance of application rate when applying crop residues, since application rates <5 mg pepper residues kg^{-1} soil do not easily release available N for plant growth, immobilizing most N as microbial biomass.

3.3.3 SOLUBLE CARBON AND BIOCHEMICAL PROPERTIES

The response observed within time was different depending on the concrete property and application rate (table 3.4). There was a general significant decrease in SC and MBC in all application rates at day 155 comparing to initial values, owing to depletion of labile organic compounds with the incubation (Cayuela et al., 2009; Geisseler et al., 2009). Decreases in microbial biomass can also be responsible for final increments in inorganic N owing to mineralization of microbial N. Amended soils showed significantly ($P<0.05$) higher contents of MBC at the end of incubation than CT, showing D3 the significantly highest value. Thus, application of plant residues contributes to maintenance of microbial biomass in soil, also previously reported (Trinsoutrot et al., 2000; Cayuela et al., 2009). TOC and TN did not significantly change with incubation (data not shown). The activity of β -glucosidase significantly decreased with time in all application rates, while arylesterase activity showed no significant shifts after incubation. Nonetheless, D3 showed significant higher arylesterase activity than the other application rates. Arylesterase activity at the end of the incubation period was positively correlated with net N mineralization ($r=0.87$; $P<0.01$) and mineralized C- CO_2 ($r=0.90$; $P<0.01$), indicating that this enzyme may be involved in the degradation of more recalcitrant organic compounds when labile substances are being depleted. Urease activity decreased with time in CT, showed no significant differences ($P>0.05$) between initial and final days of incubation period in D1 and D2, and significantly increased ($P<0.05$) in D3. Protease activity significantly decreased with time in all application rates ($P<0.05$) except for D3 which showed no significant differences between 0 and 155 d of incubation. The activities of these two enzymes at the end of the incubation period were positively correlated with net N mineralization ($r=0.86$ and $r=0.79$, for urease and protease, respectively; $P<0.01$), confirming their active role in the mineralization of organic compounds.

Table 3.4 Changes in soluble C, soil microbial biomass C and enzyme activities after 155 days of incubation. Values are mean and standard deviation (n=3).

Soil property ^a	Day ^b	Control	Dose 1	Dose 2	Dose 3	<i>F</i> ^c
SC (mg kg ⁻¹)	0	320 ± 4a	383 ± 4b	425 ± 4c	455 ± 9 d	342.14***
	155	249 ± 10	261 ± 8	256 ± 9	254 ± 2	0.74ns
	<i>t</i>	10.68**	25.73**	41.05**	51.09**	
MBC (mg kg ⁻¹)	0	556 ± 37	499 ± 8	575 ± 29	556 ± 25	3.16ns
	155	293 ± 24a	344 ± 39b	361 ± 33b	413 ± 25c	10.89*
	<i>t</i>	9.33*	4.26*	4.87*	7.39*	
β-glucosidase (μmol PNP g ⁻¹ h ⁻¹)	0	0.21 ± 0.00	0.24 ± 0.03	0.24 ± 0.00	0.25 ± 0.01	1.89ns
	155	0.14 ± 0.04	0.14 ± 0.02	0.15 ± 0.01	0.16 ± 0.02	0.56ns
	<i>t</i>	8.99*	9.29*	36.85**	6.86*	
Arylesterase (μmol PNP g ⁻¹ h ⁻¹)	0	24.55 ± 1.8	24.51 ± 1.6	24.99 ± 0.79	25.52 ± 0.99	0.344ns
	155	24.55a ± 0.49	24.06a ± 0.88	25.60a ± 0.75	28.26b ± 1.11	14.94**
	<i>t</i>	0.04ns	0.36ns	-0.74ns	-3.09ns	
Urease (μmol NH ₄ ⁺ g ⁻¹ h ⁻¹)	0	1.15 ± 0.05	1.08 ± 0.07	1.14 ± 0.04	1.18 ± 0.06	1.68ns
	155	0.82a ± 0.08	0.91a ± 0.09	0.97a ± 0.09	1.39b ± 0.08	25.59***
	<i>t</i>	7.62*	2.69ns	2.45ns	-9.08*	
Protease (μmol NH ₄ ⁺ g ⁻¹ h ⁻¹)	0	0.98 ± 0.03a	1.12 ± 0.08b	1.17 ± 0.03b	1.14 ± 0.02b	8.431**
	155	0.83a ± 0.05	0.88a ± 0.02	1.04b ± 0.02	1.16c ± 0.05	45.124***
	<i>t</i>	4.52*	4.48*	9.47*	-2.61ns	

^a SC: soluble carbon; MBC: microbial biomass carbon ; PNP : *p*-nitrophenol.

^b *t* is calculated by paired-sample t-test for initial and final day of incubation within each dose. Significant at: **P*<0.05; ***P*<0.01; ****P*>0.001; ns: non significant.

^c *F*-value. Significant at: **P*<0.05; ***P*<0.01; ****P*>0.001; ns: non significant. Different letters indicate significant differences among doses within each day of incubation (*P*<0.05).

The decrease in enzyme activities after 155 d of incubation is due to depletion of organic compounds and limitation by the low availability of N; this trend has been previously reported (Geisseler et al., 2009; Henriksen and Breland, 1999). Nonetheless, an initial increase was supposed to take place in response to new available organic compounds. Cayuela et al. (2009) observed immediate increments in β -glucosidase activity after the application of wheat straw and cotton cardings proportional to the application rate. Geisseler et al. (2009) documented increments in soil protease activity during the first week of incubation after application of wheat residues. The addition of the high application rate of crop residue was the only one to maintain high activities of arylesterase, urease and protease after the incubation period, suggesting that lower application rates do not provide enough organic material to long-term activate enzyme activities to release available inorganic nitrogen.

3.4. CONCLUSIONS

C mineralization of pepper residues as soil amendment is proportional to the applied application rate. However, to maintain this mineralization, microorganisms have to immobilize most of the inorganic N mainly the first days of incubation at low application rates. This reflects that N is likely a limiting factor for microbial growth when pepper residues are added. As a consequence, high application rates of this type of crop residue should be applied to guarantee the release of available N for plants. The activities of soil enzymes involved in C and N cycles responded differently according to the application rate, indicating that only high application rates would increase capacity of soil to perform useful ecosystem functions. The study of the mid-term impact of residues is essential to be able to judge their potential adverse effects and optimize their use for the appropriate supply of nutrients and conservation of C stocks.

CHAPTER 4

EFFECT OF CHEMICAL FERTILIZERS

AND PEPPER RESIDUES

ON C CYCLE AND

BROCCOLI YIELD

CHAPTER 4

Effects of Chemical fertilizers and pepper residues on C cycle and broccoli yield

Abstract

We conducted a two-years experiment to evaluate the effects of fresh pepper residues (CR treatment) on soil properties and broccoli yield compared with the application of chemical fertilizers (CF treatment). Soil-state ^{13}C nuclear magnetic resonance spectroscopy (^{13}C NMR) was used to characterize the organic chemical composition of added residues and surface soil. We also measured total and soluble organic carbon and different biochemical properties associated with the C cycle. The results show that large application rates of CR increased microbial biomass carbon (MBC) and arylestersase and β -glucosidase activities compared to control. There was a significant increase in MBC for the CF treatment only with the greatest application rate. No differences in soil organic carbon composition and structure were found between treatments, with soil organic C, soluble C, basal soil respiration, arilesterase and β -glucosidase activities correlated positively with the soil carboxylic region and negatively with the aromatic region of the spectra. Broccoli yield was significantly higher for the second crop cycle with maximum values for the greatest application rates of CF and CR indicating that crop residues with a minimum amount of chemical fertilizers can be used to obtain an adequate yield. The use of pepper residues as an organic amendment improved biochemical properties of the soil with no negative impact on yield.

4.1 INTRODUCTION

Vegetable crop production in greenhouses has expanded over recent decades in Campo de Cartagena (south east Spain) and has become an important source of pollution. The growing of pepper is of great economic importance in the region. At the end of the growing season, pepper crop residues (CR) from greenhouses are removed, mainly because they could be a source of pathogens for the next growing season. In 2009 the area occupied by greenhouses producing peppers in Campo de Cartagena totaled to 1447 ha with a density of 25000 plants ha⁻¹ (Econet, 2013). On average each plant weighed 2.5 kg, so that more than 90000 t of pepper residues were produced in 2009. The residues are usually burned causing pollution, while agricultural soils lose soil organic matter. A possible solution to both issues would be the use of these residues as soil amendments to act as a source of nutrients for other crops. In addition, a return of CR to soils may increase carbon sequestration (Jarecki et al., 2005).

Much research has been carried out on the use crop residues as organic amendments, including those of wheat, maize, cotton gin, as well as others (Barzegar et al., 2002; Shafi et al., 2007; Ghosh et al., 2011). However, the use of pepper crop residues as a soil amendment has not been described in the literature although they are produced at large quantities in countries such as China, Mexico, Turkey, Indonesia, Spain and USA (USDA, 2008). The influence of CR on soil properties depends mainly on the biochemical composition of the residues, location in soil at the beginning of decomposition, total amount and size (Nelson and Oades, 1998; Barzegar et al., 2002). Soil quality is influenced to a large extent by soil organic matter (SOM) (Roldan et al., 2003), and it is crucial to study the effect of organic residues on soil properties relevant to the C cycle. The return of CR to agricultural soils can sustain soil organic carbon (SOC) (Kasongo et al., 2011), enhances biological activity and nutrient content and availability (Smith et al., 1993; Shafi et al., 2007), improves water conservation and crop yield while reducing erosion and nutrient losses (Bukert et al., 2000).

Differences in soil organic carbon (SOC) as a result of different soil management are difficult to measure over short and medium terms (Saffigna et al., 1989; Power et al., 1998). Therefore, it is necessary to measure not only the amount of SOC but also its quality. Nuclear Magnetic Resonance (NMR) spectroscopy is useful to determine changes in SOC quality (Kölbl and Kögel-knabner, 2004) as well as the effect of plant litter decomposition on soil characteristics (Kögel-Knabner, 2002). Soil microbial populations and enzymes are crucial in the degradation of SOM and nutrients cycling. As a result they

can be used as sensitive indicators that reflect the effects of land management practices on soil quality (Bandick and Dick, 1999). Microbial biomass and soil enzyme activities have been used for different purposes such as indicators of productivity and soil quality or potential capacity of soil to decompose organic wastes (Paolini, 2002). Short-term measurements of biomass and enzyme activities can reflect long-term trends in soil organic matter (Powlson et al., 1987).

A study of growing broccoli using CF or CR during two crop cycles has been carried out to (1) study the effects of fresh pepper crop residues as organic amendment versus applications of mineral fertilizers on soil properties relevant to the C cycle and broccoli yield; (2) assess if the application rate of CR affects crop yield; and (3) determine relationships between soil organic functional groups and soil microbial biomass and activity following application of CF and CR.

4.2 MATERIALS AND METHODS

4.2.1 STUDY SITE AND EXPERIMENTAL DESIGN

The study was located in “Campo de Cartagena”, south east of Spain (figure 4.1) (UTM: 4191030N, 68625016E). The climate is semiarid Mediterranean, with a mean annual temperature of 17°C and a mean annual rainfall of 300 mm. The soil is a Typic Petrocalcid (Soil Survey Staff, 2010) with loam texture. Soil characteristics are shown in Chapter 3 (table 3.1)

Figure 4.1 Location of study area in Murcia Region, SE Spain.

The experiment has a randomized design with three replications and each plot was 6x4 m². The crop grown in all plots was *Brassica oleracea* (25 plants m⁻²) under drip irrigation. The experiment spanned for two crop cycles (2008-2009 and 2009-2010). Two different treatments were used: chemical fertilizers (CF) and crop residues (CR). Three different application rates per treatment were used. Nitrogen was applied at 170 (CF1), 255 (CF2) and 383 kg N ha⁻¹ (CF3) as NH₄NO₃, Ca(NO₃)₂ and KNO₃. The CR was also applied at rates corresponding to 170 (CR1), 255 (CR2) and 383 kg N ha⁻¹ (CR3).

The pepper residues were mechanically crushed to <2 cm and air-dried in a greenhouse. They were weighted, spread out over each plot and incorporated into the soil (0-30 cm) with a rotovator. For CR1, CR2 and CR3 the amounts of dry residues applied were 8.13, 12.20 and 18.33 t ha⁻¹ for the first crop season, and 7.39, 11.09 and 16.66 t ha⁻¹ for the second one, depending on the N content of the residue (table 4.1).

Table 4.1 Chemical characteristics of the pepper residues for the two studied broccoli cycles. Values are mean ± standard deviation (n=5).

Properties	1 st crop cycle	2 nd crop cycle
Nt (g kg ⁻¹)	20.9 ± 2.9	22.9 ± 2.9
TOC (g kg ⁻¹)	480 ± 1	438 ± 13
C/N	23 ± 3	19 ± 2
P (g kg ⁻¹)	7.59 ± 0.78	6.02 ± 0.37
Ca (g kg ⁻¹)	17.6 ± 0.2	23.9 ± 6.2
Mg (g kg ⁻¹)	5.36 ± 0.38	7.23 ± 1.33
Na (g kg ⁻¹)	1.26 ± 0.61	1.89 ± 0.22
K (g kg ⁻¹)	30.1 ± 2.0	40.7 ± 3.2
Cu (mg kg ⁻¹)	8.6 ± 2.0	12.5 ± 1.0
Zn (mg kg ⁻¹)	32.7 ± 5.2	77.6 ± 11.8
Fe (mg kg ⁻¹)	160 ± 9	184 ± 58
Mn (mg kg ⁻¹)	34.0 ± 2.6	87.4 ± 18.8
Cellulose (%)	-	20.0 ± 0.1
Lignin (%)	-	17.9 ± 0.5
Soluble C (%)	-	3.4 ± 0.3

Nt: Total N; TOC: Total Organic C.

The amount of carbon applied by CR doses was: 3.90, 5.86 and 8.80 t C ha⁻¹ and 3.24, 4.86 and 7.30 t C ha⁻¹ for CR1, CR2 and CR3 in 2008 and 2009, respectively. An additional 170 kg N ha⁻¹ was applied as CF to all CR plots to guarantee initial crop requirements. A negative control (CT) was used without fertilizers or amendment. The application rate corresponding to CF2 and CR2 was the N recommended for broccoli by the local law (BORM 287, 2007), while the application rates 1 and 3 were 1.5 times smaller and greater,

respectively, than the recommended N level. One hundred kg ha⁻¹ of P, 300 kg ha⁻¹ of K and 80 kg ha⁻¹ of Ca were applied as KH₂PO₄, KNO₃ and Ca(NO₃)₂ to all plots except for the CT.

4.2.2 SOIL AND BROCCOLI SAMPLING

There were two soil sampling periods per crop cycle, one before addition of CR (October 2008 and 2009 – S1 and S3, respectively) and the other one immediately after the broccoli harvest (February 2009 and 2010 – S2 and S4, respectively). All plots were sampled at 0-30 cm (plough depth). Three random soil samples per plot were taken and homogenized to obtain a composite sample.

To calculate crop yield, all marketable broccolis from each plot were collected and weighed at the end of growing season.

4.2.3 SOIL PHYSICOCHEMICAL AND BIOCHEMICAL ANALYSIS

Soil pH and electrical conductivity (EC) were measured in deionised water (1:1 and 1:5 w/v, respectively). Particle size distribution was determined using the Robinson pipette method. Soil organic carbon (SOC) was analysed using the dichromate oxidation technique, while total nitrogen (Nt) was determined using the Kjeldahl digestion method (Hoeger, 1998). For equivalent calcium carbonate the volumetric method (Bernard calcimeter) was used and cation exchange capacity (CEC) was analysed according to Chapman (1965).

Since physicochemical properties varied little between treatments and application rate in the first growing season, several biochemical parameters associated with the C cycle were evaluated in the second season as they are usually more sensitive to changes (García et al., 2000). The microbial biomass carbon (MBC) was determined using the chloroform fumigation-extraction method of Vance et al. (1984). The non-fumigated fraction was considered as soluble carbon (SC). Basal soil respiration (BSR) was determined after four days of incubation at 55% at the maximum water holding capacity, following the method of Anderson (1982). The arylesterase activity was quantified following the method of Zornoza et al. (2009b). β -Glucosidase and β -Galactosidase activities were measured according to Tabatabai (1982) and Eivazi and Tabatabai (1988), respectively. Four eco-physiological ratios were also calculated: the metabolic quotient ($qCO_2 = BSR/MBC$), MBC/SOC , BSR/SOC and SC/SOC .

4.2.4 CROP RESIDUES ANALYSIS

The characteristics of pepper residues are shown in table 4.1. The residues were dried at 55 °C for 3 days, then ground and analysed for total nitrogen (Nt) according to the Kjeldahl method (Hoeger, 1998) and total organic carbon (TOC) (Golueke, 1977). Cellulose was determined following the procedure of Brendel et al. (2000). Lignin was determined according to Hatfield et al. (1994). Soluble organic carbon was extracted in water (1:500 w/v) and determined by UV-persulfate digestion.

4.2.5 SOLID-STATE ¹³C NMR ANALYSIS

¹³C CP/MAS-Nuclear Magnetic Resonance (NMR) (Varian Unity 300 spectrometer, Germany) was applied to samples S1 and S4. Dry soils were packed into cylindrical zirconia rotors with outside and internal diameters of 7 and 5.6 mm, respectively, and sealed with Kel-F caps. Measurements were performed at magic angle spinning (MAS) rates of 4 kHz, operating at 75.42 MHz. Spectra were acquired using a contact time of 1.5 ms, 4s recycle time, a 90° pulse of 6.7 µm, a spectral width of 50000 Hz and 60000 scans, and processed using a 100 Hz line-broad. The chemical shift regions used were: 0-50 mg kg⁻¹ (alkyl); 50-60 (methoxyl); 60-98 mg kg⁻¹ (O-alkyl); 98-112 mg kg⁻¹ (di-O-alkyl); 112-145 mg kg⁻¹ (aromatic); 146-163 mg kg⁻¹ (phenolic) and 163-210 mg kg⁻¹ (carboxyl) according to Kavdir et al. (2005). Areas of the chemical shift regions were measured by integration and were expressed as percentage of total area. The assignment of peaks was developed according to Nierop et al. (2001), Kögel-Knabner (2002), Carvalho et al. (2009) and Pontevedra-Pombal et al. (2001). An index of the extent of decomposition, the alkyl C/O-alkyl C ratio (A/O-A, Eq. 1), suggested by Baldock and Preston (1995) was calculated. The aromaticity index was expressed according to Kavdir et al. (2005) (Eq. 2).

$$A/O-A = \frac{\text{Alkyl C (0-50 ppm)}}{\text{Methoxyl C + O alkyl C + Di O alkyl C (50-110 ppm)}} \quad (1)$$

$$\text{Aromaticity (\%)} = \left[\frac{\text{Aromatic C + Phenolic C (112-163 ppm)}}{\text{Alkyl C + O alkyl C + Aromatic C + Phenolic C (0-163 ppm)}} \right] \times 100 \quad (2)$$

4.2.6 STATISTICAL ANALYSIS

Data were checked to ensure normal distribution using the Kolmogorov-Smirnov test and were submitted to one-way ANOVA to assess the differences between samplings and application rates. The separation of means was made according to Tukey's at $P < 0.05$.

Relationships among properties were studied using Pearson correlation coefficients. Statistical analysis were performed with SPSS for Windows, Version 17.0.

4.3 RESULTS

4.3.1 PHYSICOCHEMICAL ANALYSIS

Soil physicochemical properties did not change greatly between treatments (table 4.2). There was no significant effect of treatment on soil pH and water holding capacity for both crop seasons. Soil organic carbon (SOC) was lower in the samples taken after harvest in the first season, compared with initial values before application of fertilizers or residues (table 4.2). In the first cycle we found that final SOC (S2) significantly decreased in CT, CR1 and CR2 compared to S1.

4.3.2 SOLID-STATE ^{13}C NMR ANALYSIS

The spectrum for fresh non-decomposed pepper residue (figure 4.2) was dominated by O-alkyl (46.0%) and alkyl regions (22.8%) followed by methoxyl (9.5%), carboxyl (7.9%), di-O-Alkyl (7.6%), aromatic (4.6 %) and phenolic (1.5%) regions. The first signal was at 20 mg kg^{-1} and it is related to acetyl groups in hemicelluloses and lipids. A signal around 30 mg kg^{-1} indicated methylene C in long chain aliphatic structures (waxes, lipids, cutins and resins). At ca. 55 mg kg^{-1} we found a peak caused by methoxilic-C (from lignin and proteins). The carbohydrate region (60-112 mg kg^{-1}) represented almost 54% of the total signal intensity. The highest three peaks were ca. 64 mg kg^{-1} (hexose-C6 and pentose-C5), a dominant signal at 71 mg kg^{-1} (celluloses and hemicelluloses) and ca. 104 mg kg^{-1} (anomeric-C of polysaccharides and lignin). The maximum signal of aromatic-C was ca. 133 mg kg^{-1} and is predominantly classified as C-atoms of lignin. The phenolic region presented a peak at 147 mg kg^{-1} , also associated with lignin. The carboxylic region related to esters, COO-, COOH and amide-C developed a double peak around 167 and 172 mg kg^{-1} .

The relative signal intensities of soil samples are shown in table 4.3. Since no differences were found between S1 and S4, only data for the last sampling is shown. Samples displayed similar distribution of functional groups independently of the treatment. The spectra were dominated by alkyl-C structures (>42%), with a wide peak ca. 15-30 mg kg^{-1} (methylene structures). The methoxyl region (>6%) presented a peak ca. 56 mg kg^{-1} associated with lignin and hemicelluloses. The O-alkyl-C was the second most abundant region (>16%) with a signal around 70 mg kg^{-1} and the di-O-alkyl region (>2%;

104 mg kg⁻¹) was associated with carbohydrates. The aromatic (>14%; 130 mg kg⁻¹) and phenolic region (>6%; 153 mg kg⁻¹) were attributed to lignin-C. The signal ca. 168 mg kg⁻¹ was attributable to carboxyl/amide groups (>9%). The Alkyl group was higher than the O-alkyl group, as also reported by Ottenhof et al. (2007). The carboxyl region revealed a slightly greater signal for CR treatments at the greatest application rates.

Table 4.2 pH, water holding capacity (WHC) and soil organic carbon content (SOC) for both broccoli cycles. Data are shown as mean $\pm$ standard deviation (n=3).

Parameter	Sampling	Control	Chemical Fertilizers			Crop Residues + Chemical Fertilizers			F ^a
		CT	CF1	CF2	CF3	CR1	CR2	CR3	
pH	S1	7.79 0.10	7.62 $\pm$ 0.23	7.77 $\pm$ 0.03	7.72 $\pm$ 0.19	7.95 $\pm$ 0.09	7.91 $\pm$ 0.05	7.83 $\pm$ 0.15	2.036 ns
	S2	7.91 0.11	7.96 $\pm$ 0.07	7.77 $\pm$ 0.10	7.92 $\pm$ 0.10	8.04 $\pm$ 0.06	7.98 $\pm$ 0.02	7.89 $\pm$ 0.10	2.853 ns
	F ^a)	4.369 ns	5.981 ns	0.013 ns	2.882 ns	1.904 ns	4.056 ns	0.343 ns	
	S3	8.28 0.05	8.21 $\pm$ 0.15	8.3 $\pm$ 0.09	8.25 $\pm$ 0.06	8.26 $\pm$ 0.04	8.22 $\pm$ 0.06	8.21 $\pm$ 0.10	0.555 ns
	S4	8.30 0.05	8.29 $\pm$ 0.07	8.31 $\pm$ 0.03	8.30 $\pm$ 0.09	8.17 $\pm$ 0.06	8.19 $\pm$ 0.12	8.20 $\pm$ 0.10	1.513 ns
	F ^a)	0.000ns	0.751 ns	0.016 ns	0.431 ns	3.956 ns	0.085 ns	0.002 ns	
WHC (%)	S1	52.76 $\pm$ 2.77	52.85 $\pm$ 0.39	51.15 $\pm$ 1.40	52.42 $\pm$ 1.55	50.92 $\pm$ 4.49	50.25 $\pm$ 2.22	49.59 $\pm$ 1.73	0.861ns
	S2	52.3 $\pm$ 2.21	53.65 $\pm$ 2.14	51.60 $\pm$ 0.95	53.41 $\pm$ 1.90	53.71 $\pm$ 2.05	54.4 $\pm$ 1.60	52.21 $\pm$ 0.98	0.913 ns
	F ^a)	0.050 ns	0.406 ns	0.211 ns	0.493 ns	0.959 ns	6.928 ns	5.208	
	S3	52.84 $\pm$ 1.08	53.50 $\pm$ 1.32	51.10 $\pm$ 1.38	53.73 $\pm$ 1.62	53.82 $\pm$ 2.10	56.62 $\pm$ 1.15	54.37 $\pm$ 3.98	1.996 ns
	S4	53.33 $\pm$ 1.46	54.83 $\pm$ 1.30	53.27 $\pm$ 1.46	53.00 $\pm$ 0.21	56.26 $\pm$ 1.43	57.26 $\pm$ 1.30	55.71 $\pm$ 1.32	2.260 ns
	F ^a)	0.220 ns	1.554 ns	3.484 ns	0.606 ns	2.758 ns	0.406 ns	0.308 ns	
SOC (g kg ⁻¹)	S1	17.84 $\pm$ 0.83	20.69 $\pm$ 3.48	19.73 $\pm$ 2.80	20.59 $\pm$ 4.30	18.92 $\pm$ 1.42	19.72 $\pm$ 0.92	18.88 $\pm$ 1.95	0.471 ns
	S2	15.06 $\pm$ 1.20	16.96 $\pm$ 1.27	15.11 $\pm$ 0.92	16.40 $\pm$ 0.89	15.28 $\pm$ 1.70	15.22 $\pm$ 1.55	18.44 $\pm$ 1.37	3.400 ns
	F ^a)	10.907 *	3.028 ns	7.387 ns	2.720 ns	8.129 *	18.738 *	0.144 ns	
	S3	17.47 $\pm$ 1.32	19.65 $\pm$ 2.17	18.37 $\pm$ 3.39	17.20 $\pm$ 3.32	19.27 $\pm$ 1.14	18.09 $\pm$ 2.05	19.40 $\pm$ 3.68	0.413 ns
	S4	16.79 $\pm$ 2.22	19.10 $\pm$ 2.71	16.36 $\pm$ 1.19	16.90 $\pm$ 1.27	18.04 $\pm$ 0.93	17.86 $\pm$ 2.17	19.50 $\pm$ 2.34	1.137 ns
	F ^a)	0.075 ns	0.075 ns	0.942 ns	0.021 ns	2.088 ns	0.018 ns	0.002 ns	

^a F-value. ns: non significant differences among treatments ($P>0.05$).

Figure 4.2 Solid-state ^{13}C NMR-spectra of pepper residues and soil samples under different treatments.

Table 4.3 Relative contributions of C species to the total signal intensity in ^{13}C NMR, alkyl C/O alkyl C ratio (A/O-A) and aromaticity index for pepper and soil under different treatments from sampling 4.

Sample	Chemical shift range (mg kg^{-1})							A/ O-A	Aromatic Index (%)
	Alkyl	Meth	O- Alkyl	Di-O- Alkyl	Arom	Phen	Carbox		
Pepper	22.84	9.54	46.02	7.67	4.55	1.53	7.85	0.36	6.60
CT	42.29	6.80	16.25	3.31	15.60	6.53	9.22	1.60	24.38
CF1	41.50	7.21	16.39	2.72	13.08	6.73	12.37	1.58	22.61
CF2	43.99	7.42	16.35	2.30	14.21	6.58	9.15	1.69	22.88
CF3	42.54	7.46	16.96	2.87	14.66	5.87	9.64	1.56	22.72
CR1	44.85	6.60	15.11	3.55	13.75	6.82	9.33	1.78	22.69
CR2	40.85	6.57	17.74	2.29	14.58	6.12	11.86	1.54	23.48
CR3	42.14	7.11	15.88	2.63	13.02	6.73	12.50	1.64	22.57

CT: control; CF: Chemical Fertilizers; CR: Crop residues + Chemical Fertilizers.

4.3.3 SOIL BIOCHEMICAL PARAMETERS

Biochemical soil properties were more sensitive to treatments than chemical characteristics. None of the measured parameters showed significant differences among application rates in S3 (table 4.4). However, in the final sampling (S4), MBC, BSR, arylesterase and β -glucosidase activities and the MBC/SOC ratio were significantly different depending on the application rate. MBC was significantly greater in CR3 compared to CF1. β -glucosidase and arylesterase values were greatest in CR3 compared with control, or with the CF treatment (β -glucosidase only). No differences among application rates were found in β -galactosidase activity or ecophysiological ratios, except for MBC/SOC

With regard to variations between the beginning and end of the growing season, MBC, arylesterase and β -glucosidase activities and MBC/SOC had the greatest values after harvest in some application rates, while SC, qCO_2 and SC/SOC tended to decrease. We observed a decrease of SC in all application rates except CT, following the pattern observed for SOC. MBC increased especially in CF3, CR2 and CR3. Arilesterase and β -glucosidase activities were higher in CR2 and CR3. qCO_2 had significantly lower values in S4 for CR3. The SC/SOC ratio decreased in CF1, CF2, CR1 and CR2 in the final sampling compared with initial values.

4.3.4 BROCCOLI YIELD

Broccoli yield in CR plots was greatest in the second growth cycle. Significant differences among application rates were only found in the second season, with values for CF3 and CR3 greater than those of control and CF1 (figure 4.3).

Table 4.4 Soil biochemical properties and eco-physiological ratios for initial (S3) and final (S4) sampling in the second crop cycle. Data are shown as mean $\pm$ standard deviation (n=3).

Parameter ^a		Control	Chemical Fertilization			Crop Residues + chemical fertilization			F ^b
		CT	CF1	CF2	CF3	CR1	CR2	CR3	
SC (mg kg ⁻¹)	S3	192 $\pm$ 35	212 $\pm$ 14	218 $\pm$ 19	230 $\pm$ 31	235 $\pm$ 24	215 $\pm$ 24	215 $\pm$ 25	0.885 ns
	S4	135 $\pm$ 40	151 $\pm$ 8	140 $\pm$ 15	143 $\pm$ 5	154 $\pm$ 8	147 $\pm$ 9	162 $\pm$ 15	2.037 ns
	F	6.939 ns	36.918 **	28.323 **	22.159 **	29.278 **	20.657 *	9.599 *	
MBC (mg kg ⁻¹)	S3	230 $\pm$ 150	154 $\pm$ 63	162 $\pm$ 104	103 $\pm$ 64	322 $\pm$ 206	153 $\pm$ 42	79 $\pm$ 60	1.558 ns
	S4	269ab $\pm$ 50	217a $\pm$ 55	259ab $\pm$ 30	259ab $\pm$ 45	347ab $\pm$ 13	318ab $\pm$ 82	373b $\pm$ 42	3.992 *
	F	0.179 ns	1.685 ns	2.375 ns	11.485 *	0.044 ns	9.489 *	25.710 **	
BSR (mg C-CO ₂ kg ⁻¹ h ⁻¹)	S3	1.26 $\pm$ 0.23	1.43 $\pm$ 0.09	1.51 $\pm$ 0.37	1.26 $\pm$ 0.56	1.40 $\pm$ 0.27	1.28 $\pm$ 0.29	1.34 $\pm$ 0.17	0.288 ns
	S4	0.89ab $\pm$ 0.31	1.36ab $\pm$ 0.15	0.70a $\pm$ 0.23	1.04ab $\pm$ 0.29	1.65b $\pm$ 0.30	1.28ab $\pm$ 0.51	1.72b $\pm$ 0.42	3.918 *
	F	2.714 ns	0.561 ns	10.474 **	0.353 ns	1.131 ns	0.000 ns	2.124 ns	
Arylesterase (μ mol PNP g ⁻¹ h ⁻¹)	S3	22.2 $\pm$ 0.82	21.91 $\pm$ 4.31	23.47 $\pm$ 2.91	23.32 $\pm$ 3.97	24.16 $\pm$ 0.60	22.33 $\pm$ 1.13	22.64 $\pm$ 2.50	0.270 ns
	S4	22.16a $\pm$ 2.86	26.87ab $\pm$ 1.52	23.36ab $\pm$ 0.70	24.10ab $\pm$ 3.77	25.98ab $\pm$ 1.23	26.27ab $\pm$ 0.56	28.38b $\pm$ 1.09	3.529 *
	F	0.000 ns	3.546 ns	0.004 ns	0.061 ns	5.293 ns	29.124 **	13.286 **	
β -Glucosidase (μ mol PNP g ⁻¹ h ⁻¹)	S3	0.48 $\pm$ 0.10	0.58 $\pm$ 0.05	0.56 $\pm$ 0.09	0.57 $\pm$ 0.05	0.52 $\pm$ 0.11	0.53 $\pm$ 0.09	0.58 $\pm$ 0.01	0.728 ns
	S4	0.48a $\pm$ 0.07	0.61a $\pm$ 0.06	0.58a $\pm$ 0.07	0.54a $\pm$ 0.04	0.63ab $\pm$ 0.10	0.68ab $\pm$ 0.02	0.85b $\pm$ 0.14	6.525**
	F	0.003 ns	0.395 ns	0.00 ns	0.696 ns	1.672 ns	8.303 *	10.421 *	
β -Galactosidase (nmol PNP g ⁻¹ h ⁻¹)	S3	8.16 $\pm$ 7.08	4.61 $\pm$ 6.67	15.99 $\pm$ 3.04	11.06 $\pm$ 7.67	7.79 $\pm$ 4.46	7.54 $\pm$ 3.67	6.11 $\pm$ 8.03	1.137 ns
	S4	17.97 $\pm$ 6.91	17.75 $\pm$ 12.30	18.20 $\pm$ 2.25	46.9 $\pm$ 17.40	30.64 $\pm$ 12.50	18.35 $\pm$ 10.19	25.35 $\pm$ 16.31	2.384 ns
	F	1.520 ns	0.377 ns	0.094 ns	6.037 ns	5.893 ns	3.546 ns	4.866 ns	
qCO ₂ (mg C-CO ₂ g ⁻¹ MBC h ⁻¹)	S3	6.82 $\pm$ 3.73	10.24 $\pm$ 3.45	11.01 $\pm$ 4.51	14.79 $\pm$ 6.76	8.62 $\pm$ 10.05	9.03 $\pm$ 3.88	22.03 $\pm$ 10.08	1.788 ns
	S4	3.26 $\pm$ 0.91	6.60 $\pm$ 2.07	2.69 $\pm$ 0.73	4.21 $\pm$ 1.84	4.77 $\pm$ 1.03	4.43 $\pm$ 2.42	4.63 $\pm$ 1.10	1.896 ns
	F	2.591 ns	2.458 ns	9.956 **	6.853 ns	0.436 ns	3.040 ns	8.653 *	
MBC/SOC (%)	S3	1.30 $\pm$ 0.77	0.77 $\pm$ 0.23	0.87 $\pm$ 0.46	0.57 $\pm$ 0.29	1.67 $\pm$ 1.07	0.87 $\pm$ 0.33	0.40 $\pm$ 0.27	1.742 ns
	S4	1.63ab $\pm$ 0.38	1.13a $\pm$ 0.12	1.58ab $\pm$ 0.13	1.53ab $\pm$ 0.16	1.93b $\pm$ 0.08	1.81b $\pm$ 0.56	1.92b $\pm$ 0.16	2.980 *
	F	0.446 ns	5.547 ns	6.603 ns	24.501 **	0.168 ns	6.288 ns	70.605 **	
BSR/SOC (g kg ⁻¹ h ⁻¹)	S3	0.072 $\pm$ 0.009	0.073 $\pm$ 0.005	0.082 $\pm$ 0.011	0.071 $\pm$ 0.024	0.073 $\pm$ 0.041	0.070 $\pm$ 0.009	0.070 $\pm$ 0.01	0.324 ns
	S4	0.075 $\pm$ 0.012	0.076 $\pm$ 0.008	0.092 $\pm$ 0.016	0.073 $\pm$ 0.029	0.078 $\pm$ 0.020	0.071 $\pm$ 0.011	0.069 $\pm$ 0.004	0.634 ns
	F	0.156 ns	0.227 ns	0.726 ns	0.008 ns	0.156 ns	0.019 ns	0.046 ns	
SC/SOC (%)	S3	1.10 $\pm$ 0.18	1.08 $\pm$ 0.05	1.21 $\pm$ 0.2	1.39 $\pm$ 0.41	1.22 $\pm$ 0.11	1.20 $\pm$ 0.13	1.14 $\pm$ 0.27	0.643 ns
	S4	0.84 $\pm$ 0.10	0.80 $\pm$ 0.08	0.86 $\pm$ 0.04	0.85 $\pm$ 0.04	0.86 $\pm$ 0.09	0.83 $\pm$ 0.05	0.84 $\pm$ 0.05	0.237 ns
	F	4.744 ns	24.165 **	9.350 **	5.138 ns	19.255 *	20.192 *	3.644 ns	

^a SC: soluble C, MBC: microbial biomass C, BSR: basal soil respiration, PNP: p-nitrophenol, SOC: soil organic C.

^b F-value. Significant at: * P <0.05; ** P <0.01; ns: non significant. Different letters indicate significant differences (P <0.05) among rates within a sampling.

Figure 4.3 Broccoli yield (kg ha⁻¹) for both crop cycles. Error bars denote standard deviation. Different letters indicate significant differences ($P < 0.05$) among application rates within each crop cycle. Means with no letter are not significantly different ($P > 0.05$).

4.4 DISCUSSION

The small effect of treatments on soil pH is likely due to the buffering capacity of calcareous soils (Nielsen et al., 1998). Although Acosta-Martinez and Tabatabai, (2000) reported that increases in pH stimulated microbial activity to result in an increase in 13 soil enzyme activities. We did not find any correlation between pH and biochemical parameters; similar results were obtained by García et al. (1994). This may be due to the absence of significant shifts in soil chemical properties with treatments.

Soil organic carbon tended to decrease with time. In semi-arid areas SOC decreases in surface layers as a result of the continuous loss of organic matter by oxidation (López-Garrido et al., 2011). Crop cultivation per se also is known to cause a net depletion of SOC in soils (Six et al., 2000) depending on intensity of cropping. Losses were greatest in soils treated with CF at the end of the experiment, suggesting that short-term application of organic amendments may be beneficial to avoid losses of organic matter in soils, as also reported by Pascual et al. (1998). The rate of decomposition of CR is low, supported by the C/N ratio of pepper residues, cellulose and lignin content and NMR results. A C/N ratio higher than 15-20 indicates a slow rate of decomposition (Brady and Weil, 2008).

Pepper residues have a high concentration of cellulose and hemicellulose, which are the most difficult compounds to degrade among the polysaccharides and lignin whose degradation is even slower (Cook and Allan, 1992). As a consequence, CR did not improve the soil C status in short-term as previously reported by Mugwe et al. (2009).

SC correlated (table 4.5) with SOC and SC/SOC ($r=0.61$ and 0.82), supporting that losses in SOC may be due to mineralization of soluble carbon sources such as soluble carbohydrates (Lorenz et al., 2006). Pepper residues also contain readily degradable compounds such as saccharides of low molecular weight (supported by a peak at 64 mg kg^{-1} in NMR analysis corresponding to hexoses and pentoses) and a small amount of SC. According to Carvalho et al. (2009) the biodegradation of the majority of hydrosoluble and readily decomposable organic material occurs during the initial 60 days and mineralization of C is higher than its accumulation.

The soil treated with the greatest level of CR had values of MBC significantly greater than CF1 indicating a positive effect of the organic amendment on soil microbial biomass in agreement with Trinsotrou et al. (2000) and Tejada et al. (2006). This confirms that in order to increase soil organic matter and maintain enzymatic activity at a high level it is necessary to add sufficient organic matter (Pascual et al., 1998). A high MBC/SOC ratio, indicating a greater population maintained per unit of organic carbon, was observed in CR application rates which had positive effects on crop yield with a significant correlation ($r=0.64$). The fact that SC decreased in the final sampling indicates mineralization of labile compounds, also supported by the negative correlations between SC and MBC ($r=-0.49$), and MBC/SOC ($r=-0.61$). SC is the most accessible source of carbon for microorganisms when microbial populations increase, and thus SC usually decreases due to the metabolic activity of microorganisms.

BSR and the metabolic quotient ($q\text{CO}_2$) reflect soil microbial activities and are affected by the environmental changes (Álvarez et al., 1995). BSR consists of heterotrophic respiration which is associated with the decomposition of soil organic matter which correlates positively with β -glucosidase activity ($r=0.52$), indicating a relationship between degradation of carbohydrates and microbial activity. Greater BSR in the final sampling in some plots amended with CR may indicate that carbohydrates from pepper could act as energy sources for microbial respiration. No correlation was found between SC and BSR, although labile carbon is the first source of carbon for microorganisms. However, BSR was positively correlated with SOC ($r=0.53$).

Table 4.5 Correlation coefficients (r values) for relationships between soil properties and broccoli yield.

Parameters ^a	WHC	SOC	SC	MBC	BSR	qCO ₂	MBC /SOC	SC/ SOC	Ariles	β-Gluc	Galact	Yield
CE	0.53*	0.40*	0.80*	-0.35	0.30	0.51 ⁺	-0.44 ⁺	0.62*	0.27	0.17	-0.51*	0.34
WHC	-	-0.43*	-0.30	0.22	-0.01	-0.16	0.19	-0.26	0.09	0.327	-0.02	-0.23
SOC		-	0.61 ⁺	0.20	0.53*	0.03	-0.04	-0.30	0.22	0.41 ⁺	-0.04	-0.07
SC			-	-0.49 ⁺	0.31	0.70*	-0.61*	0.82*	-0.22	-0.02	-0.49*	0.06
MBC				-	0.13	-0.76*	0.94*	-0.60*	0.34	0.33	0.43 ⁺	0.35
BSR					-	0.19	-0.07	-0.01	0.33	0.52*	-0.05	0.14
qCO ₂						-	-0.82*	0.69*	-0.15	0.03	-0.34	-0.01
MBC/SOC							-	-0.60*	0.27	0.13	0.41 ⁺	0.64 [•]
SC/SOC								-	0.32	-0.23	-0.47 ⁺	0.12
Ariles									-	0.55*	0.25	0.34
β-Gluc										-	0.27	0.31
Galacto											-	0.35
Yield												-

^a WHC: water holding capacity; SOC: soil organic carbon; SC: soluble carbon; MBC: microbial biomass carbon; BSR: basal soil respiration; Ariles: arilesterase activity; β-Gluc: β-glucosidase; Galacto: Galactosidase; Significant at: [•]*P*<0.05, ⁺*P*<0.01, **P*<0.001.

These findings together with the lack of differences in the BSR/SOC ratio in all application rates and sampling dates support the influence of total organic carbon content in the mineralization rates under this crop. CR application rates, expressed as applied carbon amount correlated positively with SC, MBC and BSR ($r=0.64$; 0.63 and 0.57 , respectively), suggesting that CR incorporation stimulates the microbial population by releasing easily degradable compounds. The qCO_2 is a quotient which indicates the metabolic efficiency of microbial populations. Decreases in qCO_2 are normally related to less soil disturbance and stress, and increases in microbial efficiency (Leita et al., 1999). However, here we hypothesize that decreases in qCO_2 may be mainly due to the decline in the pool of labile C substrates as described by Zornoza et al. (2007), and supported by the positive correlation with SC ($r=0.70$) and SC/SOC ($r=0.69$).

According to Pascual et al. (1998), hydrolase activities involved in the C cycle are positively influenced by large amounts of carbonated sources coming from the organic amendments. The plots amended with CR3 had greater values of β -glucosidase after harvest compared with CF. β -glucosidase catalyzes the hydrolysis of the long carbohydrate chains of β -D-glucosides to form β -D-glucose, so it is involved at the end of the process of cellulose and hemicelluloses decomposition. The increase in this activity with CR application confirms that this material contains carbohydrates, supported by a high signal of CR in the O-alkyl region. Arylesterase catalyzes the hydrolysis of phenolic esters. The pepper residue has few of these compounds (aromatic and phenolic region of spectra) and the increase of this activity was comparatively smaller than that produced in β -glucosidase activity. These two enzyme activities had greater values from the final sampling at CR2 and CR3, with a strong correlation with CR application rates ($r=0.85$ and 0.84); this confirms the positive effect of CR addition on biochemical processes. The positive correlations found between these two enzyme activities ($r=0.55$) indicate a similar response of microorganisms to these type of substrates. β -galactosidase catalyzes the hydrolysis of disaccharides, present in the pepper residue, but this activity did not increase with CR application, although a negative relationship was found between β -galactosidase activity and EC ($r=-0.51$), which may be explained by the toxicity of specific ions that may have had a significant influence on enzyme activities (García et al., 2000). No correlations were found between the three enzyme activities and MBC. Thus, it is likely that not only microorganisms but also plants are releasing enzymes to use the organic amendment.

A positive correlation between C applied as CR in the different application rates and the soil carboxylic region ($r=0.89$) was found, suggesting that application of CR may be related to increments in carboxylic functional groups. Carboxylic groups have superficial variable charges, which can adsorb and retain soil nutrients. The positive correlation between SOC, SC, BSR, qCO_2 , arilesterase and β -glucosidase activities with the soil carboxylic region (table 4.6), indicates the incorporation of easily decomposable substances like carbohydrates in the CR, with stimulation of microbial activity and the release of enzymes to decompose these compounds.

Table 4.6 Correlation coefficients (r-values) for relationships between soil properties, broccoli yield, soil chemical shift regions and CR and CF application rates.

Parameter ^a	Alkyl (0-50)	O-Alkyl (50-112)	Aromatic (112-163)	Carboxylic (163-190)	Arom index	CR	CF
CE	0.03	-0.70*	-0.32	0.15	-0.32	0.43	-0.03
WHC	-0.04	-0.33	0.20	0.08	0.31	-0.06	-0.50
SOC	-0.20	-0.34	-0.43*	0.49*	-0.27	0.48	-0.07
SC	-0.03	-0.49*	-0.52	0.46*	-0.41	0.64*	0.09
MBC	0.08	-0.52*	-0.22	0.24	-0.15	0.63*	-0.03
BSR	-0.04	-0.60+	-0.50*	0.47*	-0.38	0.57*	0.01
qCO_2	-0.24	-0.19	-0.45*	0.46*	-0.32	0.35	0.03
MBC/ SOC	0.23	-0.39	0.09	-0.09	0.06	0.31	-0.03
SC/ SOC	0.24	-0.02	0.09	-0.23	-0.02	-0.02	0.13
Ariles	-0.23	-0.40	-0.67+	0.65+	-0.49*	0.84*	0.18
β -Gluc	-0.18	-0.46*	-0.60+	0.63+	-0.41	0.85*	0.28
Galacto	0.19	0.16	-0.13	-0.20	-0.29	0.15	0.61*
Yield	0.13	-0.10	-0.38	0.09	-0.44*	0.69*	0.70*
Alkyl	-	-0.37	0.12	-0.72*	-0.27	-0.23	0.32
O-Alkyl		-	0.22	-0.18	0.19	-0.29	0.87
Aromatic			-	-0.69*	0.90*	-0.96*	-0.61*
Carboxylic				-	-0.31	0.89*	-0.04
Arom index					-	-0.80+	-0.81+
CR						-	-
CF							-

^aArom: aromaticity; CR is CR application rates expressed as C input, CF is CF application rates expressed as N input.

Significant at: * $P<0.05$, + $P<0.01$, * $P<0.001$.

The negative correlation detected for SOC, SC, BSR, qCO_2 , arilesterase and β -glucosidase activities and CR and CF application rates with the soil aromatic region (table 4.6), confirms the recalcitrant nature of aromatic compounds. Thus, the application of high

doses of fertilizers or residues contributes to the decrease in the proportion of recalcitrant soil compounds owing to the stimulation of the microbial activity which increases the proportion of labile compounds. The general low aromaticities (low soil aromatic region and aromaticity index) indicate that this soil was probably in the early stages of humification or lacked highly aromatic structures (Mathers and Xu, 2003).

3.5 CONCLUSIONS

Incorporation of pepper residues into a soil, preferably at the highest application rate (CR3: 7.2 Mg C per hectare and year), prior to planting of crop, increased the labile organic compounds available to decomposer communities. This residue also contributes to mitigating the decrease in SOC and SC, and increase microbial biomass carbon (MBC), arylestersase and β -glucosidase activities compared to control, which have no negative effects on broccoli yield.

Broccoli yield was similar with the largest application rates of CF and CR (CF3 and CR3) indicating that crop residues with minimal chemical fertilizers, can be used to obtain an adequate yield.

The use of CR can represent economic savings for farmers (more than 200 € ha⁻¹ using CR3 instead of CF3; average price of chemical fertilizers in 2009 in Spain).

CHAPTER 5

EFFECT OF CHEMICAL FERTILIZERS
AND PEPPER RESIDUES
ON SOIL FERTILITY AND
NUTRITIONAL STATUS OF CROP

CHAPTER 5

Effects of chemical fertilizers and pepper residues on soil fertility and nutritional status of crop

Abstract

Effects of pepper crop residues as amendment and their optimal application rates on an agricultural soil have been sparse studied. A comparative study of the development of a broccoli crop has been conducted using chemical fertilizers (CF treatment) and fresh pepper crop residues (CR treatment) at different application rates during two cycles. We measured soil chemical and biochemical properties and broccoli yield, quality parameters and nutritional status. We found that CR at highest application rate increased total N (Nt), potentially mineralizable N (Nmin), microbial biomass N (MBN), Nmin/Nt, Nmin/Nsol and urease and alkaline phosphatase activities compared to CF treatment and control (CT). The maximum broccoli yields were obtained in CF3 (383 kg N ha⁻¹ applied as CF) and all CR application rates, especially CR3 (383 kg N ha⁻¹); this corresponded with season available N (initial available N plus available N applied as CF or CR) of 545 - 598 kg N ha⁻¹. Results demonstrated that pepper CR at highest rate (16.7 – 19.2 t ha⁻¹), with a minimum chemical fertilization enhances crop yield and quality, with significant increments in soil quality and fertility compared to chemical fertilization.

5.1 INTRODUCTION

The *Campo de Cartagena* is a semiarid agricultural land in SE Spain and is one of the main horticulture growing areas in Europe (Álvarez-Rogel et al., 2006). In agricultural areas nutrients are removed from the soil by crop uptake, leaching and erosion (Ghosh et al., 2011). As a result of soil degradation, chemical fertilizers (CF) has become an essential input for crop productivity in most areas of the world, in an attempt to give back to the soil what was taken (Ordóñez et al., 2001). In *Campo de Cartagena*, vegetable crop yield has undergone an enormous expansion over the last decades, especially lettuce and broccoli. In greenhouses, mainly pepper is grown as monocrop. Pepper crop residues (CR) from greenhouses (leaves, roots, stems and fruits) should be removed to facilitate the continuity of crop yield. Since there are no procedures to minimize the huge amount of CR or to reuse them, the habitual alternative practice is to burn them. However, returning CR into the soil can improve soil physical properties, nutrient availability and crop yield (Lal, 1980; Aggarwal et al., 1997). Likewise, applying organic amendments to soil not only increases the organic carbon content but also has a series of positive effects on microbial proliferation and activity (Tejada et al., 2006). Decomposition of plant residues in soil releases essential nutrients required for plant and microbial growth (Roldán et al., 2003).

An extensive amount of vegetable residues has been studied as an organic amendment such as wheat, mungbean, maize, straw, among others while the use of pepper CR as a soil amendment has not been described yet, although they are produced at high quantities in countries such as China, Mexico, Turkey, Indonesia, Spain and USA (USDA, 2008).

Biochemical parameters are used because of their highest sensitivity to changes (García et al., 2000). Soil microbial populations and enzymes are crucial in the degradation of organic compounds and for the cycling of nutrients. As a result they can be used as sensitive indicators that reflect the effects of land management practices on soil fertility (Bandick and Dick, 1999). Soil microbial biomass, soil enzyme activities and biochemical quotients have been used for different purposes such as indicators of productivity and soil quality or potential rate of soil to decompose organic wastes (Paolini, 2002).

In this study, CF and pepper CR were used to cultivate broccoli. Applied N regarded as optimal for broccoli varies over a wide range depending on differences in soil fertility status, climatic conditions, plant density, harvest length or methods of cultivation among others (Zebarth et al., 1995; Vagen, 2005). Thereby, many studies showed the highest broccoli yield with an N fertilization rate ranged mainly from 224 to 558 kg N ha⁻¹ (Dufault

and Waters, 1985; Zebarth et al., 1995), but even lower values have been found. For that, N fertilization rates recommendations must be based not only on crop yield but also on crop quality and inorganic N content in soils to prevent environmental risks.

According to the previous approaches a comparative study of the development of a broccoli crop using CF or CR during two crop cycles has been carried out, with the following objectives: (1) to study the effects of fresh pepper crop residues as organic amendment on soil fertility, microbial biomass and activity compared to mineral fertilization at different application rates; (2) to assess if CR treatment affects broccoli yield and quality or crop nutritional status; (3) to determine if CR could partially replace CF with positive effects on soil and crop.

5.2 MATERIALS AND METHODS

5.2.1 STUDY SITE AND EXPERIMENTAL DESIGN

The study was located in *Campo de Cartagena*, SE of Spain (UTM: 4191030N, 68625016E). The climate is semiarid Mediterranean, with a mean annual temperature of 17°C and a mean annual rainfall of 300 mm. The soil is a Typic Petrocalcic (Soil Survey Staff, 2010) with loam texture. Soil characteristics are shown in table 5.1.

The experimental site was a randomized design with 3 replication and plot size 6x4 m². The crop grown was broccoli (*Brassica oleracea* L. var. *Italica*) cv. Marathon under drip irrigation. Broccoli was planted in October and harvested in February (between 97 and 111 days after transplanting) with density of 5 plants m⁻². The experiment spanned for two crop cycles (2008-2009 and 2009-2010). Two different treatments were used: chemical fertilization (CF) and crop residues (CR). Three different application rates per treatment were applied. Application rate 2 was the N recommended for broccoli crop suggested by the local law (BORM 287, 2007), while application rates 1 and 3 were 1.5 times smaller and greater, respectively. CF application rates were 170 (CF1), 255 (CF2) and 383 kg N ha⁻¹ (CF3) applied as Ca(NO₃)₂, KNO₃ and NH₄NO₃. We applied NH₄NO₃ in different amounts to get CF1, CF2 and CF3 rates. CR application rates were 170 (CR1), 255 (CR2) and 383 kg N ha⁻¹. (CR3) applied as crop residues. A control (CT) was used without fertilizers or amendment. According to local recommendations, 100 kg ha⁻¹ of P, 300 kg ha⁻¹ of K and 80 kg ha⁻¹ of Ca were applied as KH₂PO₄, KNO₃ and Ca(NO₃)₂ to all plots except for the CT.

Table 5.1 Soil characteristics of the study site. Values are mean $\pm$ standard deviation (n=24).

Soil properties	0 - 30 cm	30 - 60 cm
Sand (g kg ⁻¹)	407 $\pm$ 25	352 $\pm$ 32
Silt (g kg ⁻¹)	356 $\pm$ 24	399 $\pm$ 28
Clay (g kg ⁻¹)	237 $\pm$ 27	249 $\pm$ 18
Textural class	Loam	Loam
pH	7.81 $\pm$ 0.15	7.79 $\pm$ 0.34
Electrical conductivity (μ S cm ⁻¹)	921 $\pm$ 172	657 $\pm$ 145
CaCO ₃ (g kg ⁻¹)	584.4 $\pm$ 31.8	556.7 $\pm$ 15.27
Soil Organic Carbon (g kg ⁻¹)	19.34 $\pm$ 2.38	9.51 $\pm$ 1.91
Total Nitrogen (g kg ⁻¹)	1.52 $\pm$ 0.13	0.65 $\pm$ 0.18
Cation Exchange Capacity (cmol ⁺ kg ⁻¹)	7.95 $\pm$ 0.72	6.27 $\pm$ 0.80
Exchangeable Ca (g kg ⁻¹)	4.51 $\pm$ 0.27	4.06 $\pm$ 0.09
Exchangeable Mg (g kg ⁻¹)	1.70 $\pm$ 0.03	1.19 $\pm$ 0.24
Exchangeable Na (g kg ⁻¹)	0.75 $\pm$ 0.10	0.85 $\pm$ 0.45
Exchangeable K (g kg ⁻¹)	2.26 $\pm$ 0.22	1.09 $\pm$ 0.37
Available Cu (mg kg ⁻¹)	0.76 $\pm$ 0.06	0.73 $\pm$ 0.06
Available Zn (mg kg ⁻¹)	2.07 $\pm$ 0.04	0.69 $\pm$ 0.16
Available Fe (mg kg ⁻¹)	3.30 $\pm$ 0.12	2.20 $\pm$ 0.24
Available Mn (mg kg ⁻¹)	16.59 $\pm$ 0.82	8.55 $\pm$ 0.43

To get the application rates CR1, CR2 and CR3, the amounts of dry residues applied were 8.13, 12.20 and 18.33 t ha⁻¹ for the first crop season, and 7.39, 11.09 and 16.66 t ha⁻¹ for the second season, depending on the N content of the residue. Additionally, a minimal chemical fertilization was applied in CR plots consisting of 170 kg N ha⁻¹ per cycle to guarantee the initial requirements of N for the crop. Crop residues (*Capsicum annuum* L.) were broadcast evenly over each plot and incorporated with a rotovator. Characteristics of pepper residues are shown in chapter 4, table 4.1.

5.2.2 SOIL AND BROCCOLI SAMPLING

Two soil samplings per crop cycle were carried out, one before adding CR (October 2008 and 2009 – S1 and S3, respectively) and the other one after broccoli harvest (February 2009 and 2010 – S2 and S4, respectively). All plots were sampled at 0-30 cm (plow layer) and 30-60 cm. Three random soil samples per plot and depth were taken, which were homogenised to obtain a composite sample. Samples were air-dried, sieved through a 2 mm and stored at room temperature.

5.2.3 ANALYTICAL METHODS

Since physicochemical properties varied little among samplings and application rates in the first growing season, several biochemical parameters were evaluated in the second season. Soil pH and electrical conductivity (EC) were measured in deionised water (1:1 and 1:5 w/v, respectively). Particle size distribution was determined using the pipette method. Equivalent calcium carbonate was determined by the volumetric method (Bernard calcimeter). Soil organic carbon (SOC) was determined by $K_2Cr_2O_7$ oxidation, while total nitrogen (Nt) was determined using the Kjeldahl digestion method (Hoeger, 1998). Available P was measured using Olsen method (Watanable and Olsen, 1965), and cation exchange capacity (CEC) by Chapman (1965). Exchangeable cations (Ca, Mg, Na, K) were determined in the extract used to determine CEC. Available metals (Cu, Zn, Fe, Mn) were extracted using DTPA (1:2 soil-extractant ratio) (Lindsay and Norvell, 1978). Exchangeable cations and bioavailable metals concentrations were measured by ICP-MS (7500 CE, Agilent). NO_3^- and NH_4^+ contents were extracted with 2M KCl according to Keeney and Nelson (1982) in 1:10 soil:extractant ratio. Then NO_3^- was measured by UV-visible espectrophotometry according to Sempere et al. (1993) and NH_4^+ colorimetrically according to Kandeler and Gerber (1988). Available N is the sum of NO_3^- -N and NH_4^+ -N. We calculated the season available N, which is the amount of N applied as CF or CR plus the available N at the beginning of the season. Available N from CR was calculated considering a mineralization rate of 30%, according to the previous mineralization experiment (chapter 3).

The microbial biomass nitrogen (MBN) was determined using the chloroform fumigation-extraction method (Vance et al., 1984), and measured with an automatic Analyser for liquid samples (Shimadzu TOC- 5050A). The non-fumigated fraction was considered as soluble nitrogen (Nsol). Potentially mineralizable N (Nmin) was determined according to Ribó et al. (2003). Nmin is the difference between available N after and before incubation (15 days at 25°C). Urease activity was measured according to the method of Nannipieri et al. (1978). The activity of N- α -benzoyl-L-argininamide protease (BAA-protease) was assayed according to Bonmatí et al. (1988). Alkaline phosphatase activity was assayed by the method of Tabatabai and Bremner (1969). Two eco-physiological ratios were also calculated: MBN/Nt and Nmin/Nt.

The plant tissues (pepper residues and broccoli) were oven dried and ground. Samples were incinerated at 500 °C, then ashes were dissolved in 6N HNO_3 and analysed for Ca, Mg, Na and K, Fe, Cu, Mn and Zn by ICP-MS (7500 CE, Agilent). Nitrogen (N) was

determined by Kjeldhal method (Hoeger, 1998) and total organic carbon (TOC) according to Golueke (1977).

All marketable broccoli heads from each plot were collected and weighed to calculate the yield. Five heads per plot, from the two central rows, were randomly collected from each plot (on the first harvest day), dried and their nutrient content analyzed. Also we collected 20 plants (10 plants per harvest day and plot) to measure some quality parameters: hollow stem, fresh head weigh, head dry matter, leaf dry matter, head diameter (widest part), stem diameter (at the cutting point), fresh head weight/head diameter and head weight/stem diameter.

5.2.4 STATISTICAL ANALYSIS

The fitting of the data to a normal distribution for all properties measured was checked with the Kolmogorov-Smirnov test. The data were submitted to one-way ANOVA to assess the differences among application rates and samplings. The separation of means was made according to Tukey's at $P < 0.05$. Relationships among variables were studied using Pearson's correlations, while the relationship between season available N and crop yield was checked by linear regressions. Statistical analyses were performed with the software IBM SPSS Statistics 19.

5.3 RESULTS

5.3.1 SOIL PHYSICOCHEMICAL PROPERTIES

There was no significant treatment effect on soil pH, EC and CEC for both cycles (data not shown). Superficial Nt showed significant differences between application rates in S2, with higher values in CR3. We observed lower Nt declines between samplings in CR than in CF treatment, on average, for both cycles; however they were only significant in CF3 for first cycle and in CT and CF1 for second cycle (table 5.2). In depth, Nt values in CF3 were significantly higher in S4 than in S3. Available N presented significant differences among application rates for S2 in surface (with lower available N in CR1) and for S4 in depth (with values in CR higher than in CF). A decreasing trend between samplings were found, with significant depletion in first cycle (in surface) and second cycle (for both depths), for all application rates (table 5.2). For NO_3^- content, significant differences among application rates were observed in depth for S2 (with CR2 and CR3 values higher than CT) and for S4 (with CR values higher than those of CF). Between samplings, we observed the same depletion pattern for NO_3^- and available N (table 5.3). NH_4^+ content

showed, for S2 in surface, values significantly lower in CR than for CT and CF application rates. Between samplings, NH_4^+ concentrations were higher in S2 than in S1, except for CT (table 5.3). In subsurface samples, NH_4^+ remained more or less stable in both crop cycles. Available P showed, as a general trend, no significant differences between application rates or samplings. It only significantly enhanced for S2 compared with S1 in CR2 and CR3 (table 5.4).

For available metals (values not shown) no significant differences among application rates and samplings were found except for Zn and Mn. Zn at topsoil showed higher values in CR3 ($2.52 \pm 0.18 \text{ mg kg}^{-1}$) than in CF treatment and CT ($2.04 - 2.22 \text{ mg kg}^{-1}$) in S4. Mn values in surface samples during the first cycle decreased in CT and CF application rates (from 16.18 ± 0.90 to $11.99 \pm 0.69 \text{ mg kg}^{-1}$ in average).

Exchangeable cations showed no significant differences among application rates in any crop cycle (data not shown), except for superficial values of K in S2, where CR3 was higher than others application rates ($2.50 \pm 0.29 \text{ mg kg}^{-1}$ vs. $1.32-1.89 \text{ mg kg}^{-1}$). There were no significant differences between samplings at each crop cycle for any of these nutrients, except for the first cycle in K, which significantly decreased in CT, CF1 and CF2 compared with CR treatment (from 2.28 ± 0.19 to $1.78 \pm 0.24 \text{ mg kg}^{-1}$ on average), and Mg which significantly decreased in all application rates (from 1.70 ± 0.08 to $1.44 \pm 0.09 \text{ mg kg}^{-1}$ on average) but for CR3.

Table 5.2 Total and available nitrogen in surface and subsurface soil samples for both broccoli cycles. Data are shown as mean $\pm$ standard deviation (n=3).

Soil property	Depth (cm)	Sampling	Control	Chemical Fertilization			Crop Residues + Chemical fertilization			F _a
				CF1	CF2	CF3	CR1	CR2	CR3	
Nt (g kg ⁻¹)	0-30	S1	1.40 $\pm$ 0.06	1.58 $\pm$ 0.19	1.56 $\pm$ 0.2	1.59 $\pm$ 0.06	1.45 $\pm$ 0.01	1.52 $\pm$ 0.12	1.53 $\pm$ 0.10	0.685ns
		S2	1.39a $\pm$ 0.13	1.45a $\pm$ 0.07	1.34a $\pm$ 0.08	1.45a $\pm$ 0.05	1.32a $\pm$ 0.18	1.51a $\pm$ 0.11	1.76b $\pm$ 0.08	4.313*
		F _a	0.018ns	1.141ns	2.873ns	15.360*	1.727ns	0.010ns	2.298ns	
	30-60	S1	0.64 $\pm$ 0.04	0.85 $\pm$ 0.08	0.69 $\pm$ 0.08	0.74 $\pm$ 0.10	0.63 $\pm$ 0.19	0.66 $\pm$ 0.06	0.71 $\pm$ 0.21	1.070ns
		S2	0.68 $\pm$ 0.13	0.68 $\pm$ 0.19	0.51 $\pm$ 0.24	0.83 $\pm$ 0.19	0.71 $\pm$ 0.19	0.51 $\pm$ 0.19	0.73 $\pm$ 0.13	1.168ns
		F	0.247ns	2.063ns	1.434ns	0.511ns	0.254ns	1.634ns	0.027ns	
	0-30	S3	1.32 $\pm$ 0.13	1.43 $\pm$ 0.05	1.38 $\pm$ 0.2	1.39 $\pm$ 0.11	1.34 $\pm$ 0.07	1.30 $\pm$ 0.11	1.34 $\pm$ 0.18	0.365ns
		S4	1.18 $\pm$ 0.12	1.30 $\pm$ 0.04	1.19 $\pm$ 0.08	1.21 $\pm$ 0.15	1.28 $\pm$ 0.72	1.18 $\pm$ 0.62	1.36 $\pm$ 0.14	1.200ns
		F	27.078**	13.657*	2.265ns	3.007ns	1.678ns	1.135ns	0.048ns	
	30-60	S3	0.56a $\pm$ 0.061	0.84b $\pm$ 0.117	0.65a $\pm$ 0.04	0.67ab $\pm$ 0.05	0.70ab $\pm$ 0.06	0.61a $\pm$ 0.06	0.77b $\pm$ 0.18	2.992*
		S4	0.81 $\pm$ 0.08	0.91 $\pm$ 0.15	0.89 $\pm$ 0.15	0.91 $\pm$ 0.03	0.72 $\pm$ 0.05	0.62 $\pm$ 0.09	0.76 $\pm$ 0.19	2.543ns
		F	17.864*	0.451ns	6.845ns	54.244**	0.194ns	0.031ns	0.001ns	
Available N (mg kg ⁻¹)	0-30	S1	135.9 $\pm$ 9.5	163.6 $\pm$ 28.8	167.9 $\pm$ 26.6	153.7 $\pm$ 0.6	168.2 $\pm$ 45.0	180.4 $\pm$ 13.6	188.4 $\pm$ 40.3	1.141ns
		S2	63.4a $\pm$ 6.8	62.6a $\pm$ 7.4	62.4a $\pm$ 9.5	62.6a $\pm$ 4.3	44.7b $\pm$ 0.6	55.7a $\pm$ 5.3	59.9a $\pm$ 2.1	3.927*
		F	115.109***	34.750**	42.013**	1307.7***	22.625***	218.19***	30.463***	
	30-60	S1	80.0 $\pm$ 39.7	71.3 $\pm$ 28.3	57.7 $\pm$ 22.6	82.3 $\pm$ 0.3	101.8 $\pm$ 27.4	87.9 $\pm$ 34.0	98.5 $\pm$ 22.9	0.925ns
		S2	23.2 $\pm$ 8.4	22.2 $\pm$ 6.0	38.1 $\pm$ 4.3	30.4 $\pm$ 12.4	44.3 $\pm$ 21.3	60.2 $\pm$ 34.7	66.3 $\pm$ 20.4	2.690 ns
		F	5.863ns	8.607*	2.179ns	52.783**	8.218*	0.976ns	3.328ns	
	0-30	S3	61.4 $\pm$ 13.3	80.8 $\pm$ 45.0	69.5 $\pm$ 9.6	59.8 $\pm$ 7.6	88.6 $\pm$ 9.3	88.8 $\pm$ 11.0	70.1 $\pm$ 14.3	1.105ns
		S4	32.0 $\pm$ 3.6	35.7 $\pm$ 4.3	38.1 $\pm$ 2.6	35.5 $\pm$ 3.5	37.9 $\pm$ 1.7	30.9 $\pm$ 4.7	38.7 $\pm$ 4.7	2.032ns
		F	13.723*	2.983ns	29.950**	25.968**	85.351***	70.145***	13.047*	
	30-60	S3	77.4 $\pm$ 2.6	107.5 $\pm$ 16.9	70.7 $\pm$ 5.6	82.8 $\pm$ 16.7	123.2 $\pm$ 6.7	117.9 $\pm$ 9.6	116.6 $\pm$ 29.3	2.745ns
		S4	21.1ab $\pm$ 1.8	19.0a $\pm$ 0.9	19.2a $\pm$ 3.3	19.3a $\pm$ 4.0	38.0b $\pm$ 8.6	32.6bc $\pm$ 11.3	45.0c $\pm$ 9.7	7.396**
		F	917.81***	81.778***	187.787***	40.862**	182.061***	9.670*	16.120*	

S1: sampling 1; S2: sampling 2; S3: sampling 3; S4: sampling 4. CT: control.

^aF-value- Significant at: * P <0.05, ** P <0.01, *** P <0.001 ns: non significant (P >0.05). Different letters indicate significant differences among means (P <0.05).

Table 5.3 Nitrate and ammonium concentrations in surface and subsurface soil samples for both broccoli cycles. Data are shown as mean $\pm$ standard deviation (n=3).

Soil property	Depth (cm)	Sampling	Control	Chemical Fertilization			Crop Residues + chemical fertilization			F ^a
				CF1	CF2	CF3	CR1	CR2	CR3	
NO ₃ ⁻ (mg kg ⁻¹)	0-30	S1	542 $\pm$ 34	655 $\pm$ 132	674 $\pm$ 127	486 $\pm$ 5	700 $\pm$ 195	745 $\pm$ 67	784 $\pm$ 175	0.256ns
		S2	166 $\pm$ 31	144 $\pm$ 31	149 $\pm$ 32	141 $\pm$ 21	130 $\pm$ 6	164 $\pm$ 18	193 $\pm$ 20	2.227ns
		F ^a	195.293***	42.599**	47.899**	145.49***	25.518**	214.03***	33.727**	
	30-60	S1	320 $\pm$ 177	279 $\pm$ 124	214 $\pm$ 107	330 $\pm$ 0	415 $\pm$ 121	353 $\pm$ 150	398 $\pm$ 101	0.948ns
		S2	65a $\pm$ 39	50a $\pm$ 10	128abc $\pm$ 16	97ab $\pm$ 54	160abc $\pm$ 93	233bc $\pm$ 151	257c $\pm$ 89	3.048*
		F	5.932ns	10.211*	1.884ns	55.151**	8.443*	0.947ns	3.323ns	
	0-30	S3	200 $\pm$ 61	271 $\pm$ 185	232 $\pm$ 33	173 $\pm$ 34	272 $\pm$ 22	284 $\pm$ 49	194 $\pm$ 31	0.933ns
		S4	58 $\pm$ 57	71 $\pm$ 9	80 $\pm$ 19	66 $\pm$ 8	90 $\pm$ 6	70 $\pm$ 18	101 $\pm$ 24	2.705ns
		F	15.374*	3.494ns	48.445**	27.468**	195.03***	50.459**	16.446*	
	30-60	S3	294ab $\pm$ 17	411abc $\pm$ 60	257a $\pm$ 26	298ab $\pm$ 69	496c $\pm$ 32	466bc $\pm$ 188	455bc $\pm$ 129	3.190*
		S4	57ab $\pm$ 11	41a $\pm$ 2	43a $\pm$ 10	47a $\pm$ 16	125bc $\pm$ 24	101bc $\pm$ 52	156c $\pm$ 34	9.145***
		F	383.04***	114.60***	173.41***	37.363**	261.73***	10.552*	15.093*	
NH ₄ ⁺ (mg kg ⁻¹)	0-30	S1	16.5ab $\pm$ 2.2	19.0b $\pm$ 2.6	18.4ab $\pm$ 2.0	17.4ab $\pm$ 0.7	12.4c $\pm$ 1.2	14.8ac $\pm$ 2.7	13.9ac $\pm$ 0.9	4.822**
		S2	31.5a $\pm$ 1.3	36.6a $\pm$ 0.8	37.6a $\pm$ 0.1	37.4a $\pm$ 2.0	18.6b $\pm$ 1.1	22.6b $\pm$ 1.6	19.9b $\pm$ 2.9	9.811***
		F	4.619ns	124.09***	269.42***	270.53***	43.881**	19.141*	11.686*	
	30-60	S1	9.4 $\pm$ 0.7	10.0 $\pm$ 0.7	9.9 $\pm$ 1.0	9.5 $\pm$ 0.4	9.8 $\pm$ 0.3	10.0 $\pm$ 0.4	10.5 $\pm$ 0.2	1.066ns
		S2	10.3 $\pm$ 1.2	13.4 $\pm$ 5.2	11.4 $\pm$ 1.7	10.2 $\pm$ 0.2	10.0 $\pm$ 0.6	9.1 $\pm$ 1.1	10.0 $\pm$ 0.5	1.249ns
		F	1.223ns	1.279ns	1.827ns	8.109*	0.354ns	1.681ns	2.714ns	
	0-30	S3	19.7a $\pm$ 1.3	24abc $\pm$ 4.7	20.8ab $\pm$ 3.4	25.1abc $\pm$ 0.7	33.1c $\pm$ 8.0	29.8bc $\pm$ 1.7	32.1c $\pm$ 9.2	3.248*
		S4	23.0 $\pm$ 2.8	24.0 $\pm$ 5.2	24.4 $\pm$ 2.1	24.5 $\pm$ 2.9	21.3 $\pm$ 1.9	18.2 $\pm$ 1.0	19.4 $\pm$ 1.0	2.539ns
		F	3.654ns	0.000ns	2.408ns	0.122ns	6.140ns	105.602***	5.622ns	
	30-60	S3	13.4 $\pm$ 1.6	18.0 $\pm$ 4.9	15.4 $\pm$ 0.6	18.8 $\pm$ 1.9	13.5 $\pm$ 1.1	15.5 $\pm$ 4.6	16.9 $\pm$ 3.7	1.394ns
		S4	10.0 $\pm$ 1.3	11.8 $\pm$ 0.7	11.6 $\pm$ 1.4	10.6 $\pm$ 0.7	11.8 $\pm$ 4.7	12.0 $\pm$ 0.5	11.8 $\pm$ 2.6	0.374ns
		F	8.755*	4.593ns	19.951*	51.006**	0.371ns	1.678ns	3.823ns	

S1: sampling 1; S2: sampling 2; S3: sampling 3; S4: sampling 4. CT: control.

^a F-value- Significant at: *P<0.05, **P<0.01, ***P<0.001 ns: non significant (P>0.05). Different letters indicate significant differences among means (P<0.05).

Table 5.4 Available phosphorus (mg kg⁻¹) in surface and subsurface soil samples for both broccoli cycles. Data are shown as mean $\pm$ standard deviation (n=3).

Depth (cm)	Sampling	Control	Chemical Fertilizers			Crop Residues + Chemical fertilization			F ^a
			CF1	CF2	CF3	CR1	CR2	CR3	
0-30	S1	228 $\pm$ 20	253 $\pm$ 20	232 $\pm$ 25	247 $\pm$ 16	225 $\pm$ 44	225 $\pm$ 10	208 $\pm$ 11	1.240ns
	S2	239 $\pm$ 64	302 $\pm$ 85	258 $\pm$ 13	259 $\pm$ 13	265 $\pm$ 35	293 $\pm$ 36	281 $\pm$ 25	0.685ns
	F ^a	0.086ns	0.987ns	2.567ns	0.618ns	1.570ns	10.140*	20.994*	
30-60	S1	71 $\pm$ 20	92 $\pm$ 7	83 $\pm$ 11	87 $\pm$ 23	61 $\pm$ 20	65 $\pm$ 5	61 $\pm$ 12	2.011ns
	S2	69 $\pm$ 40	69 $\pm$ 18	58 $\pm$ 8	68 $\pm$ 3	63 $\pm$ 14	44 $\pm$ 15	56 $\pm$ 18	0.654ns
	F	0.185ns	0.006ns	4.248ns	10.028*	0.015ns	5.239ns	0.185ns	
0-30	S3	344 $\pm$ 28	332 $\pm$ 28	320 $\pm$ 42	345 $\pm$ 15	319 $\pm$ 37	307 $\pm$ 63	324 $\pm$ 54	0.510ns
	S4	310 $\pm$ 22	325 $\pm$ 38	312 $\pm$ 28	334 $\pm$ 19	376 $\pm$ 6	331 $\pm$ 35	352 $\pm$ 44	1.841ns
	F	2.845ns	0.071ns	0.087ns	0.649ns	6.801ns	0.323ns	0.412ns	
30-60	S3	67.4a $\pm$ 17	120.6b $\pm$ 37	68.9a $\pm$ 3	81.3a $\pm$ 9	60.3a $\pm$ 21	73.2a $\pm$ 13	81.7a $\pm$ 4	3.539*
	S4	122 $\pm$ 36	144 $\pm$ 8	126 $\pm$ 37	140 $\pm$ 25	100 $\pm$ 36	91 $\pm$ 31	96 $\pm$ 29	1.536ns
	F	5.593ns	1.192ns	7.060ns	14.483*	2.762ns	0.821ns	0.677ns	

S1: sampling 1; S2: sampling 2; S3: sampling 3; S4: sampling 4. CT: control.

^a F-value- Significant at: * P <0.05, ** P <0.01, *** P <0.001 ns: non significant (P >0.05). Different letters indicate significant differences among means (P <0.05).

5.3.2 SOIL BIOCHEMICAL PROPERTIES

The values of MBN, Nmin and urease activity in S4 were significantly higher in CR3 than in CT and CF treatment, while for protease activity and MBN/Nt ratio, CR3 showed higher values than CT. Alkaline phosphatase and Nmin/Nt ratio presented higher values in all CR application rates than in CT and CF application rates. Nsol did not show significant differences among application rates (figure 5.1).

Between samplings, MBN showed a significant increase in S4 compared with S3 for CR application rates. For Nsol, a significant decrease was found in all application rates except for CT and CF1. Urease and protease activities significantly increased in S4 for CR3 and CR2 respectively, compared with S3 (figure 5.1). Alkaline phosphatase increased its activity in S4 compared to initial values, being significant for CT, CF1 and CR3. The MBN/Nt ratio increased in all application rates except for CF1, being more intense in CR application rates, while the Nmin/Nt ratio significantly decreased in CF3.

5.3.3 BROCCOLI YIELD, QUALITY AND NUTRIENTS

Significant differences in yield among application rates were only found for the second cycle (table 5.5), being values for CF2, CF3 and CR greater than those of control and CF1. Quality parameters only showed significant differences during the second season for head weight and the ratios head weight/head diameter and head weight/steam diameter, which were lower in CT, with no significant differences between CR and CF application rates (table 5.6). Excessive N supply can increase physiological disorders like hollow stem. In the first season, a high percentage (>60%) of broccoli plants presented this disorder. As a general pattern, there was no treatment effect on broccoli nutrient levels, except for Ca, Mg and K which were significantly lower in some CR application rates for the first cycle (table 5.7). Broccoli nutrients concentrations followed the decreasing trend $N > P > K > Mg \sim Ca > Na > Fe > Zn > Mn > Cu$.

Figure 5.1 Soil microbial biomass nitrogen (a), soluble nitrogen (b), potentially mineralizable nitrogen (c), urease activity (d), protease activity (e), alkaline phosphatase activity (f), MBN/Nt ratio (g) and Nmin/Nt ratio (h) for initial (S3; gray) and final (S4; white) sampling of the second crop cycle. Data are shown as mean $\pm$ standard deviation ($n=3$). Error bars denote standard deviation. Different letters indicate significant differences ($P < 0.05$) among application rates within the same sampling. Means with no letter are not significantly different ($P > 0.05$). Asterisks denote significant differences between S3 and S4 within each application rate (significant at: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). PNP: *p*-nitrophenol.

Table 5.5 Broccoli yield in both crop cycles. Data are shown as mean $\pm$ standard deviation (n=3).

Sample	Broccoli yield (kg ha ⁻¹)			
	Crop cycle 1		Crop cycle 2	
CT	9747	± 591	9450 a	± 934
CF1	11409	± 878	9967 ab	± 652
CF2	10847	± 1555	10657 abc	± 1671
CF3	11153	± 1156	12555 c	± 1547
CR1	10110	± 659	12163 bc	± 1128
CR2	9455	± 1384	11675 abc	± 1236
CR3	10998	± 1797	12428 c	± 1116
F ^a	ns		3.104*	

CT: control; CF: Chemical Fertilizers; CR: Crop residues.

^a F-Value. Significant at: * $P < 0.05$, ns: non significant ($P > 0.05$). Different letters indicate significant differences among means ($P < 0.05$).

Table 5.6 Broccoli quality parameters in the second crop cycle. Data are shown as mean $\pm$ standard deviation (n=20).

Sample	Head weight (g head ⁻¹)	Head diam (cm)	Stem diam (cm)	Head weight/ Head diam	Head weight/ Stem diam	Head dry matter (%)	Leaf dry matter (%)
CT	189.8a $\pm$ 42.6	9.66 $\pm$ 1.06	3.09 $\pm$ 0.35	19.9a $\pm$ 2.7	59.4a $\pm$ 8.7	9.71 $\pm$ 0.34	11.38 $\pm$ 1.09
CF1	223.6ab $\pm$ 11.9	10.57 $\pm$ 0.33	3.24 $\pm$ 0.09	21.0ab $\pm$ 0.9	68.2ab $\pm$ 2.8	9.42 $\pm$ 0.25	11.14 $\pm$ 0.39
CF2	219.4ab $\pm$ 1.30	10.44 $\pm$ 0.27	3.23 $\pm$ 0.06	20.7ab $\pm$ 0.4	66.8ab $\pm$ 1.0	9.36 $\pm$ 0.07	11.04 $\pm$ 0.55
CF3	223.8ab $\pm$ 15.8	10.70 $\pm$ 0.45	3.20 $\pm$ 0.11	20.9ab $\pm$ 1.0	69.0ab $\pm$ 4.9	9.37 $\pm$ 0.07	11.23 $\pm$ 0.56
CR1	256.5b $\pm$ 18.5	11.28 $\pm$ 0.59	3.34 $\pm$ 0.06	22.7ab $\pm$ 1.1	76.3b $\pm$ 4.7	9.54 $\pm$ 0.05	11.32 $\pm$ 0.36
CR2	236.6ab $\pm$ 12.4	10.98 $\pm$ 0.60	3.37 $\pm$ 0.14	21.4ab $\pm$ 0.8	70.4ab $\pm$ 3.0	9.62 $\pm$ 0.04	10.90 $\pm$ 0.31
CR3	261.6b $\pm$ 9.7	11.09 $\pm$ 0.12	3.41 $\pm$ 0.11	23.4b $\pm$ 0.8	75.6b $\pm$ 1.8	9.37 $\pm$ 0.09	11.34 $\pm$ 0.71
F ^a	4.451*	ns	ns	3.595*	4.725 **	ns	ns

CT: control; CF: Chemical Fertilizers; CR: Crop residues; Diam: diameter.

^a F-value. Significant at: * $P < 0.05$, ** $P < 0.01$, ns: non significant ($P > 0.05$). Different letters indicate significant differences among means ($P < 0.05$).

Table 5.7 Nutrients concentrations in broccoli heads in both broccoli cycles. Data are shown as mean $\pm$ standard deviation (n=3).

Nutrient	Cycle	Control	Chemical Fertilizers			Crop residues + Chemical Fertilizers			F ^a
		CT	CF1	CF2	CF3	CR1	CR2	CR3	
N (g kg ⁻¹)	1	56.73 $\pm$ 0.24	55.34 $\pm$ 1.59	56.68 $\pm$ 1.51	56.96 $\pm$ 0.99	56.15 $\pm$ 0.7	55.48 $\pm$ 2.06	53.99 $\pm$ 0.61	2.118ns
	2	46.67 $\pm$ 1.51	48.57 $\pm$ 0.85	48.87 $\pm$ 0.64	47.86 $\pm$ 2.54	48.37 $\pm$ 3.01	46.85 $\pm$ 1.46	48.12 $\pm$ 1.37	0.651ns
P (g kg ⁻¹)	1	25.4 $\pm$ 0.82	24.52 $\pm$ 1.85	24.07 $\pm$ 0.17	26.08 $\pm$ 1.68	26.86 $\pm$ 2.34	23.67 $\pm$ 1.41	23.73 $\pm$ 1.03	2.081ns
	2	23.1 $\pm$ 0.63	24.38 $\pm$ 1.6	24.35 $\pm$ 1.02	24.56 $\pm$ 0.48	23.72 $\pm$ 1.6	22.93 $\pm$ 1.41	25.24 $\pm$ 1.2	1.411ns
Ca (g kg ⁻¹)	1	1.82a $\pm$ 0.15	1.84a $\pm$ 0.16	1.78ab $\pm$ 0.06	1.81a $\pm$ 0.09	1.55abc $\pm$ 0.2	1.41c $\pm$ 0.08	1.52bc $\pm$ 0.23	4.204*
	2	3.05 $\pm$ 0.32	3.07 $\pm$ 0.25	3.00 $\pm$ 0.2	3.08 $\pm$ 0.17	2.82 $\pm$ 0.19	2.8 $\pm$ 0.1	2.72 $\pm$ 0.19	1.510ns
Mg (g kg ⁻¹)	1	2.08ab $\pm$ 0.09	2.10ab $\pm$ 0.02	2.13b $\pm$ 0.06	2.19b $\pm$ 0.05	2.14a $\pm$ 0.09	1.96b $\pm$ 0.12	1.97b $\pm$ 0.08	3.655*
	2	2.19 $\pm$ 0.11	2.22 $\pm$ 0.2	2.15 $\pm$ 0.06	2.18 $\pm$ 0.03	2.3 $\pm$ 0.07	2.33 $\pm$ 0.12	2.36 $\pm$ 0.11	1.581ns
Na (g kg ⁻¹)	1	1.15 $\pm$ 0.11	1.08 $\pm$ 0.22	1.14 $\pm$ 0.05	1.06 $\pm$ 0.18	1.33 $\pm$ 0.04	1.21 $\pm$ 0.11	1.13 $\pm$ 0.15	1.287ns
	2	0.98 $\pm$ 0.14	0.94 $\pm$ 0.13	0.88 $\pm$ 0.21	1.02 $\pm$ 0.14	1.09 $\pm$ 0.09	1.2 $\pm$ 0.04	1.12 $\pm$ 0.2	1.710ns
K (g kg ⁻¹)	1	36.01ab $\pm$ 2.08	35.59ab $\pm$ 0.5	36.14ab $\pm$ 1.01	37.73a $\pm$ 0.86	34.48b $\pm$ 0.7	32.11c $\pm$ 1.5	31.78c $\pm$ 0.52	10.824**
	2	36.94 $\pm$ 1.39	38.26 $\pm$ 1.71	38.06 $\pm$ 1.41	38.67 $\pm$ 1.63	38.08 $\pm$ 1.08	36.46 $\pm$ 2.00	37.33 $\pm$ 1.5	0.784ns
Cu (mg kg ⁻¹)	1	5.52 $\pm$ 0.45	5.14 $\pm$ 0.34	5.62 $\pm$ 0.37	5.32 $\pm$ 0.35	6.15 $\pm$ 0.63	5.62 $\pm$ 0.37	5.11 $\pm$ 0.7	1.205ns
	2	8.98 $\pm$ 1.83	8.73 $\pm$ 1.34	7.72 $\pm$ 2.26	9.68 $\pm$ 3.3	10.08 $\pm$ 2.07	9.01 $\pm$ 3.08	9.18 $\pm$ 1.66	0.312ns
Zn (mg kg ⁻¹)	1	41.32 $\pm$ 1.66	42.02 $\pm$ 2.83	37.48 $\pm$ 4.42	42.43 $\pm$ 0.3	41.52 $\pm$ 7.06	37.48 $\pm$ 4.42	36.61 $\pm$ 2.41	1.324ns
	2	36.64 $\pm$ 1.53	35.18 $\pm$ 1.57	34.39 $\pm$ 1.27	24.8 $\pm$ 0.63	36.31 $\pm$ 2.97	34.53 $\pm$ 3.48	36.84 $\pm$ 2.23	0.696ns
Fe (mg kg ⁻¹)	1	74.85 $\pm$ 23.19	50.29 $\pm$ 5.32	36.95 $\pm$ 0.63	45.13 $\pm$ 2.06	58.01 $\pm$ 21.2	36.95 $\pm$ 0.63	42.84 $\pm$ 7.47	1.138ns
	2	44.82 $\pm$ 5.73	47.99 $\pm$ 3.41	47.13 $\pm$ 10.78	44.27 $\pm$ 10.18	37.45 $\pm$ 3.63	40.17 $\pm$ 4.61	38.58 $\pm$ 5.39	1.123ns
Mn (mg kg ⁻¹)	1	22.82 $\pm$ 0.29	21.45 $\pm$ 0.88	23.36 $\pm$ 2.36	22.94 $\pm$ 1.65	25.74 $\pm$ 1.36	23.36 $\pm$ 2.36	21.88 $\pm$ 0.93	2.431ns
	2	28.4 $\pm$ 0.84	25.81 $\pm$ 3.84	24.35 $\pm$ 1.29	25.65 $\pm$ 1.42	24.81 $\pm$ 3.84	27.21 $\pm$ 0.81	25.17 $\pm$ 2.18	1.719ns

^a F-value. Significant at: * P <0.05, ** P <0.001, ns: non significant (P >0.05). Different letters indicate significant differences among means (P <0.05).

5.4 DISCUSSION

A decrease of Nt was observed during the experiment, also observed by Melero et al. (2005) in Southern Spain under semiarid conditions. This is likely due to N-mineralization of organic N present in the soil. However, the lowest superficial loss of Nt in CR treatment and the increase in Nt for CR3 in both cycles suggest a positive effect of CR addition compared to CF, in agreement with Mugwe et al. (2009).

The similar decreasing trend presented for available N and NO_3^- is because for initial sampling, 29- 82% of available N is actually NO_3^- , supporting the fact that losses in available N may be mainly due to NO_3^- losses (correlation coefficient between these properties $r=0.98$; $P<0.001$). This decline is mainly explained by mineral N uptake by plants during the growing season. NO_3^- concentrations at depth were higher for CR treatment. This could be likely due to a displacement of NO_3^- from the upper layer (with higher initial content). Available N and NO_3^- losses between samplings in surface layer were lower at greatest application rate in both treatments and second cycle. This could be related to a higher amount of NO_3^- coming from chemical fertilizers and a higher mineralization in CF3 and CR3 respectively, which compensate losses by crop uptake, immobilization or lixiviation. Another possible explanation to the N loss is denitrification favoured by oxygen-deprived soils, which is often the case when irrigation is used. In irrigated soils, 10 to 30% of applied mineral nitrogen is subjected to denitrification (Tremblay et al., 2001). We found a lower amount of available N in surface at the end of the harvest sampling compared with the initial sampling, in both cycles, for all application rates, reducing the potential leaching after harvest.

No correlation between available N and NH_4^+ content was found. Lower values of NH_4^+ in CR treatment compare with CF and its negative correlation with CR application rates ($r=-0.65$; $P<0.01$) may indicate that the application of residues stimulates N immobilization and/or nitrifying microorganisms in soil. Another explanation to the loss of NH_4^+ is volatilization, favoured by dry soils (Zhou et al., 2006) but is less likely because dryness was not experienced in our study. Increasing nitrification rates under this management practice is supported by a positive correlation between CR application rates and NO_3^- content ($r=0.61$; $P<0.01$). Increased nitrification by microbial activation is a positive factor, enhancing soil fertility, since NO_3^- can be taken up by plants roots since it is soluble in water (Mellgren, 2008).

The available P trend in soil is towards superficial accumulation because of its low mobility and the extraction from deepest roots (Mackay et al., 1987). In calcareous soils it

is common that Ca-ion activity in the liquid phase forms insoluble Ca-phosphate mineral phases (Tunessi et al., 1999). Nonetheless, organic matter addition of soil reduces P insolubilization and increase extractable P (Braschi et al., 2003). The amount of P added as chemical fertilizer was the same in all plots, however, a significant increase for CR2 and CR3 in S2 indicates the positive effect of addition of organic material. This fact is supported by the positive correlation of available P with SOC and Nt ($r=0.86$ and 0.83 ; $P<0.01$, respectively).

Plots where CR3 was applied showed higher MBN and microbial and enzymatic activity. Thus, the application of high rates of crop residues promotes the immobilization of higher amounts of N in terms of microbial biomass which can degrade the organic compounds to release available nutrients for vegetation uptake, in agreement with Pascual et al. (1998). Furthermore, positive correlations between rates applied as CR and MBN, Nmin and MBN/N were found ($r=0.75$, 0.70 and 0.69 ; $P<0.01$, respectively), confirming the direct effect of organic residues content on microbial biomass and activity.

Nsol in this study is likely mainly composed by NO_3^- , supported by the strong positive correlation between Nsol and NO_3^- ($r=0.96$; $P<0.01$). Thus, decreases in Nsol are mainly due to crop uptake. Nevertheless, because of Nsol is the most accessible source of N for microorganisms, Nsol can also decrease due to the metabolic activity of microorganisms, supported by the negative correlations between Nsol and MBN ($r=-0.53$; $P<0.01$), and MBN/Nt ($r=-0.64$; $P<0.01$). This N immobilization, utilization of mineral N by soil microorganisms competing for N with cultivated plants (Gros and Domínguez, 1992), did not affect negatively broccoli yield or quality parameters.

Regarding the use of inorganic fertilizers, there are evidences that the presence of available nutrients as inorganic forms inhibits the synthesis of soil enzymes (Olander and Vitousek, 2000). Dick et al. (1988) reported a decrease, between final and initial sampling, in urease activity with increasing inputs of ammonia-based N fertilizer, although we did not find decreases in this activity with fertilization. A negative correlation ($r=-0.70$; $P<0.01$) was detected between phosphatase activity and N applied as CF, indicating that chemical fertilization may have negative effects on this enzyme. The addition of CR favoured the increment in enzyme activities, compared with CT, as previously reported Melero et al. 2005. The positive effect could be explained by the addition of microbial cells or enzymes with the amendment (Tejada et al., 2006). However, is more likely that organic residues enhance labile compounds which may stimulate microbial activity in soil in response, supported by the positive correlation between urease and phosphatase

activities and MBN ($r=0.50$; $P<0.01$ and 0.62 ; $P<0.01$, respectively). Increases in phosphatase activity indicate changes in the quantity and quality of soil phosphoryl substrates (Rao and Tarafdar, 1992). The positive correlations found between urease and phosphatase activities ($r=0.59$; $P<0.01$) indicate a similar response of microorganisms to these type of substrates. The positive correlation between Nsol, MBN, Nmin and MBN/Nt ratio with CR application rates, confirms that the incorporation of decomposable substances stimulates microbial growth and activity, increasing soil quality, since these parameters are considered to be direct indicators of soil quality because they integrate information both about microbial status and soil physico-chemical conditions (Aon et al., 2001). There is a positive correlation between CR application rates and urease and protease activities ($r=0.70$; $P<0.01$ and 0.63 ; $P<0.05$, respectively), indicating that CR stimulates the release of enzymes to decompose them. No significant correlation was found between CR application rates and phosphatase activity.

Although most nutrients were not affected by treatments, the highest application rates of CR maintained high levels of K, Mg, Zn and Mn. The high content of these nutrients in the pepper residue (table 4.1) could explain these increments, which are released after microbial mineralization. The fact that pepper residues had high content of Fe, but no significant increase with CR application rates was observed, may be due to rapid precipitation and immobilization of this nutrient in alkaline environments (Ylivainio, 2010). Concentrations of Zn, Fe and Mn were correlated with SOC and Nt ($r>0.76$; $P<0.01$). This could be attributed to the fact that an important exchangeable fraction of these nutrients is adsorbed by soil organic matter (Acosta et al., 2011). There was a good correlation between Zn, Fe and Mn concentrations ($r>0.84$; $P<0.01$), indicating a similar behaviour in soil.

The reduced response of broccoli yield, quality and nutrients content to the treatments in the first cycle was indeed expected due to a narrow range of mineral N amounts at planting time (residual fertility) as consequence of an excessive previous fertilization, also reported by Zebarth et al. (1995). Nonetheless, with a second crop cycle, it was possible to distinguish treatments and application rates effects, with CR3 and CF3 supporting the highest yield. Yield was enhanced in the second cycle with increasing levels of CF rates. This trend is in agreement with Zebart et al. (1995) and Babik and Elkner, (2002) who reported increasing broccoli yields with increasing N application rates. However, others authors (Belec et al., 2001; Thompson et al., 2002) found that excessive N fertilization had detrimental effect on marketable yield. N fertilization rates recommendations must be based, not only on fertilization application rates and crop yield,

but also on current soil available N. In figure 5.2 we have represented the relationship between season available N and the relative yield (plot yield-to-maximum season yield ratio). Relative yield increased up to the season available N value of 545 kg N ha⁻¹, coinciding with the yield data of CR application rates and CF3 for the second season. Above that value in season available N, no increments in crop yield were recorded. Thus, application rates which surpass this threshold in season available N (all application rates in first season except CT) would suppose not effective source of N. This is in agreement with Babik and Elkner (2002) who found that yield enhanced for every increased in N application rates up to 600 kg N ha⁻¹.

Figure 5.2 Relationship between relative broccoli yield (plot yield-to-maximum season yield ratio) and season available N (initial soil available N plus available N applied as CF or CR) for both crop cycles. Black symbols indicate CR treatment, white symbols indicate CF treatment and gray symbols indicate control. Application rates: 1(squares), 2 (circles) and 3 (diamonds)

Excessive N supply can produce negative effects not only on yield but also on crop quality parameters. We found a high percentage of hollow stem during the first season, just when N was highly available, as previously reported by Belec et al. (2001) and Tremblay (1989). CR application had positive effects on broccoli quality parameters, with a positive significant correlation between CR application rates and all quality parameters ($r > 0.57$; $P < 0.05$). The fact that CF1 and CF2 supported lower broccoli yield, indicates that mineralization of organic residues supplies available nutrients more efficiently than

controlled chemical fertilization. The N_{min}/N_t ratio, which is an indicator of N mineralization potential of a soil in terms of total nitrogen content, showed positive correlations with yield ($r=0.51$; $P<0.05$), head weight ($r=0.53$; $P<0.05$), head diameter ($r=0.42$; $P<0.05$), stem diameter ($r=0.48$; $P<0.05$), head weight/head diameter ($r=0.53$; $P<0.05$) and head weight/stem diameter, ($r=0.48$; $P<0.05$), indicating that increased microbial activity which release available N favours yield and crop quality

The absorption rate of mineral nutrients by aboveground plant parts considerably differs among plant species but also among varieties (Wojcik, 2004). N uptake by broccoli was lower in the second cycle, likely be due to lower N_t and available N contents during the second cycle; this is supported by the significant positive correlations of broccoli N with N_t ($r=0.50$; $P<0.01$) and available N ($r=0.52$; $P<0.01$). Broccoli N was positively correlated with soil exchangeable K and Mg ($r=0.50$; $P<0.01$ and 0.70 ; $P<0.01$, respectively), and negatively correlated with soil available P ($r=0.70$; $P<0.01$). Despite CR treatment had a positive effect on exchangeable K and Mg, lower broccoli K and Mg contents were found for first year in CR treatment compared with CF. Even so, K and Mg contents in broccoli were really similar to those obtained by Rincón et al. (1999) with the same variety in the same agricultural area. Mineral content (N, P, K, Mg, Na, Fe, Mn, and Cu) levels were within or even higher than sufficiency range for broccoli reported by Hanlon and Hochmuth (2000). However, broccoli Ca contents were lower than those found by Hanlon and Hochmuth (2000) and Rincón et al. (1999) for both cycles and treatments. These decreased Ca contents are not usual for a calcareous soil, with a large reservoir of exchangeable calcium and it is likely due to an uptake problem. Cation competition with high levels of other cations tends to reduce the uptake of Ca, which presented a negative correlation with exchangeable Na, K and Mg ($r>-0.51$; $P<0.01$). Nevertheless, all nutrient contents were higher than mean values for *Brassica oleracea* var. *italic* reported by USDA (U.S. Department of Agriculture, 2012).

5.5 CONCLUSIONS

Maximum yields were obtained in the second year when the season available N content (N applied as CF or CR plus initial available N) was between 545 and 598 kg N ha⁻¹. That corresponds to CF3 (383 kg N ha⁻¹ applied as CF) and all CR application rates, especially CR3 (383 kg N ha⁻¹ applied as CR). Thus, under our growing conditions, pepper crop residues, mainly at a high rate (16.7 – 19.2 t ha⁻¹), with a minimum chemical fertilization, is an integrated nutrient supply from both organic and inorganic N sources

which maintains crop yield and quality, with significant increments in soil quality and fertility compared to chemical fertilization. This management practices is more suitable for a long-term sustainable agriculture.

CHAPTER 6

USE OF ^{13}C -GLUCOSE TO STUDY

THE C TURNOVER

UNDER THE INFLUENCE OF

DIFFERENT PLANT MATERIALS

CHAPTER 6

Use of ^{13}C -glucose to study the C turnover under the influence of different plant materials

Abstract

We used ^{13}C -glucose to label the soil microbial biomass and subsequently investigate the fate and turnover of microbial biomass carbon in an arable soil under organic and conventional management. The amount of C derived from glucose and incorporated into different soil organic C pools was measured under the influence of three plant amendments (mature wheat, immature wheat and vetch) in an incubation experiment of 64 days. Findings indicated that at the end of incubation the original input of glucose was much more assimilated into microbial biomass in plant-amended samples. However, plant addition also caused a positive priming effect, enhancing mineralization of organic C, which led to overall less glucose-derived C being incorporated into soil labile carbon pool. This was more pronounced in the organically-managed soil. Recovery of the original glucose input as recalcitrant C ranged between 6.0 (wheat treatment) and 7.1% (organic control), although no significant differences were found among plants materials or soil management.

6.1 INTRODUCTION

It is recognized that the mineralization rate of organic material is a slow process mainly because soil microbial biomass (SMB) is the eye of the needle through which all that material enters the soil must pass to mineralize (Jenkinson, 1978). The SMB represents only a small fraction of the total amount of soil C and N, but it has a relatively rapid turnover. Although abiotic factors such as pH, temperature or moisture affect C turnover (Blagodatskaya and Kuzyakov, 2008), the size of microbial biomass, the microbial structure and its activity are key factors controlling the amounts of C and N mineralized (Fontaine and Barot, 2005)

Use of ^{13}C -labeled substrates is an established method for assessment of soil microbial populations after applications of substrates (Crossman et al., 2004; Evershed et al., 2006). Several studies have used this approach to quantify the C input from labelled litter material to soil organic matter (SOM) (Rasmussen et al., 2008). Some other laboratory incubation experiments with labelled glucose added to soil have tracked microbial consumption of that substrate, studying the modification and accumulation of substrate C and the stabilization of C through time (Aoyama et al., 2000; Fontaine, 2004; Evershed et al., 2006; Dungait et al., 2011). The SMB represents a significant compartment of the terrestrial biomass and microbial residues in soil are an important parent material for humus formation (Haider, 1992). For the current study, SMB was labelled via addition of ^{13}C -glucose to assess the influence of adding plant materials with different quality on the microbially-mediated processes of decomposition and stabilization of organic C, including C present in the SMB itself. The limitation of this method is related to the knowledge of the quantity of C necessary to be incorporated into the soil without promoting significant microbial growth (Fournier et al., 1992). In addition, many field and controlled observations have demonstrated that despite the incorporation of organic inputs into the soil in large quantities, soil total organic C content does not necessarily increase, although microbial biomass and activity rapidly respond to the new soil conditions (Fontaine, 2004). Addition of C substrates to soil can cause a change in the turnover of soil organic matter resulting in more or less $\text{CO}_2\text{-C}$ evolved than C contained in the original substrate. This acceleration or retardation of mineralization of organic C with the supply of organic inputs is called a positive or negative priming effect (PE). When extra $\text{CO}_2\text{-C}$ evolved following addition of organic substrates comes directly from an acceleration of microbial biomass turnover, it is called apparent PE, while if it comes from mineralization of soil organic matter by stimulating the microbial growth (co-

metabolism), it is termed real PE, both being governed by microbial activity (Bladogatskaya and Kuzyakov, 2008).

We pre-incubated one arable soil under two different management systems (organic and conventional fertilization) with ^{13}C -glucose for 8 days, and used tracer techniques to examine C cycling through microbial biomass. According to Gregorich et al. (1991), around 96% of the added labelled-glucose disappeared from soil in the first day. It is assumed that glucose is completely metabolized and uniformly incorporated into the SMB during the first days, except for that C-glucose released as CO_2 . After pre-incubation, three different plant materials (immature wheat, mature wheat and vetch) were applied to soil, which was incubated under laboratory conditions during 64 days for monitoring of CO_2 -C release and different soil C pools.

Our aim was to determine the influence of management history and addition of plant residues of varying composition on (1) the incorporation of microbial biomass C into stable soil organic matter, and (2) the priming effect on soil organic matter mineralization resulting from the addition of plant material.

6.2 MATERIALS AND METHODS

6.2.1 SOIL AND PLANT MATERIALS USED

Soil was taken from the Russell Ranch Sustainable Agricultural Facility in Davis, CA. The site has a mean annual temperature of 17 °C and a mean annual rainfall of 460 mm. Samples were collected from the same soil under two contrasting management systems: a conventional fertilized system, and an organic system which uses cover crops and compost. The soil is a silty clay loam and classified as Mollic Haploxeralf (Soil Survey Staff, 2010). Three random soil samples per management were taken from the upper 20 cm layer, which were homogenized to obtain a composite sample. Samples were sieved <4 mm and stored at 4°C. Roots and visible plants residues were removed where possible.

The plant materials consisted of the aboveground part of mature wheat (*Triticum aestivum* L.), immature wheat and vetch (*Vicia sativa* L.). The residue was dried at 55 °C and ground to less than 0.5 mm before application.

6.2.2 SOIL LABELLING AND PLANTS AMENDMENTS

The optimal amount of ^{13}C labelled glucose amendment was determined as the minimum concentration of glucose that produces the maximal respiration rate (Anderson

and Domsch, 1978). This ensures the most efficient incorporation of ^{13}C into the standing microbial biomass, while at the same time minimizing its growth. We tested a range of glucose concentrations: 84, 169, 253, 337, 422, 843, 1686 and 3372 $\mu\text{g C g}^{-1}$ soil. The respiratory response ($\text{mg CO}_2 \text{ h}^{-1}$) was determined by CO_2 evolution using an Infrared Gas Analyzer (IRGA, S151, Qubit systems). The minimum concentration of glucose invoking maximal respiratory response without an increase in CO_2 production (interpreted as new biomass synthesis) was 169 $\mu\text{g C g}^{-1}$ soil. This level was indicated by the asymptote in the curve of CO_2 evolution rate versus time (Horwath and Paul, 1994). Thus, we added the ^{13}C labelled glucose solution (169 $\mu\text{g C g}^{-1}$ dry soil) to samples of 22 g of dry soil and were maintained at 21 °C and 55% water holding capacity. N was also added as ammonium sulfate (17 $\mu\text{g N g}^{-1}$, C:N ratio = 10) to ensure no limitation of N. We left a conditioning period of 8 days before the incorporation of the different plant materials.

For each soil management, organic (Org) and conventional (Conv), three different plant materials were applied: mature wheat (MW), immature wheat (IW) and vetch (V). For each soil management, a control (CT) receiving the initial ^{13}C -glucose input but without any plant material addition was included. In total, 8 treatments with three replicates per treatment were assayed. The plants were thoroughly mixed at rates of 1% of dry mass soil (4.23, 4.09 and 4.29 g C kg^{-1} for MW, IW and V treatments, respectively). Soil samples were incubated for 64 days at 21°C into previously weighed 60 mL plastic cups. Moisture levels were gravimetrically maintained every 3 days using deionised water.

6.2.3 ANALYTICAL METHODS

The plant materials were analyzed for lignin by a two stage sulphuric acid hydrolysis (Hatfield et al., 1994), cellulose content was measured following the procedure of Brendel et al. (2000) and soluble C was extracted in water (1:500 w/v) and determined by UV-persulfate digestion (Teledyne-Tekmar Phoenix 8000). Characteristics of plant materials are shown in table 6.1.

Soil total organic C and ^{13}C content following incubation was determined by combustion GC (Elementar Vario EL Cube) interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (IRMS). Recalcitrant C and ^{13}C was determined at day 22 and day 64 following the acid hydrolysis method of Rovira and Vallejo (2003). Labile C was calculated as the difference between total organic C and recalcitrant C. Recalcitrance index was calculate for both total C and ^{13}C as the ratio of recalcitrant C to organic C.

Microbial biomass C (C_{bio}) was measured at days -8, 0, 7, 14, 22, 29, 37, 50 and 64 using the chloroform fumigation-extraction method proposed by Vance et al. (1987). We applied a KEC = 2.64 factor to the difference in extractable C between the fumigated and non-fumigated samples. K₂SO₄-extractable C was measured with UV-persulfate digestion (Teledyne-Tekmar Phoenix 8000) while the ¹³C content of these extracts was determined using an O.I. Analytical Model 1030 TOC Analyzer interfaced to a PDZ Europa 20-20 IRMS

To estimate the rate of CO₂ mineralization by microbial biomass, the soil vials were placed in a 500 mL mason jar during several hours and CO₂ concentration was measured by injectable IRGA (S151, Qubit systems). Three jars without soil or plant residue were considered as blanks. The total amount of C mineralized during the incubation time (64 days) was calculated by integrating these data over the course of the incubation, taking the rate measured at a given date to be the average rate for the interval represented by that date. The ¹³C-CO₂ was measured using a ThermoScientific PreCon-GasBench system interfaced to a ThermoScientific Delta V Plus IRMS.

Table 6.1 Chemical composition of plants materials used for incubation.

Plant properties	Vetch	Mature Wheat	Immature wheat
N (%)	3.1	0.29	1.75
C (%)	42.9	42.3	40.9
C/N	13	145	23
Cellulose (%)	19	38	24
Lignin (%)	13	25	15
Soluble C (%)	14	2.5	15
Lignin:N	4	86	9

6.2.4 DATA ANALYSIS AND STATISTICAL PROCEDURES

Data were checked to ensure normal distribution using the Kolmogorov-Smirnov test and transformed when necessary to ensure normal distribution. The ¹³C labelling of glucose allowed the separation of C respiration derived from the original glucose addition from respiration derived from soil or plant residue. The priming effect (PE, mg ¹³C-CO₂ g⁻¹ soil) induced by the addition of plant materials was calculated as the difference between ¹³C-CO₂ released from soils amended with plant materials and ¹³C-CO₂ released from control receiving no addition of plant materials, but pre-incubated with ¹³C-glucose. The

relative intensity of PE was estimated as a percentage of changes relative to the ^{13}C - CO_2 production with and without plant materials addition by:

$$\text{PE (\%)} = \frac{{}^{13}\text{C} - \text{CO}_{2\text{amended}} - {}^{13}\text{C} - \text{CO}_{2\text{control}}}{{}^{13}\text{C} - \text{CO}_{2\text{control}}} * 100 \quad (1)$$

In order to calculate the effect of plant material addition on CO_2 -C evolved from soil and the PE, the cumulative values were fitted versus time to different kinetics functions. The coefficients of regression (R^2) and assumption of normal distribution of residuals were used as criteria to decide the best fit. A one-way ANOVA was carried out to assess differences among plant materials and soil management systems separately for each sampling day. The comparison of means was made according to Tukey's Honestly significant difference test ($P < 0.05$). Relationships among properties were studied using Pearson correlation coefficients. The fits and kinetic parameters were carried out using the SigmaPlot 10.0 software. ANOVA and correlations were performed with the software SPSS for Windows, Version 19.

6.3 RESULTS

6.3.1 SOIL ORGANIC C DYNAMICS

Total organic C was significantly greater in Org than in Conv soil, with higher values in the plants-amended soils compared to their CT ($\text{MW} > \text{IW} \approx \text{V} > \text{CT}$) (figure 6.1a). For recalcitrant C, Conv soil showed no significant differences among treatments at either 22 or 64 days, and showed values lower than those of the Org soil (figure 6.2a). Org soil showed significant higher values ($P < 0.001$) in the three plant-amended soils compared with CT at day 22; this trend was not observed at day 64, since recalcitrant C concentration significantly increased in CT from day 22 to day 64 (figure 6.2a). Addition of plant materials generally increased labile C contents in both management systems, with higher values in MW under Conv management (figure 6.2b). The recalcitrance index for total C (data not shown) presented no significant differences between treatments for any date.

Glucose addition did not cause an increase in Cbio for any soil management from day -8 to day 0 (figure 6.1b), as it was expected. However, an increase in microbial activity, measured as CO_2 released, was detected in the first hours (data not shown) after applying labelled glucose in trace amount termed "trigger" effect (De Nobili et al., 2001; Mondini et al., 2006). Cbio concentrations during the pre-incubation were significantly higher in Org soil. Plant materials addition significantly increased Cbio from day 0 to day 7, compared

with CT, mainly for IW and V treatments regardless of the management system. This newly formed biomass was not sustainable and, after day 7, C_{bio} significantly decreased in all treatments, with a slight increase from day 37. Throughout the incubation, plant-amended soils showed C_{bio} values greater than those of the controls, in the order IW > V > MW.

6.3.2 RECOVERY OF ¹³C IN SOIL ORGANIC C POOLS

The soil under Org management showed the highest significant ($P < 0.05$) content in total organic ¹³C at the beginning of the incubation. The Org-CT maintained these higher values along the entire incubation (figure 6.1c). As a general trend, total organic ¹³C contents significantly decreased as incubation progressed from day 0 to day 64 for all treatments in both soil managements. IW and V at Conv soil showed the lowest values in total organic ¹³C. The recovery of the ¹³C added from glucose in total organic C decreased during incubation being slightly higher in CT than plants-amended soil within same management, especially in Org soil. The lowest recovery was usually found in IW and V, especially for Conv soil (table 6.2).

The results for recalcitrant ¹³C did not show significant differences among treatments in any date (figure 6.2c). At the end of the incubation, the average recovery of ¹³C derived from glucose was 6.6 and 6.2%, for Org and Conv, respectively, with no significant differences among treatments (table 6.2). Labile ¹³C contents tended to significantly decrease with time in all treatments (figure 6.2d), with lowest values in IW and V. The percentage of the original input of glucose C recovered in the labile fraction at the end of incubation averaged 35.3 and 32.4% for Org and Conv management, respectively (table 6.2). All Org treatments and Conv-CT showed the highest recovery ratios. The recalcitrance index for ¹³C (data not showed) presented no significant differences among treatments for any date, with an average value of 0.15.

The ¹³C content of the microbial biomass was similar at day 0 for both managements, without significant differences between them. After plant materials addition, the amount of ¹³C incorporated in the microbial biomass reached the maximum at day 7, being significantly higher in the plants-amended soils (IW ≈ V > MW) compared to CT regardless of the management system (figure 6.1d). The ¹³C of the microbial biomass significantly decreased in all treatments from day 7 to day 14; from that date forward it remained more or less stable until day 37, after which an increasing trend was observed. The presence of C derived from glucose (recovery) in C_{bio} reached a maximum 7 days after incorporation of plants (table 6.3). At the end of incubation, it was significantly

higher for plant-amended treatments, with highest values in Org-MW, Conv-MW and Org-IW (5.56%, 4.81% and 4.73%, respectively).

Figure 6.1 Total organic C (a), microbial biomass C (b), total organic ¹³C (c), and microbial biomass ¹³C (d). Data are mean (g C kg⁻¹) ± standard deviation (n=3). Gray arrow shows timing of ¹³C-glucose addition, while white arrow shows plant materials addition. Solid line and orange symbols indicate organic management; open line and blue symbols indicate conventional management. Treatments: control (crosses), mature wheat (squares), immature wheat (triangles) and vetch (circles). Significant differences at *P<0.05; **P<0.01 and ns: non significant among treatments within the same day. If nothing is indicated treatments showed significant differences at P<0.001.

Figure 6.2 Recalcitrant C (a), labile C (b), recalcitrant ¹³C (c) and labile ¹³C (d) at days 22 and 64. Data are mean (g C kg⁻¹) ± standard deviation (n=3). Management practices: organic (orange bars) and conventional (blue bars). Treatments are CT: control; M: mature wheat; I: immature wheat and V: vetch. Significant differences at *P<0.05; **P<0.01; ***P<0.001 and ns: non significant among treatments within the same day. Different letters indicate significant differences among treatments within same day at P < 0.05.

Table 6.2 Percentage of ^{13}C -glucose recovery in soil organic C pools. Values are mean $\pm$ standard deviation (n=3).

C pool	Day	Organic management								Conventional management								F ^a
		CT		MW		IW		V		CT		MW		IW		V		
Total	0	55.4a	±1.60							52.2b	±0.19							6.590***
	7	54.5a	±0.54	53.5ab	±1.09	50.7b	±0.14	51.3ab	±0.98	48.6bc	±2.35	47.7bc	±1.40	45.4c	±0.33	45.1c	±1.91	21.555***
	14	52.3a	±1.70	48.7ab	±1.68	47.3b	±0.66	47.4b	±0.68	46.2bc	±1.05	45.3bc	±1.64	42.7c	±1.70	43.2c	±0.45	16.885***
	22	51.3a	±0.34	51.0a	±0.75	47.0b	±0.52	47.9b	±0.66	48.2b	±0.49	45.5b	±0.42	43.4c	±0.76	43.4c	±1.05	63.764***
	29	50.9a	±0.71	49.2ab	±2.13	46.3b	±0.79	47.9b	±0.41	46.2b	±0.62	45.5bc	±1.21	42.7c	±0.44	43.2c	±0.26	23.783***
	37	49.5a	±0.66	48.3ab	±0.53	45.4ab	±0.45	43.9bc	±2.46	43.5bc	±0.79	44.5bc	±2.08	40.3c	±0.57	42.8bc	±2.37	11.662***
	50	46.5a	±0.82	44.8a	±0.63	42.3b	±0.52	43.9ab	±0.53	43.1ab	±1.04	40.6bc	±0.70	37.3c	±0.25	38.7bc	±0.84	58.311***
Recalc	64	44.3a	±1.62	41.6ab	±1.18	40.4b	±0.48	41.7ab	±0.69	41.5ab	±0.70	39.7b	±1.69	35.8c	±0.86	37.2bc	±0.79	18.593***
	22	6.5	±0.35	7.2	±0.70	6.8	±0.17	7.2	±0.76	7.3	±0.33	7.0	±0.32	6.9	±0.20	7.4	±0.82	0.829ns
	64	7.1	±0.28	6.9	±0.75	6.3	±0.89	6.0	±0.64	6.4	±0.25	6.1b	±0.51	6.0	±0.77	6.2	±0.40	1.351ns
Labile	22	44.8a	±0.18	43.9ab	±1.45	40.2b	±0.66	40.7b	±1.31	41.0b	±0.21	38.5bc	±0.66	36.5c	±0.59	36.0c	±1.85	25.575***
	64	37.3a	±1.90	34.7ab	±0.71	34.1b	±1.03	35.7ab	±0.27	35.1ab	±0.83	33.6b	±1.38	29.8c	±0.96	31.0bc	±0.72	15.469***

Recalc: recalcitrant C. CT: control; MW: mature wheat; IW: immature wheat; V: vetch. ^a F-value: *** indicate significant differences at $P < 0.001$. ns: non significant. Different letters indicate significant differences among treatments.

Table 6.3 Percentage of ^{13}C -glucose recovery in soil microbial biomass. Values are mean $\pm$ standard deviation (n=3).

Day	Organic management				Conventional management				F^a
	CT	MW	IW	V	CT	MW	IW	V	
0	10.1 $\pm$ 0.65				10.2 $\pm$ 0.32				0.034ns
7	7.23bc $\pm$ 0.17	9.11b $\pm$ 0.89	13.8a $\pm$ 0.94	13.1a $\pm$ 1.23	7.12c $\pm$ 0.97	9.66b $\pm$ 0.70	12.7a $\pm$ 0.62	13.2a $\pm$ 1.15	31.147***
14	1.47bc $\pm$ 0.20	1.88ab $\pm$ 0.06	2.24a $\pm$ 0.20	1.99ab $\pm$ 0.15	1.43c $\pm$ 0.07	1.63bc $\pm$ 0.22	2.02ab $\pm$ 0.12	2.28a $\pm$ 0.16	12.618***
22	2.66c $\pm$ 0.20	4.04ab $\pm$ 0.45	5.08a $\pm$ 0.21	4.04ab $\pm$ 0.43	2.69c $\pm$ 0.31	3.36bc $\pm$ 0.07	3.42bc $\pm$ 0.39	3.02c $\pm$ 0.37	16.651***
29	0.68 $\pm$ 0.21	1.06 $\pm$ 0.19	1.07 $\pm$ 0.17	1.01 $\pm$ 0.11	0.75 $\pm$ 0.16	1.26 $\pm$ 0.68	1.12 $\pm$ 0.18	1.04 $\pm$ 0.39	1.028ns
37	1.11 $\pm$ 0.11	1.76 $\pm$ 0.20	1.76 $\pm$ 0.18	1.13 $\pm$ 0.40	1.52 $\pm$ 0.18	1.50 $\pm$ 0.49	1.61 $\pm$ 0.36	1.40 $\pm$ 0.32	1.675ns
50	2.64c $\pm$ 0.07	3.68b $\pm$ 0.27	3.68a $\pm$ 0.07	4.20ab $\pm$ 0.17	2.75c $\pm$ 0.30	3.84ab $\pm$ 0.38	4.14ab $\pm$ 0.41	4.25ab $\pm$ 0.80	15.088***
64	3.73bc $\pm$ 0.06	5.56a $\pm$ 0.34	4.73ab $\pm$ 0.23	4.29b $\pm$ 0.61	3.56bc $\pm$ 0.31	4.81ab $\pm$ 0.41	4.00bc $\pm$ 0.18	3.22c $\pm$ 0.44	12.551***

CT: control; MW: mature wheat; IW: immature wheat; V: vetch. ^a F -value: *** indicate significant differences at $P < 0.001$. ns: non significant. Different letters indicate significant differences among treatments.

6.3.3 C MINERALIZATION DYNAMICS

The cumulative quantities of total CO₂-C evolved from soils under the different treatments were fitted to different kinetic models (figure 6.3). CT and soils amended with MW showed the best fit with a zero-order model:

$$C_{min} = kt + C_0 \quad (2)$$

where C_{min} is the carbon mineralized from soil (mg C g⁻¹) in a given time t (d), k is a zero-order rate constant (d⁻¹) and C_0 represents a pool of highly mineralizable C (mg C g⁻¹). Soils amended with IW and V were best fitted to a double first-order exponential model:

$$C_{min} = C_1 (1 - e^{-k_1 t}) + C_2 (1 - e^{-k_2 t}) \quad (3)$$

where C_{min} is the carbon mineralized from soil (mg C g⁻¹) in a given time t (d), C_1 and C_2 represent the sizes of the active and slow pools of mineralizable C (mg C g⁻¹), respectively, and k_1 and k_2 are the corresponding mineralization rate constants for each pool (d⁻¹).

Figure 6.3 Cumulative values of CO₂-C mineralized (symbols are experimental data and lines represent the fitting of zero-order or the double first-order model). Values from -8 to 0 days indicate the pre-incubation of soils with ¹³C-glucose. The addition of plant materials was carried out at day 0. Solid line and black symbols indicate organic management (Org); open line and white symbols indicate conventional management (Conv). Treatments: control (CT), mature wheat (MT), immature wheat (IW) and vetch (V). Error bars denote standard deviation.

These models explained more than 96% of the experimental data for all treatments (table 6.4). The mineralizable C pool, in general, was lower for CT, mainly under Conv management, compared with the other treatments. MW showed greater C_0 values compared to CT, being slightly lower under Conv management (table 6.4). Values of rapidly mineralizable C (C_1) indicated a much larger presence of easily biodegradable organic compounds in V treatment for Org soil, with 45% of the total potentially mineralizable C ($C_1 + C_2$). Rapidly mineralizable C pool in V treatment was higher for Org (45%) than Conv soil (37%). The IW showed similar values in both management systems (26 and 28%). These values are higher than those found in literature for composted materials (Fernández et al., 2007; Turrión et al., 2012). Since C_2 values are higher than C_1 , most of the organic C in the samples is slowly mineralized, except for Org-V. This slow phase is particularly predominant in the case of IW amendments. Rate mineralization constants for CT were the lowest among all treatments, being 3.4 and 4.2 times higher in Org-MW and Conv-MW, respectively. Rate constants corresponding to the first stage (k_1) showed values between 0.48 to 0.68 d^{-1} , higher than those obtained for composted materials (Fernández et al., 2007; Turrión et al., 2012), with a decreasing trend as Org-IW > Conv-IW > Org-V $\approx$ Conv-V. According to the rate constant of second stage (k_2), this step is faster in Conv-IW, followed by Conv-V $\approx$ Org-V > Org-IW.

Table 6.4 reports data relative to the initial potential rate of C mineralization ($C_n \cdot k_n$), a product considered more accurate to explain the quality of organic matter than either one separately (Saviozzi et al., 1993). The lowest values of this potential rate were obtained in CT samples, followed by MW, mainly in Conv soil, indicating high stability of the organic matter. The Org-IW and Org-V had the highest initial potential rate of C mineralization at first stage, which highlights the importance of the labile compounds and their intense degradation of these plant materials under organic management. The degradability of these materials is more reduced under conventional management. During the second phase, the quality of the organic matter present in soil under conventional management favours an enhanced microbial attack, reflected by the higher $C_2 \cdot k_2$ product.

Table 6.4 Parameters of zero-order model fitted to the cumulative values of CO₂-C mineralized from control (CT) and soil amended with mature wheat (MW), and parameters of double first order kinetic model fitted to the cumulative values of CO₂-C mineralized from soil amended with immature wheat (IW) and vetch (V) for both organic and conventional managements.

Model	Parameter	Organic management				Conventional management			
		CT	MW	IW	V	CT	MW	IW	V
Zero-order	C ₀ (mg C g ⁻¹)	0.1666	0.4906	-	-	0.1026	0.4418	-	-
	k (d ⁻¹)	0.0114	0.0390	-	-	0.0082	0.0347	-	-
	C ₀ *k (mg C g ⁻¹ d ⁻¹)	0.0019	0.0191	-	-	0.0008	0.0153	-	-
Double first-order	C ₁ (mg C g ⁻¹)	-	-	1.4286	2.1187	-	-	1.2570	1.5868
	C ₂ (mg C g ⁻¹)	-	-	4.1351	2.5724	-	-	3.2517	2.6550
	%C ₁	-	-	26	45	-	-	28	37
	%C ₂	-	-	74	55	-	-	72	63
	k ₁ (d ⁻¹)	-	-	0.6789	0.4933	-	-	0.5433	0.4777
	k ₂ (d ⁻¹)	-	-	0.0187	0.0229	-	-	0.0354	0.0243
	C ₁ *k ₁ (mg C g ⁻¹ d ⁻¹)	-	-	0.9699	1.0452	-	-	0.6829	0.7580
	C ₂ *k ₂ (mg C g ⁻¹ d ⁻¹)	-	-	0.0773	0.0589	-	-	0.1151	0.0645
R ² adj		0.982	0.984	0.999	0.999	0.967	0.974	0.999	0.998
F ^a		1161	912	7545	11687	474	562	6116	2757

^a F-value. Significant at $P < 0.001$

The addition of plant materials to soil provoked positive PEs on the native SOM mineralization (figure 6.4; table 6.5). The labelled ¹³C-CO₂ production after addition of plant materials over time was fitted to a double first-order exponential model (Eq.3) for all treatments, suggesting the presence of two different pools of mineralizable organic ¹³C. These models explained more than 99% of the experimental data. Values of C₁ indicated a much larger presence of easily biodegradable organic compounds in Org-V. Percentages of rapidly mineralizable ¹³C pools (table 6.5) were similar to those observed for total C-CO₂ emissions (table 6.4), suggesting that newly formed native ¹³C organic compounds derived from ¹³C-glucose have similar structure and biodegradability than oldest native SOM. As C₂ values indicate, except for V treatment, most of the organic ¹³C in the samples is slowly mineralized, particularly for MW. Rate constants corresponding to the first stage (k₁) showed similar values (table 6.5) as those achieved for total C-CO₂ emissions (table 6.4). However, k₂ for ¹³C-CO₂ production is higher than for total C-CO₂ production, suggesting higher rates of decomposition for the second phase of mineralization of newly formed ¹³C compounds. The highest values of the C₁*k₁ product were observed for IW and V in both

management systems, while the highest values of the $C_2 \cdot k_2$ product were found in IW and V under conventional management. The PE at the end of the incubation was higher in soil under conventional management with $IW > V > MW$ (figure 6.4; table 6.5).

Figure 6.4 Cumulative values of $^{13}\text{C-CO}_2$ mineralized where control values have been subtracted to assess the priming effect (symbols are experimental data and lines represent the fitting of the double first-order kinetic model). Solid line and black symbols indicate organic management (Org); open line and white symbols indicate conventional management (Conv). Treatments: mature wheat (MT), immature wheat (IW) and vetch (V). Error bars denote standard deviation.

Table 6.5 Parameters of double first order kinetic model fitted to the cumulative values of ^{13}C - CO_2 mineralized from soils amended with mature wheat (MW), immature wheat (IW) and vetch (V) for both organic and conventional managements.

Parameters	Organic management			Conventional management		
	MW	IW	V	MW	IW	V
C_1 (mg C g $^{-1}$)	0.0017	0.0052	0.0066	0.0015	0.0060	0.0059
C_2 (mg C g $^{-1}$)	0.0178	0.0132	0.0069	0.0119	0.0153	0.0110
% C_1	9	28	49	11	28	35
% C_2	91	72	51	89	72	65
k_1 (d $^{-1}$)	0.6354	0.6763	0.5139	0.6956	0.5729	0.5016
k_2 (d $^{-1}$)	0.0100	0.0292	0.0503	0.0292	0.0553	0.0632
C_1*k_1 (mg C g $^{-1}$ d $^{-1}$)	0.0011	0.0035	0.0034	0.0010	0.0034	0.0030
C_2*k_2 (mg C g $^{-1}$ d $^{-1}$)	0.0002	0.0004	0.0003	0.0003	0.0008	0.0007
PE (%)	23	38	30	28	50	39
R^2 adj	0.993	0.997	0.994	0.989	0.999	0.996
F_a	466	1254	568	308	4594	828

^a F -value. Significant at $P < 0.001$

PE: priming effect

6.4 DISCUSSION

6.4.1 TOTAL, RECALCITRANT AND MICROBIAL BIOMASS C

Initial content of total organic C and C_{bio} were higher in the organic management system since using organic residues has been shown to maintain soil organic matter at higher levels than inorganic fertilization (Edmeades, 2003). With the addition of plant materials, greater values of total organic C were achieved, although this increase was smaller in the soils amended with IW and V, owing to the high degradability of these materials. Since total recalcitrance index (0.62 and 0.60 on average) was similar among treatments and dates, higher values of both recalcitrant and labile C pools for Org management were likely due to their higher inherent organic matter content, also supported by the positive correlation with organic C ($r=0.76$ and 0.70 ; $P < 0.001$, respectively). Recalcitrant C increased while labile C decreased significantly in Org-CT upon incubation maybe because of a more mature microbial biomass owing to the history of organic farming, compared to conventional management, and that no plants materials, a source of labile compounds, were applied. The increase in C_{bio} was greater in plants-amended soils, also observed by De Nobili et al. (2001) and Hoyle et al. (2008) in soils amended with cellulose-C, and more intense with more easily mineralizable plant materials (IW and W treatments). This observation was supported by the negative

correlation between C_{bio} and cellulose and lignin, and a positive correlation with soluble C and N plants content ($r = -0.93, -0.93, 0.67$ and 0.87 ; $P < 0.01$, respectively). The increase during the first week may represent the initial response of a fast-growing microbial community to the rapid mineralization of the more labile plant material. The highest increments are attributable to the first stage of faster mineralization for IW and V treatments. After that, C_{bio} rapidly decreases by exhaustion of easily available compounds (Fontaine et al., 2004). By day 22 we observed a new slight increment in C_{bio} that could be a consequence of labile C available from microbial necromass. The increase by day 37 could indicate mineralization of more resistant fractions corresponding to a slow mineralization for CT and MW and a second stage of slower C mineralization for IW and V treatments, with a parallel growth of K-strategists (slow-growing organism) (Cayuela et al., 2009; Fontaine et al., 2003).

6.4.2 FATE OF INITIAL ADDITION OF GLUCOSE

While total organic C maintained more or less stable with time, total organic ^{13}C significantly decreased as incubation progressed. Since $^{13}\text{C}_{\text{bio}}$ stabilized or even slightly increased at the end of the incubation, losses in total organic ^{13}C only could be caused by mineralization of the non-biomass organic ^{13}C , particularly of the new labile pool, as shown by the decline in labile ^{13}C . This is supported by the strong positive correlation between organic ^{13}C and labile ^{13}C ($r = 0.99$; $P < 0.001$). The fact that plant-amended soils suffered a larger decrease in total organic ^{13}C compared to CT confirms a positive priming effect (PE) caused by plant addition. The lowest recovery of ^{13}C derived from the initial glucose addition in all measured soil organic pools (total, recalcitrant and labile C) occurred with the addition of plant material, especially with more labile plant materials (IW and V) and under conventional management is likely due to their higher mineralization of organic matter as a result of the priming effect (table 6.5).

The $^{13}\text{C}_{\text{bio}}$ showed no significant differences between both Org and Conv management by day 0, which indicates that efficiency of glucose utilization by microbial biomass was very similar in both systems. Glucose, a soluble low-molecular substrate, presumably diffuses relatively readily within the soil matrix to sites of location of decomposer organisms, where it is utilised rapidly and with high efficiencies (Amato and Ladd 1991). The amount of glucose derived C in the microbial biomass followed a very similar trend to the overall size of the biomass throughout the incubation ($r = 0.83$; $P < 0.001$). At the beginning of incubation the ^{13}C assimilation by microbial biomass was

very influenced by plant composition, supported by its negative correlation with plant cellulose and lignin contents ($r=-0.88$; $P<0.001$ for both), and positive correlation with plant soluble C and N contents ($r=0.72$ and 0.80 ; $P<0.001$). As such, IW and V treatments showed a similar and significantly higher ^{13}C microbial immobilization than MW which was more recalcitrant, owing to increased microbial activity in response to easily degradable compounds in IW and V. However, with incubation progressing, this trend reverted and ^{13}C recovery in microbial biomass for MW treatment was significantly higher. This was likely due to lower losses of ^{13}C such as $^{13}\text{CO}_2$ by mineralization (more intense in IW and V) which makes ^{13}C more available in soil for biomass assimilation.

6.4.3 C MINERALIZATION

The rapid increment in C_{bio} in plant-amended soils during the first week of incubation (mainly for IW and V) led to an acceleration of SOM mineralization which was measured as a significant increased in CO_2 evolution. The CO_2 -C emission data for CT and MW in both Org and Conv correctly fit a zero-order model, which may be due to the very short initial phase of rapid degradation in these samples, characteristic of a well-mature organic matter. In contrast, IW and V amended soils with more intense mineralization activity, show better fits as the complexity of the models increases. The double first-order model fitted to IW and V soils appears to be consistent with some mechanistic models that divide SOM into active pool, rapidly mineralized, and slow pool which breaks down slowly during a second phase (Kätterer et al., 1998; Fernández et al., 2007; Turrión et al., 2012). The resistant pool is not included in the equation, assuming that it does not contribute significantly to C mineralization in a relatively short period (Turrión et al., 2012). As shown in this study, Fernández et al. (2007) found that soils amended with sewage sludge fitted well a double first-order model, but control and soil amended with composted materials fitted to zero-order models, owing to higher maturity of organic materials in the latter. C mineralization was lower in CT and MW treatment owing to its more recalcitrant nature, supported by the negative correlation between C mineralization and cellulose and lignin contents ($r=-0.99$; $P<0.001$), and the positive correlation with plant soluble C and N contents ($r=0.59$ and 0.95 ; $P<0.001$, respectively). Buchanan & King (1993) also reported that a high initial Lignin/N ratio (like MW) reflects a high resistance to decomposition.

6.4.4 PRIMING EFFECT

Increased production of ^{13}C -CO₂ in soils amended with plant material directly indicates greater mineralization of newly-formed organic matter derived from the ^{13}C -glucose addition and by extension, initially present as microbial bodies. This could be explained by the plants materials causing an accelerated turnover or *priming effect* (PE) of the organic ^{13}C . An overproduction of enzymes degrading cellulose and lignin by stimulating decomposers was hypothesized to be the cause of the real PE because these enzymes are also involved in the SOM decomposition (Fontaine and Barot, 2005). Guenet et al. (2010) found also positive priming effect when straw and cellulose were added to soil. We also observed that the plant material type impacts the intensity of PE with higher mineralization of native organic C especially for soils receiving more labile residues (IW and V). Thus, applying fresh organic inputs that act as a source of energy-rich substrates the microbial biomass increases its activity and growth (Fontaine et al., 2004; Bladogatskaya and kuzyakov, 2008). This confirms that PEs are related to the energy or ease of metabolism of inputs. Similar results were found by Toosi et al. (2012) who found lower positive PEs amending with rye-grass residue compare with its more labile water extract.

The management system is also an important factor contributing to ^{13}C priming effect, with higher mineralization of native organic matter under Conv management, coinciding with lower recoveries of ^{13}C -glucose. This is most likely due to a different microbial community structure and activity as a consequence of contrasting managements.

Since ^{13}C mineralization represented a very small portion of total C mineralized during incubation time we can deduce that most of the C mineralized was not derived from the original glucose addition, although it is not possible to distinguish if this was derived from the added plant materials and/or the priming effect on native soil organic C.

6.5 CONCLUSIONS

The application of plants materials to soil increased the size of microbial biomass, especially under organic management. An addition of glucose was much more assimilated into microbial biomass with the addition of plant residues compared to non-amended soils. Among plant materials, immature wheat and vetch showed higher mineralization rates than mature wheat because of their greater lability. However, addition of plant material also stimulated a higher mineralization of newly formed organic carbon (positive

priming effect). This was again most pronounced for the more labile immature wheat and vetch and under conventional management. As a consequence, at the end of incubation, less of the initial glucose input was incorporated into soil organic C (all C pools) in plant-amended soils compare with non-amended soils. Regardless of the treatment and management system, 6.4% on average of the C added as glucose was incorporated into the soils as recalcitrant organic carbon.

Changes in the quality and quantity of plant material input by changes in land use may change the proportion of soil organic carbon release through priming effect and therefore the C balance in the soil.

CHAPTER 7

CONCLUSSIONS

CONCLUSIONS

1. The rate of carbon mineralization for pepper residues used as soil amendment was very similar in the three application doses essayed (about 30%) showing that carbon mineralization was irrespective of the dose applied. It was highly affected by the initial soluble carbon content. Incorporation of organic residues increased microbial biomass carbon in comparison to unamended soils.
2. Net nitrogen mineralization of pepper crop residues indicated that there were large differences in the extent of the decomposition between application rates. N mineralization was negative for the lowest rate applied, indicating mainly a N immobilization and denitrification. Mineralization was positive from day 50 for the intermediate dose and was always positive for the highest dose. Lower application rates do not provide enough organic material to long-term activate enzyme activities and release available inorganic nitrogen. Thus, it is necessary to apply high rates of residues to avoid deficiencies of available N for plants by microbial immobilization.
3. Losses in soil organic carbon were greatest in soils treated only with chemical fertilizers at the end of the field experiment, suggesting that short-term application of pepper residues helped to mitigate the overall decline of soil organic carbon and soluble carbon.
4. The use of pepper crop residues at the highest application rate showed a positive effect on total nitrogen, microbial biomass carbon and potentially mineralizable nitrogen and arylesterase, β -glucosidase, urease, protease and alkaline phosphatase activities compared to chemical fertilizers and control confirming the direct effect of crop residues increasing soil quality.
5. The nuclear magnetic resonance ^{13}C technique showed that pepper residues contain a high amount of carbohydrates which stimulated microbial activity and the release of enzymes to decompose them. Pepper residues application was correlated with increments in carboxylic functional groups which is very positive due to their superficial charges that may adsorb and retain more nutrients.
6. Broccoli yield showed similar values with the largest application rate of both chemical fertilizer and crop residues. However, the use of crop residues maintains broccoli

quality with no negative effect on broccoli nutritional status using less chemical fertilizers. Thus, crop residues with minimal amount of chemical fertilizers can be used to obtain an adequate yield.

7. Excessive available nitrogen had detrimental effect on marketable yield. Maximum yields were obtained when the season available nitrogen content (nitrogen applied as chemical fertilizer or crop residues plus initial soil available nitrogen) was between 545 and 598 kg N ha⁻¹. Thus, application rates which surpass this threshold would suppose not effective source of nitrogen. Hence, nitrogen fertilization rates recommendations must be based, not only on fertilization application rates and crop yield, but also on current soil available nitrogen.

8. The amount of ¹³C-glucose incorporated into the soils as recalcitrant organic carbon did not show significant differences between crop residues and management practices, and ranged between 6.0 and 7.1%.

9. Carbon mineralization was lower in control and soil amendment with mature wheat treatment owing to its more recalcitrant nature, and especially in conventional management. Mineralization data for both treatments correctly fitted a zero-order model, which may be due to the very short initial phase of rapid degradation in these samples, characteristic of a well-mature organic matter. In contrast, immature wheat and vetch amended soils with more intense mineralization activity, showing better fits with more complex models.

10. Addition of crop residues caused an accelerated organic ¹³C turnover or priming effect. Crop residue type impacted the intensity of priming effect with higher mineralization for soils receiving the most labile residues (immature wheat and vetch) due to a higher stimulation of microbial biomass. The management system, organic or conventional, is also an important factor contributing to ¹³C priming effect, presenting higher mineralization of native organic matter under conventional management coinciding with lower recoveries of ¹³C-glucose.

CHAPTER 8

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PHOTOGRAPHIC ANNEX

Pictures 1 & 2. Pepper greenhouses, with and without soil (substrate).

Pictures 3 & 4. Crushing machine and pepper crop residues ready to be crushed.

Pictures 5 & 6. Crop residues application and incorporation into the soil.

Pictures 7 & 8. Broccoli crop essay. Initial and final stages.

Pictures 9 & 10. Chemical fertilizers applied and drip irrigation system detail.

Pictures 11 & 12. Broccoli yield and calibration of broccoli heads.

Pictures 13 & 14. Initial and final soil sampling at two depths (0-30 and 30-60cm).

Pictures 15 & 16. Broccoli samples grinding.

Pictures 17 & 18. Soil sieving.

Pictures 19 & 20. Detail of soil texture determination (treatment with H_2O_2) and cation exchange capacity analysis.

Pictures 21 & 22. Atomic absorption equipment (AAnalyst 800, Perkin Elmer) and nitrogen distillation unit (K-360, Büchi).

Pictures 23 & 24. Details of total Kjeldahl nitrogen determination.

Pictures 25 & 26. Crop residues and crop residues application in experiment with ^{13}C -labelled glucose.

Picture 27. ^{13}C -CO₂ samples in vials for measuring by Isotope ratio mass spectrometer (IRMS).