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RESEARCH ARTICLE

Pepper crop residues and chemical fertilizers effect on soil fertility, yield and nutritional status in a crop of *Brassica oleracea*

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Abstract

Effects of pepper crop residues as amendment and their optimal application rates on an agricultural soil have been sparsely studied. A comparative study of the development of a broccoli crop has been conducted using chemical fertilizers (CF) and fresh pepper crop residues (CR) at different application rates during two crop 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, 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 applied at the 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.

Keywords: Nutrients recycling, crop residues, microbial biomass, enzyme activities, broccoli

1. Introduction

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) have become an essential input for crop productivity in most areas of the world. In the Mediterranean region, irrigated vegetable crop production has undergone an enormous expansion over the last decades. In greenhouses, pepper is mainly grown as monocrop. Pepper crop residues (CR) (leaves, roots, steams and fruits) should be removed to facilitate the continuity of crop production. 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, crop yield, organic carbon and microbial activity (Tejada *et al*., 2006). An extensive amount of vegetable residues has been studied as an organic amendment such as wheat, mungbean, maize, straw, among others (Tejada *et al*., 2006; Ghosh *et al*., 2011), 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 (Sinsha *et al*., 2011).

Soil microbial populations and enzymes are crucial in the degradation of organic compounds and for the cycling of nutrients. As a consequence, soil microbial biomass, soil enzyme activities and biochemical quotients have been used as indicators of productivity and soil quality, or potential rate of soil to decompose organic wastes, owing to their high sensitivity (Pascual *et al*., 1998).

In this study, CF and pepper CR were used to cultivate broccoli. Many studies showed the highest broccoli production with an N fertilization rate ranging mainly from 224 to 558 kg N ha^{-1} (Dufault and Waters, 1985; Zebarth *et al*., 1995), but even lower values have been found (Babik and Elkner, 2002; Belec *et al*., 2001). For that, N fertilization rates recommendations must be based not only on crop production but also on crop quality and inorganic N content in soils to prevent environmental risks. Plants can take up nitrogen as nutrient in either of two forms, nitrate and ammonium, although ammonium is subject to conversion to nitrate by nitrification. Nitrate can be converted to N_2O and lost to the atmosphere contributing to climate warming or can be easily leached through soil contaminating groundwater (Babik and Elkner, 2002; Mugwe *et al*., 2009).

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 totally or partially replace CF with positive effects on soil and crop.

2. Materials and Methods

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 17ºC and a mean annual rainfall of 300 mm. The soil is a Typic Petrocalcid (Soil Survey Staff, 2014) with loam texture. Soil characteristics are shown in Table 1.

Table 1. Soil characteristics of the study site. Values are mean ± standard deviation (n=24)

Soil properties	0 - 30 cm	30 - 60 cm
Sand (g kg $^{-1}$)	407 ± 25	352 ± 32
Silt (g kg $^{-1}$)	356 ± 24	399 ± 28
Clay (g kg $^{-1}$)	237 ± 27	249 ± 18
Textural class	Loam	Loam
pH	7.81 ± 0.15	7.79 ± 0.34
Electrical conductivity (µS cm^{-1})	921 ± 172	657 ± 145
$CaCO_3$ (g kg $^{-1}$)	584.4 ± 31.8	556.7 ± 15.27
Soil Organic Carbon (g kg^{-1})	19.34 ± 2.38	9.51 ± 1.91
Total Nitrogen (g kg^{-1})	1.52 ± 0.13	0.65 ± 0.18
Cation Exchange Capacity (cmol$_+$ kg^{-1})	7.95 ± 0.72	6.27 ± 0.80
Exchangeable Ca (g kg^{-1})	4.51 ± 0.27	4.06 ± 0.09
Exchangeable Mg (g kg^{-1})	1.70 ± 0.03	1.19 ± 0.24
Exchangeable Na (g kg^{-1})	0.75 ± 0.10	0.85 ± 0.45
Exchangeable K (g kg^{-1})	2.26 ± 0.22	1.09 ± 0.37
Available Cu (mg kg^{-1})	0.76 ± 0.06	0.73 ± 0.06
Available Zn (mg kg^{-1})	2.07 ± 0.04	0.69 ± 0.16
Available Fe (mg kg^{-1})	3.30 ± 0.12	2.20 ± 0.24
Available Mn (mg kg^{-1})	16.59 ± 0.82	8.55 ± 0.43

The experiment was set as randomized design with three replications. Each plot size was 6x4 m². The crop grown was broccoli (*Brassica oleracea* L. var. *Italica*) cv. Marathon under drip irrigation. Broccoli was grown between October and February with density of 5 plants m^{-2}. The experiment spanned for two crop cycles. 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, 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 $Ca(NO_3)_2$, KNO_3 and NH_4NO_3 to achieve N rates, and Ca and K requirements by the crop according to the local law. CR application rates (CR1, CR2 and CR3) were the same than for CF but applied as crop residues. The pepper residues were mechanically crushed < 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. 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 KH_2PO_4, KNO_3 and $Ca(NO_3)_2$ to all plots except for CT. An additional 170 kg ha^{-1} of N was applied as CF to all CR plots to guarantee initial crop requirements. Characteristics of pepper residues are shown in Table 2.

Table 2. Chemical characteristics of the pepper residues for the two studied broccoli cycles. Values are mean ± standard deviation (n=5)

Properties	1st crop cycle	2nd 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 (%)	n.d.	20.0 ± 0.1
Lignin (%)	n.d.	17.9 ± 0.5
Soluble C (%)	n.d.	3.4 ± 0.3

Nt: Total N; TOC: Total Organic C; n.d.: not determined

2.2. Soil and broccoli sampling

Two samplings per crop cycle were carried out, one before adding CR in October (S1 and S3) and the other one after broccoli harvest in February (S2 and S4). 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.

All marketable broccoli heads from each plot were collected and weighed to calculate the yield. Five heads per plot were randomly collected from each plot and their nutrient content analyzed. Twenty plants were also collected to measure quality parameters: hollow steam, fresh head weigh, head dry matter, leaf dry matter, head diameter, stem diameter, fresh head weight:head diameter and head weight:stem diameter, based on marketable standards.

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, since they are highly sensitive and rapidly respond to changes in management practices (Pascual *et al*., 1998). 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.

$CaCO_3$ 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 by Kjeldahl digestion (Hoeger, 1998). Available P was measured using the Olsen method, and cation exchange capacity (CEC) by use of Ba^{2+} as exchangeable cation (Roig *et al*., 1980). Exchangeable Ca, Mg, Na and K were determined in the CEC extract. Available Cu, Zn, Fe and Mn were extracted using DTPA (1:2 soil-extractant ratio) (Lindsay and Norvell, 1978). Nutrients (exchangeable Ca, Mg, Na, K and available Cu, Zn, Fe and Mn) were measured by ICP-MS (7500 CE, Agilent). NO_3^- and NH_4^+ contents were extracted with 2M KCl in 1:10 soil:extractant ratio. Then, NO_3^- was measured by UV-visible espectrophotometry according to Sampere *et al*. (1993) and NH_4^+ colorimetrically according to Kandeler and Gerber (1988). Available N was 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 a previous mineralization experiment (data not shown).

Microbial biomass nitrogen (MBN) was determined using the chloroform fumigation-extraction method (Vance *et al*., 1987), and measured with an automatic Analyser (Shimadzu TOC-5050A). The non-fumigated fraction was considered as soluble nitrogen (Nsol). Potentially mineralizable N (Nmin) was determined as the difference between available N after and before incubation (15 days at 60% water holding capacity and 25ºC). 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*. (2003). 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.

Plant tissues (pepper residues and broccoli) were oven dried and ground. Samples were incinerated at 500 ºC, 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) by combustion.

2.4. Statistical analyses

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.

3. Results

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. Nt tended to decrease between samplings in each season, but lower declines were observed in CR than in CF (Table 3). At depth, Nt values in CF3 were significantly higher in S4 than in S3. Available N showed significant differences among application rates for S2 in surface (with lower available N in CR1) and for S4 at depth (with values in CR higher than in CF). Available N showed a decreasing trend between samplings, being significant in the first cycle (in surface) and second cycle (for both depths), for all application rates (Table 3). Available P showed, as a general trend, no significant differences between application rates or samplings, with values ~250 mg kg^{-1} in surface and 75 mg kg^{-1} in subsurface (data not shown). For micronutrients (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 ± 0.18 mg kg^{-1}) than in CF treatment and CT (2.04-2.22 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 ± 0.69 mg kg^{-1} in average).

Exchangeable Ca, Mg, K and Na 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 other application rates (2.50 ± 0.29 cmol kg^{-1} vs. 1.32-1.89 cmol 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 ± 0.24 cmol kg^{-1} on average), and Mg which significantly decreased in all application rates (from 1.70 ± 0.08 to 1.44 ± 0.09 cmol kg^{-1} on average) except for CR3.

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

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

[a] Significant at: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ ns: non significant ($P > 0.05$). Different letters indicate significant differences among means ($P < 0.05$)

S1: sampling 1; S2: sampling 2; S3: sampling 3; S4: sampling 4. CT: control; CF: Chemical Fertilizers; CR: Crop residues

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, while for protease activity and MBN/Nt ratio, CR3 showed higher values than CT. Alkaline phosphatase and Nmin/Nt ratio showed higher values in all CR application rates than in CT and CF application rates. Nsol did not show significant differences among rates (Figure 1).

Between samplings, MBN showed a significant increase in S4 compared with S3 for CR application rates (Figure 1). 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 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.

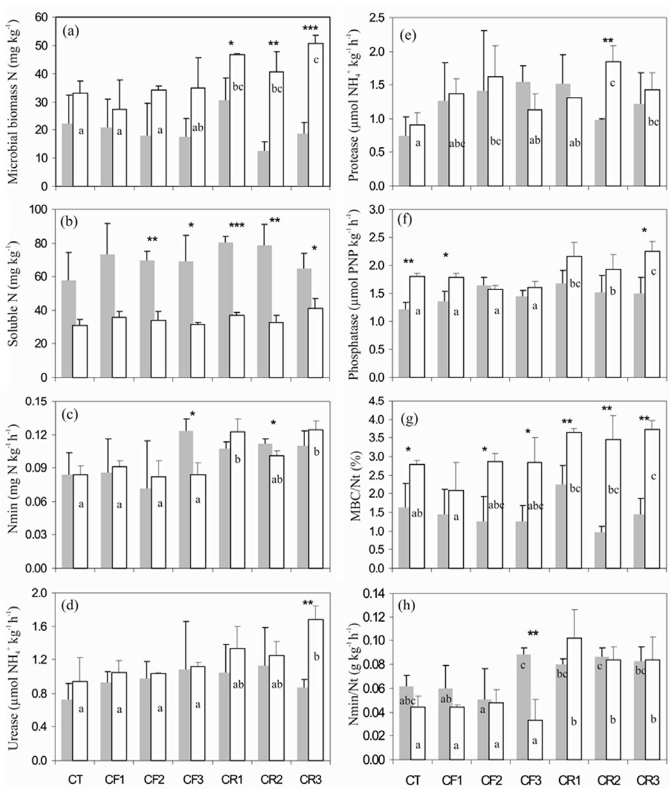

Figure 1. Soil microbial biomass nitrogen (a), soluble nitrogen (b), potentially mineralizable nitrogen (c), urease activity (d), protease activity (e), alkaline phosphatase activity (f), MBN/Nt ratio (g) and Nmin/Nt ratio (h) for initial (S3; gray colour) and final (S4; white colour) sampling of the second crop cycle. 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.

3.3. Broccoli yield, quality and nutrients

Significant differences in yield among application rates were only found for the second cycle (Table 4), being values for CF2, CF3 and CR higher than those of CT and CF1. Quality parameters only showed significant differences during the second season for head weight, head weight:head diameter and head weight:steam diameter, which were lower in CT, with no significant differences among CR and CF application rates (Table 4). In the first season, a high percentage (> 60%) of broccoli plants showed the hollow stem 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). Broccoli nutrients concentrations followed the decreasing trend N>P>K>Mg~Ca>Na>Fe>Zn>Mn>Cu.

Table 4. Broccoli yield and quality parameters in the second crop cycle. Data are shown as mean ± standard deviation (n=20).

Sample	Broccoli yield (kg ha^{-1})	Head weight (g head^{-1})	Head diam (cm)	Steam diam (cm)	Head weight: Head diam	Head weight :Stem diam	Head dry matter (%)	Leaf dry matter (%)
CT	9450a ± 934	189.8a ± 42.6	9.66 ± 1.06	3.09 ± 0.35	19.9a ± 2.7	59.4a ± 8.7	9.71 ± 0.34	11.38 ± 1.09
CF1	9967ab ± 652	223.6ab ± 11.9	10.57 ± 0.33	3.24 ± 0.09	21.0ab ± 0.9	68.2ab ± 2.8	9.42 ± 0.25	11.14 ± 0.39
CF2	10657abc ± 1671	219.4ab ± 1.30	10.44 ± 0.27	3.23 ± 0.06	20.7ab ± 0.4	66.8ab ± 1.0	9.36 ± 0.07	11.04 ± 0.55
CF3	12555c ± 1547	223.8ab ± 15.8	10.70 ± 0.45	3.20 ± 0.11	20.9ab ± 1.0	69.0ab ± 4.9	9.37 ± 0.07	11.23 ± 0.56
CR1	12163bc ± 1128	256.5b ± 18.5	11.28 ± 0.59	3.34 ± 0.06	22.7ab ± 1.1	76.3b ± 4.7	9.54 ± 0.05	11.32 ± 0.36
CR2	11675abc ± 1236	236.6ab ± 12.4	10.98 ± 0.60	3.37 ± 0.14	21.4ab ± 0.8	70.4ab ± 3.0	9.62 ± 0.04	10.90 ± 0.31
CR3	12428c ± 1116	261.6b ± 9.7	11.09 ± 0.12	3.41 ± 0.11	23.4b ± 0.8	75.6b ± 1.8	9.37 ± 0.09	11.34 ± 0.71
F[a]	3.104*	4.451*	ns	ns	3.595*	4.725 **	ns	ns

[a] Significant at: *P < 0.05, **P < 0.01, ns: non significant (P >0.05). Different letters indicate significant differences among means. (P < 0.05). CT: control; CF: Chemical Fertilizers; CR: Crop residues; Diam: diameter

Table 5. Nutrients concentrations in broccoli heads in both broccoli cycles. Data are shown as mean ± 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 ± 0.24	55.34 ± 1.59	56.68 ± 1.51	56.96 ± 0.99	56.15 ± 0.7	55.48 ± 2.06	53.99 ± 0.61	2.118ns
	2	46.67 ± 1.51	48.57 ± 0.85	48.87 ± 0.64	47.86 ± 2.54	48.37 ± 3.01	46.85 ± 1.46	48.12 ± 1.37	0.651ns
P (g kg^{-1})	1	25.4 ± 0.82	24.52 ± 1.85	24.07 ± 0.17	26.08 ± 1.68	26.86 ± 2.34	23.67 ± 1.41	23.73 ± 1.03	2.081ns
	2	23.1 ± 0.63	24.38 ± 1.6	24.35 ± 1.02	24.56 ± 0.48	23.72 ± 1.6	22.93 ± 1.41	25.24 ± 1.2	1.411ns
Ca (g kg^{-1})	1	1.82a ± 0.15	1.84a ± 0.16	1.78ab ± 0.06	1.81a ± 0.09	1.55abc ± 0.2	1.41c ± 0.08	1.52bc ± 0.23	4.204*
	2	3.05 ± 0.32	3.07 ± 0.25	3,00 ± 0.2	3.08 ± 0.17	2.82 ± 0.19	2.8 ± 0.1	2.72 ± 0.19	1.510ns
Mg (g kg^{-1})	1	2.08ab ± 0.09	2.10ab ± 0.02	2.13b ± 0.06	2.19b ± 0.05	2.14a ± 0.09	1.96b ± 0.12	1.97b ± 0.08	3.655*
	2	2.19 ± 0.11	2.22 ± 0.2	2.15 ± 0.06	2.18 ± 0.03	2.3 ± 0.07	2.33 ± 0.12	2.36 ± 0.11	1.581ns
Na (g kg^{-1})	1	1.15 ± 0.11	1.08 ± 0.22	1.14 ± 0.05	1.06 ± 0.18	1.33 ± 0.04	1.21 ± 0.11	1.13 ± 0.15	1.287ns
	2	0.98 ± 0.14	0.94 ± 0.13	0.88 ± 0.21	1.02 ± 0.14	1.09 ± 0.09	1.2 ± 0.04	1.12 ± 0.2	1.710ns
K (g kg^{-1})	1	36.01ab ± 2.08	35.59ab ± 0.5	36.14ab ± 1.01	37.73a ± 0.86	34.48b ± 0.7	32.11c ± 1.5	31.78c ± 0.52	10.824**
	2	36.94 ± 1.39	38.26 ± 1.71	38.06 ± 1.41	38.67 ± 1.63	38.08 ± 1.08	36.46 ± 2,00	37.33 ± 1.5	0.784ns
Cu (mg kg^{-1})	1	5.52 ± 0.45	5.14 ± 0.34	5.62 ± 0.37	5.32 ± 0.35	6.15 ± 0.63	5.62 ± 0.37	5.11 ± 0.7	1.205ns
	2	8.98 ± 1.83	8.73 ± 1.34	7.72 ± 2.26	9.68 ± 3.3	10.08 ± 2.07	9.01 ± 3.08	9.18 ± 1.66	0.312ns
Zn (mg kg^{-1})	1	41.32 ± 1.66	42.02 ± 2.83	37.48 ± 4.42	42.43 ± 0.3	41.52 ± 7.06	37.48 ± 4.42	36.61 ± 2.41	1.324ns
	2	36.64 ± 1.53	35.18 ± 1.57	34.39 ± 1.27	24.8 ± 0.63	36.31 ± 2.97	34.53 ± 3.48	36.84 ± 2.23	0.696ns
Fe (mg kg^{-1})	1	74.85 ± 23.19	50.29 ± 5.32	36.95 ± 0.63	45.13 ± 2.06	58.01 ± 21.2	36.95 ± 0.63	42.84 ± 7.47	1.138ns
	2	44.82 ± 5.73	47.99 ± 3.41	47.13 ± 10.78	44.27 ± 10.18	37.45 ± 3.63	40.17 ± 4.61	38.58 ± 5.39	1.123ns
Mn (mg kg^{-1})	1	22.82 ± 0.29	21.45 ± 0.88	23.36 ± 2.36	22.94 ± 1.65	25.74 ± 1.36	23.36 ± 2.36	21.88 ± 0.93	2.431
	2	28.4 ± 0.84	25.81 ± 3.84	24.35 ± 1.29	25.65 ± 1.42	24.81 ± 3.84	27.21 ± 0.81	25.17 ± 2.18	1.719ns

[a] Significant at: *P < 0.05, **P< 0.001, ns: non significant (P >0.05). Different letters indicate significant differences among means (P < 0.05). CT: control; CF: Chemical Fertilizers; CR: Crop residues

4. Discussion

The decrease trend observed for Nt in both cycles and treatments was 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 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 available N decline is mainly explained by mineral N uptake by plants during the growing season. Available N losses between samplings in the topsoil were lower with the highest application rate in both treatments in the second cycle. This could be related to a higher amount of NO_3^- coming from CF and CR with increasing application rates, which compensate losses by crop uptake, immobilization or leaching. Another possible explanation for N loss is denitrification favored 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 to the initial sampling, in both cycles, for all application rates, reducing the potential leaching after harvest. 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; Table 6). Increased nitrification by microbial activation is a positive factor, enhancing soil fertility, since NO_3^- can be taken up by plant roots (Scotti *et al*., 2015).

Table 6. Correlation coefficients (r values) for relationships between different soil and crop properties

	SOC	Nt	N_{av}	NO_3^-	Nsol	P	Nmin	MBN	MBN /Nt	Nmin /Nt	Ure	Pho	Pro	Yield	HW	HD	SD	HW/HD	HW/SD	NBroc
CR rate	0.28	0.35	0.42	0.61**	0.41	0.32	0.70**	0.75**	0.69**	0.28	0.70**	0.39	0.63**	0.48	0.42	0.40	0.28	0.38	0.36	0.27
CF rate	0.18	0.39	0.27	0.43	0.28	0.25	0.17	0.22	0.28	0.24	0.35	-0.70**	0.14	0.38	0.39	0.24	0.31	0.28	0.33	0.35
SOC		0.93**	0.23	0.15	0.37	0.86**	0.28	0.12	0.08	0.17	0.39	0.24	0.18	0.08	0.18	0.20	0.16	0.09	0.31	0.17
Nt			0.32	0.18	0.28	0.07	0.25	0.17	0.39	0.27	0.41	0.22	0.38	0.33	0.28	0.15	0.18	0.21	0.11	0.50**
N_{av}				0.95**	0.56*	0.25	0.21	0.18	-0.52*	0.27	0.18	0.22	0.12	0.28	0.31	0.28	0.33	0.17	0.16	0.52**
NO_3^-					0.96**	0.18	0.12	0.14	-0.56*	0.28	0.14	0.18	0.11	0.15	0.25	0.19	0.27	0.14	0.11	0.42
Nsol						0.08	0.28	-0.53*	-0.64**	0.18	0.24	0.14	0.22	0.12	0.14	0.08	0.11	0.14	0.21	0.17
P							0.21	0.18	0.11	0.14	0.07	0.08	14	0.24	0.14	0.18	0.12	0.08	0.14	0.04
Nmin								0.21	0.15	0.77**	0.22	0.25	0.17	0.40*	0.10	0.19	0.08	0.11	0.17	0.08
MBN									0.99**	0.08	0.59**	0.62**	0.21	0.28	0.22	0.18	0.27	0.23	0.23	0.17
MBN /Nt										0.17	0.29	0.63**	0.17	0.15	0.11	0.08	0.04	0.07	0.08	0.09
Nmin /Nt												0.22	0.30	0.51*	0.53*	0.42*	0.48*	0.53*	0.48*	0.22
Ure												0.59**	0.28	0.17	0.11	0.23	0.17	0.09	0.14	0.08

CR rate: crop residues application rate; CF rate: chemical fertilizers application rate; SOC: soil organic carbon; Nt: total nitrogen; N_{av}: available nitrogen; Nsol: soluble nitrogen; Nmin: potentially mineralizable nitrogen: MBN: microbial biomass nitrogen; Ure: urease activity; Pho: phosphatase activity; Pro: protease activity; HW: brcooli head weight; HD: broccoli head diameter; SD: broccoli steam diameter; NBrco: nitrogen content in broccoli crop.

*P<0.05; *P<0.01.

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 (Tunesi *et al*., 1999). Nonetheless, organic matter addition of soil reduces P insolubilization and increase extractable P (Scotti *et al*., 2015). 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; Table 6).

Plots where CR3 was applied showed higher MBN and microbial 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; Table 6), confirming the direct effect of organic residues content and microbial biomass and activity.

Nsol was likely mainly composed by NO_3^-, supported by the strong positive correlation between Nsol and NO_3^- (r=0.96; P<0.01; Table 6). 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 Dominguez, 1992), did not affect broccoli yield or quality parameters. Decreases in Nsol can be also associated to soil leaching, since NO_3^- is not retained by soil colloids in neutral/basic soils and rapidly moves with water (Mugwe *et al*., 2009).

With regard to 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) observed a decrease in urease activity with increasing inputs of ammonia-based N fertilizer; nonetheless, we did not find decreases in this activity with addition of fertilizers. 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 favored the increase in enzyme activities, compared with CT. This positive effect could be explained by the addition of microbial cells or enzymes with the amendment (Tejada *et al*., 2006). However, it is more likely that organic residues provide labile compounds which may stimulate microbial activity, 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). 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 physicochemical conditions (Aon *et al*., 2001). There was 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 stimulated the release of enzymes to decompose them.

Although most nutrients were not affected by treatments, the highest application rates of CR maintained high levels of soil K, Mg, Zn and Mn. The high content of these nutrients in the pepper residue (Table 2) could explain these increments, which were released after microbial mineralization. The fact that pepper residues had high content of Fe, but no significant increase in soil 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).

The absence of differences in broccoli yield, quality and nutrients content with treatments in the first cycle was expected due to residual fertility, as consequence of an excessive previous fertilization. Nonetheless, with a second crop cycle, it was possible to distinguish treatments and rate effects, with CR3 and CF3 supporting the highest yield. Yield was enhanced in the second cycle with increasing levels of CF rates; similar results were previously reported (Zebarth *et al*., 1995; Babik and Elkner, 2002). However, other authors (Belec *et al*., 2001; Thompson *et al*., 2002) found that excessive N fertilization had a detrimental effect on marketable yield and could increase physiological disorders like hollow stem, as observed in broccoli crop during the first season. Recommendations for N fertilizer rates must be also based on current soil available N. In Figure 2 we have represented the relationship between seasons 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, no increments in crop yield were recorded. Thus, application rates which surpasses this threshold in season available N (all application rates in first season except for CT) would not pose an effective source of N. This is in agreement with Babik and Elkner (2002) who found that yield increased with N application rates up to 600 kg N ha^{-1}.

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 Nmin/Nt 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:steam diameter, ($r=0.48$; $P<0.05$), indicating that increased microbial activity which release available N favors yield and crop quality. N uptake by broccoli was lower in the second cycle, likely be due to lower Nt and available N contents during the second cycle; this is supported by the significant positive correlations of broccoli N with Nt ($r=0.50$; $P<0.01$) and available N ($r=0.52$; $P<0.01$). Furthermore, 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). In addition, the nutrient content trend observed in this study was also observed by Llona and Faz (2006) in broccoli cultivated in southern Spain fertilized with pig slurry.

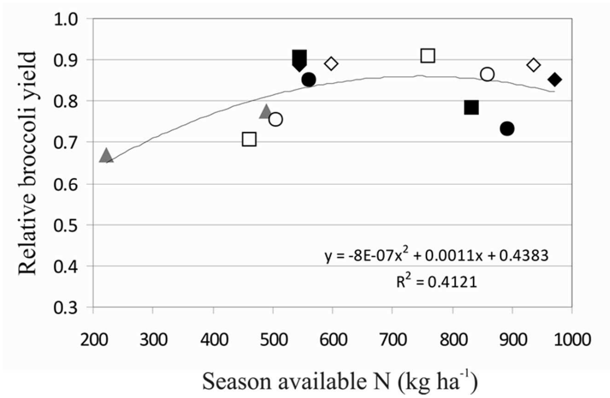

Figure 2. Relationship between relative broccoli yield (plot yield-to-maximum season yield ratio) and season available N (initial soil available N plus available N applied as CF or CR) for both crop cycles. Black symbols indicate CR treatment, white symbols indicate CF treatment and gray symbols indicate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

5. Conclusions

Highest 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 corresponded 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, pepper crop residues, mainly applied at a high rate (16.7 – 19.2 t ha^{-1}), with a minimum chemical fertilization, is an integrated nutrient supply which maintains crop yield and quality, with significant increments in soil quality and fertility compared to chemical fertilization. These management practices are therefore more suitable for a long-term sustainable agriculture.

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