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Effects of different green manures on soil biological properties and maize yield

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Abstract

The utilization of green manures as alternatives to reduce the use of mineral fertilizers is considered a good agricultural practice. However, the effect of each green manure on soil properties and crop yield depends upon its chemical composition. The main objective of this work was to study the effect of incorporating three green manures originating from residues of *Trifolium pratense*, L. (TP), *Brassica napus*, L. (BN), and the mixture of TP + BN at rates of 5384 and 8973 kg C ha^{-1}, on soil biological properties (soil microbial biomass-C, soil respiration and soil enzymatic activities), nutrition (leaf N, P and K concentration, pigments and soluble carbohydrate concentrations) and yield parameters of maize (*Zea mays* cv. Tundra) crop for four years on an Typic Xerofluvent located near Sevilla (Guadalquivir Valley, Andalusia, Spain). All green manures had a positive effect on the soil biological properties, plant nutrition an crop yield parameters, although at the end of the experimental period and at the high organic matter rate, the soil microbial biomass and dehydrogenase, urease, β-glucosidase, phosphatase and arylsulfatase activities increased more significantly in the TP amended soils (79.2%, 92.1%, 93.9%, 99.3%, 87.9% and 96%, respectively) respect to the control soil, followed by TP + BN amended soils (77.3%, 90.9%, 92.8%, 99.1%, 84.4% and 95.7%, respectively) and BN amended soils (76%, 90.1%, 91.7%, 99%, 83.2% and 95.2%, respectively). Since these soil enzymatic activities measured are responsible for important cycles such as C, N, P and S, an increase of leaf N, P an K contents and pigments and soluble carbohydrate contents were highest in TP amended soils, followed by TP + BN and BN treatments. The application of TP in soils at high doses increased the grain protein concentration, number of grains corncob^{-1} and crop yield 44.6%, 6.3% and 22.1%, respectively, compared with the control soil, followed by TP + BN treatment (41.7%, 5.7% and 20.8%, respectively) and BN treatment (39%, 5.3% and 20%, respectively). The explanation of these results can be a consequence to the different chemical composition of the green manures applied to the soils and its mineralization, aspect controlled by the soil C/N ratio.

Keywords: Green manure; Soil biological properties; Soil enzymatic activities; Zea mays

1. Introduction

Maintenance of soil organic matter is important for the long-term productivity of agroecosystems (Goyal et al., 1999). For this reason, the application of organic wastes rich in organic matter to soil, such as animal manure (Haynes and Naidu, 1998), sewage sludge (Fließbach et al., 1994; Albiach et al., 2001), compost (Sikora and Enkiri, 1999; Tejada and Gonzalez, 2003), crop residues (De Neve and Hofman, 2000; Trinsoutrot et al., 2000), by-products with high organic matter content (Madejon et al., 2001; Tejada and Gonzalez, 2004), etc. is a current environmental and agricultural practice for maintaining soil organic matter, reclaiming degraded soils and supplying plant nutrients.

The application of green manures to soil is considered a good management practice in any agricultural production

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system because can increase cropping system sustainability by reducing soil erosion and ameliorating soil physical properties (MacRae and Mehuys, 1985; Smith et al., 1987), by increasing soil organic matter and fertility levels (Doran and Smith, 1987; Power, 1990), by increasing nutrient retention (Drinkwater et al., 1998; Dinnes et al., 2002), and by reducing global warming potential (Robertson et al., 2000).

Leguminous and non-leguminous plants are used in the production of green manures. Leguminous plants form symbiotic associations with Rhizobium bacteria in order to fix N_2. This fact causes that the green manures, which their principal component are leguminous plant debris, supply to the soil important amounts of N in relation to the green manures obtained from non-leguminous plants. However, the influence of this organic matter on soil properties depends upon amount, type, size and dominant component of the added organic materials. In this respect, Becker et al. (1994) studying green manures and green manures–straw mixtures, identified the lignin to nitrogen (L/N) ratio as a significant factor controlling N release. Clément et al. (1995) studying different green manures identified the lignin + polyphenol to nitrogen [(L + PP)/N] and the tannin to nitrogen (T + N) ratios controlling N release. Also, green manure decomposition and subsequent nutrients release depend largely on soil physical (moisture, temperature, texture, mineralogy and acidity), chemical (C/N ratio, presence of nutrients) and biological (biological activity) (Myers et al., 1994). Of all these parameters, possibly the soil C/N ratio is the parameter that better controls the mineralization of the organic matter after its incorporation to the soil. In this respect, Tejada and Gonzalez (2006) studying the effect of a crushed cotton gin compost with and without N on a rice crop, found that the soil C/N ratio is the soil parameter most significant for controlling nutrient release. Also, decomposition rates of incorporated green manure differed less between seasons and locations than for mulched green manure. Incorporated residues are in a generally more favourable environment for microbial decomposition (e.g., close soil contact, adequate soil moisture, etc.) (Wilson and Hargrove, 1986).

Soil microbial biomass and soil enzyme activities respond much more quickly to the changes in soil management practices as compared to total soil organic matter (Goyal et al., 1999; Garcia et al., 2000). Enzymes may react to changes in soil management more quickly than other variables and therefore may be useful as early indicators of biological changes (Bandick and Dick, 1999; Masciandaro et al., 2004). In fact, they may also indicate the soil's potential to sustain microbiological activity (Paul and Clarck, 1989). Therefore, measurement of microbial biomass and enzymatic activities provides a sensitive indication of organic matter turnover.

In view of the above, the objective of this study was to evaluate the effects of different green manures amendment at different rates on some biological soil properties, such as soil microbial biomass, soil respiration and soil enzymatic activities (dehydrogenase, urease, β-glucosidase, phosphate and arylsulfatase) and their repercussion in the nutrition and yield of maize grown in a semiarid Mediterranean agro-ecosystem.

2. Methods

2.1. Site and properties of green manures

The study was conducted from March 2002 to September 2005 near Sevilla city (Andalusia, Spain). The soil of the field experiment was a Typic Xerofluvent and general properties (0–25 cm) are shown in Table 1. Soil pH was determined in distilled water with a glass electrode (soil:H_2O ratio 1:1). Soil texture was determined by the Robinson's pipette method (SSEW, 1982) and dominant clay types were determined by X-ray diffraction. Soil total N was determined by the Kjeldahl method (MAPA, 1986). Soil organic carbon was determined by oxidizing organic matter in soil samples with $K_2Cr_2O_7$ in sulphuric acid (96%) for 30 min, and measuring the concentration of Cr^{3+} formed (Yeomans and Bremner, 1988).

Green manure residues consisted on leguminous (*Trifolium pratense*, L.) and non-leguminous (*Brassica napus*, L.) plants and mixing of the both residues (*T. pratense*, L. + *B. napus*, L. at a ratio 1:1). Before soil application, the residues plants were composted. Prior to composting, the residues were crushed using a shredder (Bione sgon 2.0, Sandri Garden srl). Each residue (*T. pratense*, L., *B. napus*, L. and *T. pratense*, L. + *B. napus*, L.) was composted in trapezoidal piles (2 m high by 2 m width by 3 m bug) and under aerobic conditions. The piles were turned every 10 days during the maturation phase in order to improve the O_2 level inside the pile and increase the temperature which is necessary to eliminate pathogen and phytotoxic compounds. During the thermophilic phase, the piles were watered regularly to maintain moisture contents at around 55%. To assure an adequate composting process, 50–60% is the recommended range for moisture content (McKinley et al., 1985). The composting process was carried on 179 days for *T. pratense*, L. residues, 210 days for *B. napus*, L residues and 201 days for the mixing *T. pratense*, L. + *B. napus*, L. residues, when the C/N ratio and the temperature had become constant. The general properties of the green manures at the end of the composting process are shown in Table 2.

Table 1
Initial soil characteristics and standard error in parenthesis (data are the means of four samples)

pH (H_2O)	6.3 (0.2)
Clay (g kg^{-1})	125 (17)
Silt (g kg^{-1})	147 (16)
Sand (g kg^{-1})	728 (29)
Total N (g kg^{-1})	0.61 (0.09)
Total C (g kg^{-1})	3.3 (0.8)
C/N ratio	5.4 (0.3)

Table 2
Characteristics of green manures and standard error in parenthesis (data are the means of seven samples)

	TP	BN	TP + BN
Dry weight (%)	16.8 (0.6)	30.4 (1.4)	21.7 (1.0)
Total organic-C (g kg^{-1} DM)	358.9 (1.7)	405.8 (3.6)	680.4 (2.1)
Total N (g kg^{-1} DM)	40.8 (1.7)	8.5 (0.9)	37.4 (0.8)
C/N ratio	8.8 (1.1)	47.7 (4)	18.2 (2.6)
Humic acid-C (g kg^{-1}DM)	1.3 (0.1)	63.6 (1.4)	59.5 (2.8)
Fulvic acid-C (g kg^{-1}DM)	51.4 (1.2)	2.5 (0.2)	53.8 (2.1)
Lignin (g kg^{-1} DM)	5.3 (0.6)	27.8 (1.1)	29.8 (0.9)
Cellulose (g kg^{-1} DM)	4.7 (0.5)	20.4 (1.6)	18.3 (0.5)
Hemicellulose (g kg^{-1} DM)	9.8 (0.6)	34.6 (1.4)	30.5 (1.0)

DM: dry matter.

Organic matter was determined by dry combustion (MAPA, 1986) and organic C was calculated dividing organic matter value by 2. To determine humic and fulvic acids-C, green manures were extracted with a mixture of 0.1 M sodium pyrophosphate and 0.1 M sodium hydroxide at pH 13 (Kononova, 1966). The supernatant was acidified to pH 2 with HCl and allowed to stand for 24 h at room temperature. To separate humic acids from fulvic acids, the solution was centrifuged and the precipitate containing humic acids was dissolved with sodium hydroxide. The carbon content of humic acid and fulvic acids was determined by the method of Yeomans and Bremner (1988). Structural carbohydrates were determined sequentially as neutral detergent and acid detergent fibres and lignin was determined through permanganate oxidation (Goering and van Soest, 1970). The neutral detergent procedure gives the total fibre content of cell walls. The acid detergent fibre is mainly composed of lignin, cellulose and insoluble minerals (Goering and van Soest, 1970). The hemicellulose fraction was calculated by subtracting the acid detergent fibre from the neutral detergent values. The acid detergent fibre and neutral detergent values were corrected for residual ash.

2.2. Experimental layout and treatments

The experimental layout was in a randomized complete block with a total amount of 28 plots (10 × 7 m). Seven treatments were used (four replicates per treatment): (1) C, control soil (no organic amendment); (2) TP1, soil fertilized with 15 t ha^{-1} of *T. pratense*, L. green manure (TP) (dry weight) (5384 kg C ha^{-1}); (3) BN1, soil fertilized with 13.267 t ha^{-1} of *B. napus*, L. green manure (BN) (dry weight) (5384 kg C ha^{-1}); (4) (TP + BN)1, soil fertilized with 14.154 t ha^{-1} of the mixture of *T. pratense*, L. + *B. napus*, L. green manure (TP + BN) (dry weight) (5384 kg C ha^{-1}); (5) TP2, soil fertilized with 25 t ha^{-1} of TP (dry weight) (8973 kg C ha^{-1}); (6) BN2, soil fertilized with 22.11 t ha^{-1} of BN (dry weight) (8973 kg C ha^{-1}) and (7) (TP + BN)2, soil fertilized with 23.59 t ha^{-1} of the mixture of TP + BN (dry weight) (8973 kg C ha^{-1}).

The green manures was surface broadcast on 17 March 2002, 16 March 2003, 15 March 2004, and 11 March 2005, respectively and incorporated to a 25-cm depth by chisel plowing and disking the day after application. The same green manures were used for in all experimental seasons. The compost was stored at 0 °C after application in the first experimental season to avoid mineralization.

Table 3
Irrigation plan carried out for each experimental season

Week	No. irrigation	m^3
1 (sowing)	3	42
2	3	42
3	3	52
4	3	88
5	3	120
6	3	150
7	3	165
8 (maize was 40 cm high)	3	185
9	3	190
10	3	230
11	3	250
12	3	230
13 (maize was 70 cm high)	3	220
14	3	200
15	3	192
16	3	192
17	3	192
18	3	192
19	3	190
20 (tasseling)	3	190
21	3	160
22	3	140
23	3	120
24	3	100
25	2	95
26	2	90
27	2	80
28	2	70
29 (harvest)	1	65

Maize (*Zea mays* cv. Tundra) was chosen as the test crop, and seeded at a rate of 100,000 plants ha^{-1} in 75-cm rows, which is a common practice in the area. Sowing dates were 21 March 2002, 20 March 2003, 18 March 2004, and 15 March 2005, respectively. Crop yield (kg ha^{-1}) and number of grains per corncob were determined for samples collected from each plot on 29 September 2002, 30 September 2003, 28 September 2004, and 30 September 2005, respectively.

Table 3 shows the irrigation plan carried out during each experimental season and for all treatments (common practice in the area).

2.3. Soil and plant sampling and analytical determinations

Soil samples (0–25 cm) were collected 29 September 2002, 30 September 2003, 28 September 2004, and 30 September 2005, respectively from each plot (4 replicates per plot) with a gauge auger (30-mm diameter) at harvest.

After drying soil samples at room temperature, they were sieved (2 mm) and stored at 4 °C.

Soil microbial biomass was determined using the $CHCl_3$ fumigation-extraction method (Vance et al., 1987). The activity levels of six soil enzymes were measured. Dehydrogenase activity was measured by reduction of 2-*p*-iodo-3-nitrophenyl 5-phenyl tetrazolium chloride to iodonitrophenyl formazan (Garcia et al., 1993). Urease activity was determined by the buffered method of Kandeler and Gerber (1988) using urea as substrate. β-glucosidase activity was determined using *p*-nitrophenyl-β-D-glucopyranoside as substrate (Masciandaro et al., 1994). Alkaline phosphatase activity was measured using *p*-nitrophenyl phosphate as substrate (Tabatabai and Bremner, 1969). Arylsulfatase activity was determined using *p*-nitrophenylsulphate as substrate (Tabatabai and Bremner, 1970).

At the end of the each experimental season, soil respiration for all soil samples was measured by incubation for 3, 7, 15, 30, 60 and 90 days. Total C–CO_2 collected in the NaOH flasks was determined by the addition of an excess of 1.5 M $BaCl_2$ followed by titration with standardized HCl using a phenolphthalein indicator (Zibilske, 1994).

Plant samples were taken from each plot at harvest (29 September 2002, 30 September 2003, 28 September 2004, and 30 September 2005, respectively) by selecting the ear leaf (Tejada et al., 1992). The lyophilized samples were assayed for N (Kjeldahl method), and after mineralization (CIITDF, 1969), for P (by the method of Williams and Stewart as described by Guitian and Carballas (1976)), and for K (by atomic emission spectrophotometer).

Chlorophylls and total carotenoids in the lyophilized leaf samples were measured by extraction with methanol and quantified by the Lichtenthaler method (1987). Leaf soluble carbohydrate contents were measured using the anthrone method (Yemm and Willis, 1954). Fifty grams of samples were collected from each plot. Dried leaf samples were extracted in 5 ml 80% (v/v) ethanol (30 min, 30 °C). The extract was centrifuged (10 min, 2650*g*) and the pellet was extracted again with ethanol. After centrifugation, chlorophyll was removed from the combined supernatants by chloroform extraction. The samples were analyzed colorimetrically for soluble carbohydrates using the anthrone method.

Grain protein was determined as the product of grain N content, determined by the Kjeldahl method, and 6.25, a constant factor for the N in protein (MAPA, 1986).

2.4. Statistical analysis

Analysis of variance (ANOVA) was performed using the Statgraphics v. 5.0 software package (Statiscal Graphics Corporation, 1991). ANOVA was based on the HSD criterion (honestly significant difference), considering a significance level of $P < 0.05$ throughout the study.

3. Results

3.1. Soil analysis

The soils respiration values (CO_2 evolved during 90 days incubation experiment) in soil samples at the end of each experimental year are shown in Fig. 1. CO_2 emissions were highest in the organically-amended soils than for control soil. For control soil, soil respiration decreased for each experimental year probably due to a decrease of soil organic matter due to its mineralization. However, this CO_2 increase depends on the chemical composition of the organic matter applied to the soil. In this respect and for both rates studied, soil respiration was higher in TP amended soils, followed by TP + BN and BN amended soils. The statistical analysis showed important significant differences for all treatments during incubation period.

Soil microbial biomass-C increased in the plots amended with green manures (Table 4). When highest doses of organic matter were applied to the soil, soil microbial biomass carbon increased progressively during the experimental period. Also, control soil had the lowest values of this parameter and like for soil respiration, soil microbial biomass-C decreased for each experimental year. This increase was different depending of the green manure type applied to soil. In this respect and at the end of the experimental period, soil microbial biomass carbon increased 79.2%, 77.3%, 76%, 73.4%, 72.6% and 68.9%, respectively, in soils amended with TP2, (TP + BN)2, BN2, TP1, (TP + BN)1, and BN1, respectively, compared to the control soil. Again, the statistical analyses showed important significant differences for all treatments during the experimental period.

The activity of the dehydrogenase enzyme was significantly stimulated during the experimental period in the green manures amended soils (Table 4). This stimulation was higher when highest doses of organic matter were applied to the soil. However, this stimulation depends of the green manure type applied to the soil. In this respect and at the end of the experimental period, soil dehydrogenase activity increased 92.1%, 90.9%, 90.1%, 88.9%, 87.6% and 84.8%, respectively, in soils amended with TP2, (TP + BN)2, BN2, TP1, (TP + BN)1, and BN1, respectively, compared to the control soil.

Also, urease activity was strongly stimulated by green manures addition to the soil, principally at a higher rate of organic matter (Table 4). Also and at the end of the experimental period, the stimulation of this enzyme was higher in TP2 amended soils (93.9%), followed by (TP + BN)2 (92.8%), BN2 (95.2%), TP1 (94.4%), (TP + BN)1 (93.4%), and BN1 (92%) treatments respect to the control soil. Again, the statistical analyses showed important significant differences for all treatments during the experimental period.

At the end of the experimental period, the evolution of β-glucosidase activity was very similar than the others enzyme studied (Table 5). In this respect, the activity of this enzyme increased 99.3%, 99.1%, 99%, 99%, 98.9% and 98.6%, respectively, in TP2, (TP + BN)2, BN2, TP1, (TP + BN)1, and BN1 amended soils respect to the control soil.

The evolution of phosphatase and arylsulfatase activities during the experimental period were similar to the other enzyme activities studied, emphasizing the highest values in the plots amended with highest doses of organic

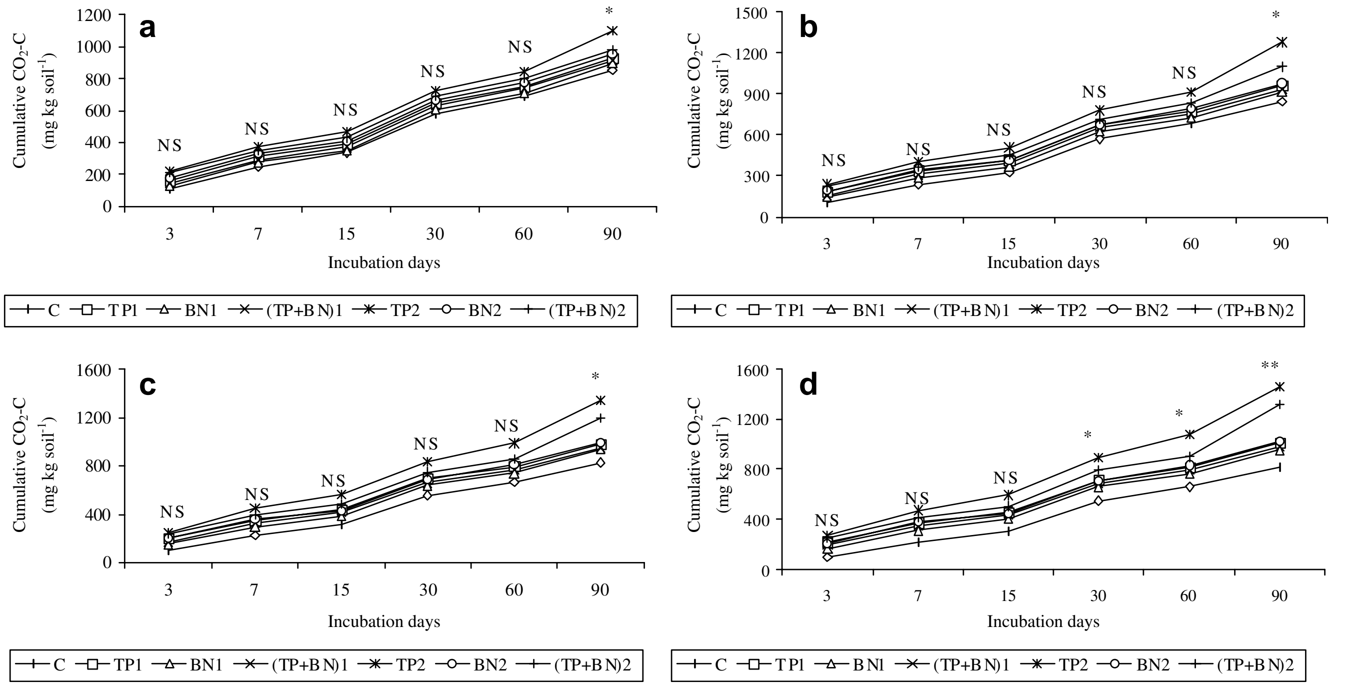

Fig. 1. Cumulative CO_2–C (mg kg soil^{-1}) during incubation in soils affected by application of green manures and for 2002 (a), 2003 (b), 2004 (c) and 2005 (d). NS, *, **, ***, non-significant or significant at $p < 0.05$, 0.01 or 0.001, respectively.

Table 4
Microbial biomass-C and dehydrogenase and urease activities in soils during the experimental period

	2002	2003	2004	2005
Soil microbial biomass (μg C g^{-1} dry soil)				
C	118a[A] (12)	109a (10)	114a (11)	104a (12)
TP1	140a (15)	222ab (12)	305b (13)	391b (25)
BN1	129a (12)	201ab (18)	282ab (17)	335b (24)
(TP + BN)1	132a (12)	210ab (18)	295ab (17)	379b (24)
TP2	215ab (20)	301b (24)	406bc (21)	499c (23)
BN2	179ab (16)	267ab (15)	368b (15)	433bc (26)
(TP + BN)2	199ab (17)	280ab (16)	392b (10)	458c (29)
Dehydrogenase activity (μg INTF g^{-1} h^{-1})				
C	15.2a[A] (0.4)	14.7a (0.3)	14.1a (0.3)	13.6a (0.5)
TP1	47.6b (1.2)	74.6bc (0.9)	100.1b (1.8)	122.3bc (1.7)
BN1	31.4b (1.8)	50.6b (1.6)	70.1b (1.8)	89.6b (1.3)
(TP + BN)1	40.5b (1.6)	65.3b (1.1)	86.4b (1.6)	109.7b (1.9)
TP2	70.2b (2.0)	111.8bc (2.2)	145.7bc (2.3)	173.2c (1.7)
BN2	59.8b (1.9)	83.6b (1.6)	115.1bc (2.0)	137.8bc (2.2)
(TP + BN)2	64.3b (1.2)	99.8b (1.4)	130.1bc (1.8)	149.8bc (1.9)
Urease activity (μmol NH_4 g^{-1} h^{-1})				
C	1.2a[A] (0.1)	1.0a (0.2)	0.95a (0.24)	0.91a (0.17)
TP1	4.2b (0.5)	5.5b (0.4)	6.9b (0.4)	8.8b (0.3)
BN1	2.7b (0.2)	3.5b (0.3)	4.6b (0.3)	6.1b (0.4)
(TP + BN)1	3.6b (0.3)	4.7b (0.4)	5.8b (0.5)	7.7b (0.5)
TP2	6.9b (1.4)	9.5bc (1.6)	11.6bc (1.9)	14.8c (1.6)
BN2	5.5b (0.7)	6.7b (0.5)	8.1b (0.8)	10.9bc (1.0)
(TP + BN)2	6.1b (1.1)	8.3b (1.4)	10.0bc (1.5)	12.7c (1.6)

Standard error in parenthesis. INTF: 2-p-iodo-3-nitrophenyl formazan.
[A] Different letters following the figures indicate a significant difference at $P < 0.05$.

matter applied to the soil (Table 5). In this respect and at the end of the experimental period, the stimulation of this phosphatase was higher in TP2 amended soils (87.9%), followed by (TP + BN)2 (84.4%), BN2 (83.2%), TP1 (81.4%), (TP + BN)1 (79.4%), and BN1 (73.1%) treatments respect to the control soil, whereas for arylsulfatase activity its stimulation was higher in TP2 amended soils (96%), followed by (TP + BN)2 (95.7%), BN2 (95.2%), TP1

Table 5
β-Glucosidase, phosphatase and arylsulfatase activities in soils during the experimental period

	2002	2003	2004	2005
β-Glucosidase activity (μmol PNP g^{-1} h^{-1})				
C	2.2a[A] (0.4)	1.5a (0.2)	0.97a (0.14)	0.68a (0.12)
TP1	15.9b (1.3)	26.2b (1.8)	52.8b (2.0)	66.6bc (2.7)
BN1	10.9b (1.2)	21.6b (1.5)	34.9b (2.0)	49.7b (2.6)
(TP + BN)1	14.6b (1.1)	24.0b (1.7)	49.6b (2.1)	61.4bc (2.7)
TP2	23.7b (1.5)	38.9b (1.8)	70.8bc (2.1)	100.3c (2.8)
BN2	17.3b (1.2)	28.7b (1.4)	55.9b (1.9)	69.8bc (2.5)
(TP + BN)2	19.2b (1.1)	30.9b (1.6)	59.8bc (2.0)	72.6bc (2.8)
Phosphatase activity (μmol PNP g^{-1} h^{-1})				
C	15.4a (1.1)	14.1a (1.1)	12.8a (1.0)	11.2a (0.8)
TP1	30.2b (2.1)	36.9b (2.5)	47.8b (2.1)	60.3b (2.6)
BN1	20.5a (1.7)	26.4ab (1.9)	34.7ab (2.0)	41.6ab (2.1)
(TP + BN)1	27.1ab (2.5)	32.8b (2.1)	42.0b (2.4)	54.3b (2.6)
TP2	51.4b (2.3)	63.2b (2.5)	76.1bc (2.2)	92.7c (2.8)
BN2	35.6b (2.4)	43.8b (2.2)	51.9b (2.5)	66.6b (2.5)
(TP + BN)2	40.1b (2.1)	46.7b (2.4)	55.8b (2.2)	71.9bc (2.6)
Arylsulfatase activity (μmol PNF g^{-1} h^{-1})				
C	4.3a[A] (0.9)	3.7a (0.7)	3.2a (0.7)	2.8a (0.6)
TP1	20.6b (1.8)	32.9b (1.9)	40.8b (2.1)	49.7b (2.4)
BN1	13.8b (1.2)	21.1b (1.5)	28.4b (1.9)	35.0b (2.1)
(TP + BN)1	17.4b (1.6)	26.9b (1.8)	34.4b (2.2)	42.3b (2.5)
TP2	33.8b (2.0)	51.2b (2.1)	62.5bc (2.6)	70.8c (2.8)
BN2	25.8ab (1.8)	39.9b (1.9)	49.2b (2.0)	58.6bc (2.4)
(TP + BN)2	29.4b (1.9)	45.6b (2.0)	56.8bc (2.4)	64.7bc (2.6)

Standard error in parenthesis. PNP: *p*-nitrophenol; PNF: *p*-nitrophenyl.
[A] Different letters following the figures indicate a significant difference at $P < 0.05$.

Table 6
Soil C/N ratio evolution during the experimental period

	2002	2003	2004	2005
C	5.3a[A] (0.2)	5.2a (0.1)	5.1a (0.2)	5.0a (0.2)
TP1	7.4a (0.2)	8.6a (0.3)	9.6a (0.2)	10.7a (0.3)
BN1	17.9b (0.3)	19.0b (0.3)	20.6b (0.4)	22.0b (0.4)
(TP + BN)1	11.9b (0.3)	13.1b (0.2)	14.5b (0.3)	16.3b (0.3)
TP2	8.3a (0.2)	9.7a (0.2)	10.9b (0.2)	12.0b (0.2)
BN2	19.1b (0.4)	20.9b (0.4)	22.1b (0.5)	24.2b (0.3)
(TP + BN)2	13.6b (0.3)	15.8b (0.4)	17.9b (0.3)	20.4b (0.2)

Standard error in parenthesis.
[A] Different letters following the figures indicate a significant difference at $P < 0.05$.

(94.4%), (TP + BN)1 (93.4%), and BN1 (92%) treatments respect to the control soil.

Table 6 shows soil C/N ratio evolution during the four experimental seasons and for all treatments. At the end of the experimental period, the lowest values were observed for control soil. TP2 and TP1 have optimum values (10–12), however for (TP + BN)1, (TP + BN)2, BN1 and BN2 amended soils C/N ratio have highest values.

3.2. Plant analysis

When highest doses of organic matter were applied to the soil, leaf N, P and K contents and pigments and soluble carbohydrate contents increased progressively during the experimental period (Tables 7 and 8). The statistical analysis indicated significant differences respect to fertilizer treatments. The lowest values are presented for control soil. The highest values were obtained for TP2 amended soils, followed by (TP + BN)1, BN2, TP1, (TP + BN)1 and BN1 treatments. At the end of the experimental period, chlorophyll A increased 47.5%, 44.5%, 42.2%, 40%, 39% and 37.4% in TP2, (TP + BN)1, BN2, TP1, (TP + BN)1 and BN1 treatments respect to the control soil. Also, chlorophyll B increased 56.1%, 27.2%, 22.2%, 17.7% and 2.9% in TP2 amended soils respect to the control soil, BN1, (TP + BN)1, TP1, BN2 and (TP + BN)2 treatments. Respect to the carotenoids, these pigments increased 29.7%, 28.2%, 26.8%, 26%, 24.4% and 22.9% in TP2, (TP + BN)1, BN2, TP1, (TP + BN)1 and BN1 treatments respect to the control soil. These results are in line with the data obtained for soil microbial biomass, soil respiration and soil enzymatic activities. Also, the highest values were observed for the fourth experimental season than that the third, second and the first experimental season, respectively.

Table 7
N, P and K contents in maize at harvest (on a dry matter basis)

	N (g kg^{-1})	P (g kg^{-1})	K (g kg^{-1})
2002			
C	15.3a[A] (0.4)	1.4a[A] (0.1)	14.1a[A] (0.5)
TP1	18.0a (0.5)	1.7a (0.2)	15.9a (0.9)
BN1	16.8a (0.3)	1.6a (0.2)	14.8a (0.7)
(TP + BN)1	17.3a (0.3)	1.7a (0.1)	15.4a (0.8)
TP2	20.3a (0.4)	1.9a (0.3)	17.8a (0.7)
BN2	18.5a (0.5)	1.8a (0.2)	16.3a (0.6)
(TP + BN)2	19.6a (0.3)	1.8a (0.2)	16.9a (0.5)
2003			
C	14.9a (0.3)	1.3a (0.1)	13.8a (0.3)
TP1	19.7a (0.2)	2.0a (0.3)	17.2a (0.4)
BN1	17.7a (0.3)	1.7a (0.2)	15.3a (0.4)
(TP + BN)1	18.5a (0.4)	1.9a (0.2)	16.1a (0.5)
TP2	22.5ab (0.5)	2.4ab (0.4)	20.2ab (0.5)
BN2	20.4ab (0.3)	2.1ab (0.2)	18.0a (0.3)
(TP + BN)2	21.3ab (0.5)	2.3ab (0.3)	19.0a (0.4)
2004			
C	14.7a (0.3)	1.2a (0.1)	13.2a (0.3)
TP1	20.8ab (0.4)	2.3ab (0.2)	18.1a (0.4)
BN1	18.8a (0.2)	1.9a (0.2)	15.9a (0.4)
(TP + BN)1	19.6a (0.3)	2.1ab (0.1)	17.2a (0.6)
TP2	23.7ab (0.5)	2.7ab (0.3)	21.5ab (0.5)
BN2	21.5ab (0.4)	2.4ab (0.2)	19.2a (0.3)
(TP + BN)2	22.4ab (0.5)	2.5ab (0.2)	20.4ab (0.4)
2005			
C	14.4a (0.2)	1.1a (0.1)	12.7a (0.2)
TP1	22.7ab (0.4)	2.4ab (0.3)	19.1a (0.3)
BN1	20.2a (0.3)	2.0a (0.2)	16.5a (0.3)
(TP + BN)1	21.8ab (0.3)	2.2ab (0.1)	17.9a (0.4)
TP2	26.0b (0.5)	2.9b (0.3)	23.0ab (0.3)
BN2	23.6ab (0.4)	2.6ab (0.2)	20.3b (0.4)
(TP + BN)2	24.7ab (0.4)	2.8ab (0.3)	21.6b (0.5)

Standard error in parenthesis.
[A] Different letters following the figures indicate a significant difference at $P < 0.05$.

Table 9 shows the grain protein content and crop yield parameters for the different treatments. Control soil had

Table 8
Leaf pigments and soluble carbohydrate contents in maize at harvest

	Chlorophyll A (mg kg^{-1})	Chlorophyll B (mg kg^{-1})	Carotenoids (mg kg^{-1})	Soluble carbohydrates (mg kg^{-1})
2002				
C	2367a[A] (36)	1143a[A] (17)	820a[A] (10)	90.3a[A] (0.9)
TP1	2778a (45)	1338a (25)	1011ab (15)	109.6a (1.2)
BN1	2583a (51)	1207a (18)	967a (14)	105.8a (1.1)
(TP + BN)1	2655a (28)	1292a (15)	992a (12)	107.9a (0.8)
TP2	3132b (34)	1457a (21)	1059b (13)	114.2ab (1.1)
BN2	2849a (21)	1602ab (24)	1026 (15)	111.1a (0.9)
(TP + BN)2	3013b (29)	1549ab (26)	1042ab (13)	112.7a (0.8)
2003				
C	2285a (32)	1098a (15)	809a (11)	88.3a (0.8)
TP1	3037b (41)	1575ab (19)	1030a (14)	111.4a (1.1)
BN1	2719a (21)	1295a (14)	980a (13)	107.5a (0.7)
(TP + BN)1	2843a (28)	1449a (13)	1012a (15)	109.9a (0.8)
TP2	3462b (27)	1861b (20)	1071b (16)	117.1ab (0.9)
BN2	3145b (30)	1763ab (17)	1040ab (12)	112.4a (0.7)
(TP + BN)2	3269b (26)	1830ab (15)	1060b (13)	114.1a (1.1)
2004				
C	2194a (23)	991a (10)	790a (12)	85.1a (0.5)
TP1	3119b (25)	1604ab (16)	1041ab (17)	112.9ab (0.9)
BN1	2888a (29)	1401a (14)	998a (10)	109.8a (1.1)
(TP + BN)1	3011ab (27)	1502a (15)	1023a (14)	110.7a (1.2)
TP2	3653b (32)	1956b (20)	1092b (16)	119.0ab (1.3)
BN2	3312ab (41)	1802ab (21)	1052ab (13)	113.4a (0.7)
(TP + BN)2	3430ab (38)	1911b (18)	1073b (15)	115.0ab (0.9)
2005				
C	2099a (27)	908a (11)	779a (11)	83.2a (0.4)
TP1	3501b (33)	1703ab (15)	1053ab (14)	114.0ab (0.8)
BN1	3351b (29)	1505a (13)	1010a (16)	111.4a (0.7)
(TP + BN)1	3442b (37)	1610ab (19)	1031a (12)	112.1a (1.1)
TP2	4001b (45)	2069b (21)	1109b (10)	120.6b (0.9)
BN2	3631b (34)	1906ab (23)	1064b (15)	115.1ab (0.6)
(TP + BN)2	3785b (28)	2008b (16)	1085b (19)	116.2ab (1.1)

Standard error in parenthesis.
[A] Different letters following the figures indicate a significant difference at $P < 0.05$.

Table 9
Protein content and maize yield parameters

	Protein content (g kg^{-1})	No. grains corncob^{-1} (No)	Yield (kg ha^{-1})
2002			
C	95.6a[A] (2.5)	452a[A] (4)	11,249a[A] (42)
TP1	113a (3.1)	463a (5)	12,481ab (53)
BN1	105a (1.9)	460a (5)	11,892a (59)
(TP + BN)1	108a (1.9)	462a (3)	12,108a (68)
TP2	127a (2.5)	469ab (4)	12,512ab (70)
BN2	116a (3.1)	466b (6)	12,314a (64)
(TP + BN)2	123a (1.9)	468b (5)	12,403a (51)
2003			
C	93.1a (1.9)	450a (4)	11,188a (39)
TP1	123a (1.3)	465a (4)	12,952ab (59)
BN1	111a (1.9)	462a (3)	12,093a (44)
(TP + BN)1	116a (2.5)	463a (5)	12,576ab (74)
TP2	141b (3.1)	472ab (6)	13,325ab (68)
BN2	128ab (1.9)	468b (5)	12,724ab (55)
(TP + BN)2	133ab (3.1)	470ab (4)	13,101ab (43)
2004			
C	91.9a (1.9)	448a (5)	11,135a (38)
TP1	130ab (2.5)	468ab (4)	13,472ab (49)
BN1	118a (1.3)	464a (4)	12,336a (61)
(TP + BN)1	123a (1.9)	466b (6)	13,012ab (55)
TP2	148b (3.1)	473ab (5)	13,824b (49)
BN2	134ab (2.5)	470ab (4)	13,343ab (72)
(TP + BN)2	140ab (3.1)	472ab (5)	13,622ab (68)
2005			
C	90a (1.2)	447a (4)	11,076a (46)
TP1	142ab (2.5)	470ab (5)	13,671ab(71)
BN1	126a (1.9)	465a (5)	12,710ab (79)
(TP + BN)1	136ab (1.9)	468b (6)	13,497ab (82)
TP2	163b (3.1)	477b (5)	14,224b (55)
BN2	148b (2.5)	472ab (5)	13,851b(62)
(TP + BN)2	154b (2.5)	474ab (4)	13,986b (76)

Standard error in parenthesis.
[A] Different letters following the figures indicate a significant difference at $P < 0.05$.

the lowest grain protein content and crop yield parameters. Grain protein concentration increased 44.6%, 41.7%, 39%, 36.6%, 33.9% and 28.7%, respectively, in TP2, (TP + BN)2, BN2, TP1, (TP + BN)1 and BN1 amended soils respect to the control soil. Number of grains cornob^{-1} increased 6.3%, 5.7%, 5.3%, 4.9%, 4.5% and 3.9%, respectively, in TP2, (TP + BN)2, BN2, TP1, (TP + BN)1 and BN1 amended soils respect to the control soil. Finally, crop yield increased 22.1%, 20.8%, 20%, 19%, 17.9% and 12.8%, respectively, in TP2, (TP + BN)2, BN2, TP1, (TP + BN)1 and BN1 amended soils respect to the control soil. Again, the statistical analysis indicated significant differences within fertilizer treatments, highlighting the highest values for the fourth experimental season than that the third, second and the first experimental season.

4. Discussion

Our data indicated that during the experimental period, the application of different green manures to soil increased microbial biomass-C and soil respiration. These results are in agreement with those of Goyal et al. (1999), Fontaine et al. (2003) and Stark et al. (2007) who found that soil microbial biomass and soil respiration responds rapidly, in terms of activity, after the addition of different green manures to soil. This increase in soil microbial biomass carbon and soil respiration can be attributed to the incorporation of easily degradable materials, which stimulate the authoctonous microbial activity and to the incorporation of exogenous microorganisms (Blagodatsky et al., 2000; Tejada et al., 2006). Soil microbial respiration, measured through CO_2 production is a direct indicator of microbial activity, and indirectly reflects the availability of organic material (Gomez et al., 2001; Tejada and Gonzalez, 2003; Tejada et al., 2006).

Soil microorganisms degrade organic matter through the production of diverse extracellular enzymes and for this reason after the application of green manures to soil, soil enzymatic activities increased. These results are in agreement with those of Goyal et al. (1999) and Kautz et al. (2004) who found an increase in enzymatic activities after the addition of different green manures to soil. Soil enzymes act as biological catalysts of specific reactions that depend on a variety of factors (Burns, 1978), such as the presence or absence of inhibitors, amendment type, crop type, etc. and can be considered as early indicators of biological changes (Bandick and Dick, 1999). The incorporation of organic amendments to soil influences soil enzymatic activities because the added material may contain intra- and extracellular enzymes and may also stimulate microbial activity in the soil (Goyal et al., 1993; Pascual et al., 1998).

Dehydrogenase activity has been proposed as a measure of overall microbial activity (Masciandaro et al., 2001), since it is an intracellular enzyme related to oxidative phosphorylation processes (Trevors, 1984). Garcia et al. (1997) found that dehydrogenase activity is a good index of the soil microbial biomass in semiarid Mediterranean areas. The greater dehydrogenase activity observed at the high dose of green manures suggests that these did not include toxic compounds to microorganisms (Tejada and Gonzalez, 2003, 2006). The high level of dehydrogenase activity in the soil treated with TP respect to the TP + BN and BN suggests the more easily decomposable components of TP and hence an improvement in their microbial activity.

Respect to the hydrolases enzymes, the highest values of soil urease, β-glucosidase, alkaline phosphatase and arylsulfatase activities for green manures amended soils, are in agree with the results obtained from other experiments carried out by us (Tejada and Gonzalez, 2003, 2006; Tejada et al., 2006) when incorporating diverse sources of organic matter to soil, indicating that the organic amendment had a positive effect on the activity of these enzymes, particularly when the amendment was at the high dose, probably due to the higher microbial biomass produced in response.

However, under field conditions, the decomposition of green manures is complex, and is controlled by numerous factors such as availability of carbon and nitrogen, the biochemical nature of the plant residue, contact between soil and compost and soil and climatic factors, etc. According to Hadas and Portnoy (1994) and Tejada and Gonzalez (2006), the C/N ratio of the organic wastes will largely determine the balance between mineralization and immobilization. The C/N ratio was the best predicting parameter for the potential amount of N that can mineralize from a crop residue (Chaves et al., 2004). In this respect, Maikstenienе and Arlauskiene (2004) found a higher mineralization of clover and lucerne green manures after the application of soil (C/N ratio 12 and 10, respectively) that vetch and oat mixture (C/N ratio 31) and straw wheat (C/N ratio 55).

For this reason, the increase in the soil microbial biomass-C as well as in the soil enzymatic activities it is very different depending from the type of green manure applied to the soil.

This difference in the C/N ratio of the different green manures is manifested in the different evolution of the soil C/N ratio. In this respect and at the end of the experiment, the results reveal that the soils amended with TP had optimum values in this soil C/N ratio. As consequence of it, the mineralization of the organic matter applied to the soil will be carried out under good conditions, prevailing the mineralization versus the immobilization processes, aspect that is manifested in the highest values in soil microbial biomass and the soil enzymatic activities. The obtained values of the soil C/N ratio for the soils amended with BN is the highest, being very superior to the good values (10–12) indicated for Tejada and Gonzalez (2006). As consequence of it, the securities soil microbial biomass and soil enzymatic activities values they are the lowest respect to the TP amended soils. According to Tejada and Gonzalez (2006), the immobilization processes it prevails versus the mineralization. Nevertheless, the results indicate that for TP and BN mixture the results they reveal soil C/N ratio values very near to optimum values (10–12), aspect that impacts in an increase of the soil microbial biomass and soil enzymatic activities with respect to the BN amended soils (Tejada and Gonzalez, 2006).

Since soil enzymatic activities measured are responsibles for important cycles such as C, N, P and S, leaf pigments and soluble carbohydrate contents during the maize growth cycle increased significantly during each experimental year for TP amended soils than for TP + BN and BN amended soils. This may be due to better mineralization of organic matter for TP amended soils than for TP + BN and BN amended soils. These results are of great importance, because the photosynthesis could be increased over a longer period of time as the levels of pigments in the leaf increase, resulting in a higher production of soluble carbohydrates and thereby increased grain quality and crop yield. These parameters of the fourth experimental season were better than those of the third, second and first experimental season, due to the residual effect of the organic matter after their application in the first season.

Since soil microbial biomass-C and soil enzymatic activities increased, the bioavailability of N, P and S by plants increased, and therefore maize yield parameters increased significantly when a higher dose of TP were applied to the soil with respect to the same dose of TP + BN and BN treatments.

5. Conclusions

The application of green manures to the soil produced an improvement in the soil biological properties as well as in the nutrition, production and quality of the obtained maize. Nevertheless, these improvements depend on the chemical composition of the green manures applied to the soil. The difference in the C/N ratio of the different green manures is manifested in the different evolution of the soil biological properties and maize yield. In this respect, the application to the soil of green manures with high or low contents in their C/N ratio imply high or low contents in the soil C/N ratio and therefore an optimal mineralization of the organic matter do not occur. Nevertheless, when green manures with a relation C/N next to 20 are applied to the soil, which facilitate an optimal degradation of the organic matter, an increase of soil microbial activity and in the quality and productivity of maize was observed.

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