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Agricultural use of digestate for horticultural crop production and improvement of soil properties

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ABSTRACT

The usefulness of a digestate from an anaerobic codigestion process as a fertiliser product was evaluated in a field experiment using two horticultural crops (watermelon and cauliflower), during two successive growing seasons. The effects of the digestate were compared with those of a traditional organic amendment (cattle manure) and a conventional mineral fertiliser. Digestate addition to soil provided a source of available nutrients (nitrogen and phosphorus) in the short-term and had positive effects on soil biological properties such as microbial biomass and enzyme activities, compared to the non-amended soil. The digestate application to soil led to yields comparable to the mineral fertilisation for the summer watermelon crop. However, for the winter cauliflower crop, only plots treated with the mineral fertiliser had good production. Nitrogen from the digestate is rapidly and highly available for plant growth in the short-term but also can be easily lost, together with a slow rate of microbial processes due to low temperatures, could reduce the fertilising capacity of the digestate. This seemed to be the main limiting factor for the winter cauliflower crop, where digestate or cattle manure, used as basal dressing, were not enough to satisfy the crop demand for nitrogen during its whole growth cycle.

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1. Introduction

The large quantities of biodegradable wastes produced by the intensive livestock production systems can have a negative impact on the environment, if they are not managed adequately. The anaerobic digestion of wastes for biogas production is of great interest for livestock waste management and energy recovery, according to the European policies concerning renewable energy production (Holm-Nielsen et al., 2009). This is clearly evidenced in Spain by the Slurry Biodigestion Plan (BOE, 2009), which promotes the treatment of animal manures and slurries through anaerobic digestion. The main benefits of anaerobic digestion are: energy savings through production of a renewable energy source (biogas); reduction in greenhouse gas emissions and air and water pollution; sanitisation of wastes and preservation of natural resources by using the end-products as soil amendments and fertilisers (Möller and Stinner, 2009; Stinner et al., 2008).

Together with biogas, anaerobic digestion produces a residual material (digestate), whose adequate management or disposal must be addressed in order to avoid any constraint to the development of anaerobic digestion systems. The legislative trends in the field of wastes management are based on integrated management, adding value to these by-products; thus, digestate addition to soil – resulting in benefits for agriculture and/or ecological improvement – is considered an appropriate option (Directive, 2008/98/EC). For the sustainable recycling of digestates in agriculture, they must satisfy certain quality characteristics such as stability and hygiene (Alburquerque et al., 2012; BSI, 2010; Siebert et al., 2008).

Also, intensive agriculture has promoted soil degradation and loss of organic matter and fertility, increased production costs (to maintain productivity) and contributed to CO_2 emissions (European Environment Agency, 2010). In this context, the recycling of digestates in agricultural systems has an important role, by reducing the use of mineral fertilisers, which leads to positive effects with respect to resource conservation (less fossil fuel and mineral resource consumption), climate change mitigation and soil quality maintenance. Northern European countries such as Denmark, Sweden, Scotland or Germany have used digestate in agriculture, mainly for cereal production (Möller and Stinner, 2009; Ortenblad, 2002; Rodhe et al., 2006; Smith et al., 2007). But these results cannot be extrapolated directly to Spanish intensive

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Table 1
Main characteristics of the digestate and the cattle manure used in the experiment on a fresh and dry weight basis for digestate and cattle manure, respectively.

Parameter	Digestate	Cattle manure
Dry matter (%)	1.9	47.1
pH	8.3	8.4
Electrical Conductivity (dS m^{-1})	30.5	11.9
Total organic carbon (%)	0.47	35.8
Total nitrogen (%)	0.38	2.61
C/N ratio	1.2	13.7
P_2O_5 (%)	0.05	0.73
K_2O (%)	0.24	2.21
CaO (%)	0.07	6.4
MgO (%)	0.03	1.0
Na (mg kg^{-1})	524	5190
Fe (mg kg^{-1})	20	3222
Cu (mg kg^{-1})	4	166
Mn (mg kg^{-1})	3	164
Zn (mg kg^{-1})	30	249

Digestate: Cd 0.01, Ni 0.2, Pb 0.04, Cr 0.1 and Hg <0.5 mg kg^{-1} fresh weight. Cattle manure: Cd 1.1, Ni 8, Pb 82, Cr 10 and Hg <0.5 mg kg^{-1} dry weight.

crop production systems, characterised by high fertiliser demand and short intercrop period under Mediterranean climate conditions. Therefore, there is a need for research in order to assess the adequate agronomic use of digested materials in Mediterranean intensive agriculture.

In the present study, the suitability of a digestate for use as fertiliser under field conditions has been evaluated for two horticultural crops over a two-year period, by analysing the effects of digestate addition on soil fertility and crop production and by comparing the fertilising capacity of the digestate with those of a mineral fertiliser and a traditional organic fertiliser (cattle manure).

2. Materials and methods

2.1. Materials

The study was carried out in an experimental field, belonging to "Fundación Ruralcaja Grupo CRM" situated in Paiporta (Valencia, eastern Spain). The main characteristics of the soil classified as a Typic Xerofluvent (Soil Survey Staff, 2010) were: sandy loam texture, pH 8.0, electrical conductivity (EC) (1:5) 0.12 dS m^{-1}, total organic carbon 0.89%, C/N 8.3, $CaCO_3$ 23.2%, available-P 34 mg kg^{-1} and available-K 442 mg kg^{-1}.

The digestate was collected from an industrial anaerobic co-digestion plant, which treated a mixture of pig slurry with 1.0% sludge from a slaughterhouse wastewater treatment plant and 6.5% biodiesel wastewaters, at a temperature of 37 °C and with a hydraulic residence time of 21 days. The cattle manure was collected from a farm close to the experimental site. Both the digestate and cattle manure were stored (<4 °C) and characterised rapidly, in order to determine the application rate based on crop nitrogen demand before each application to the field.

The collected digestate was a liquid material and the cattle manure was solid; both had alkaline pH and high EC. The cattle manure showed higher C/N ratio and contents of organic carbon and nutrients than the digestate (Table 1). The mineral treatment consisted of a NPK 15–15–15 complex for basal fertilisation, while NH_4NO_3 and K_2SO_4 were added by fertigation.

The tested crops in the present study were watermelon (*Citrullus lanatus* var. lanatus) cultivar (cv.) 'Precious Petite' (Syngenta), as a summer crop, and cauliflower (*Brassica oleracea* var. botrytis) cv. 'Meridien' (Clause-Tezier), as a winter crop. Watermelon and cauliflower seeds were sown in a seedbed and seedlings of uniform size were transplanted to the field after one month (2.5 × 0.8 m spacing for watermelon and 0.64 × 0.5 m for cauliflower), leading to a plant density of 5000 and 31,250 plants per ha for watermelon and cauliflower, respectively.

2.2. Experimental design and layout

The experiment was a field assay, having a fully randomised design with three replication plots of 32 m^2 each per treatment. Four treatments were established: control soil without fertilisation, mineral fertilisation, digestate and cattle manure (the traditional organic fertiliser in this area). Successive crops of watermelon and cauliflower (watermelon–cauliflower–watermelon–cauliflower) were grown for two consecutive growing seasons during 2009 and 2010.

The organic amendments (digestate and cattle manure) were added manually to the plots and immediately incorporated into the soil using a rotavator (depth of 30–40 cm), to ensure their uniform distribution and avoid ammonia volatilisation. Digestate (64 and 66 m^3 ha^{-1}, on average, for the first and the second crop seasons for watermelon and cauliflower, respectively) and cattle manure (20 and 22 Mg ha^{-1}, on average, for the first and the second crop seasons for watermelon and cauliflower, respectively) were added as the basal fertilisation between four and eight weeks before planting. This stabilisation period in soil was used to reduce or avoid potential detrimental effects associated to immature organic materials. For the mineral fertiliser treatment the N–P–K complex was applied two weeks before planting as a basal dose (647 and 646 kg ha^{-1}, on average, for the first and the second crop seasons for watermelon and cauliflower, respectively). In addition, a standard fertilisation programme was applied through a drip system, considering different sectors for each treatment, as is normally done in fertigation trials with watermelon and cauliflower (Table 2). Crop management followed the standard agronomic practices used in the area (soil preparation, crop cycles, fertilisation and phytosanitary treatments, etc.). The amount of both digestate and cattle manure applied was calculated according to their total-N concentration, adjusting the other macronutrients (P and K) with mineral fertiliser during the crop development, by drip irrigation. Thus, for the digestate, cattle manure and mineral fertiliser treatments, the same amount of N, P and K was applied to the experimental plots for each crop (240 N, 90 P_2O_5 and 250 K_2O kg ha^{-1} for watermelon and 280 N, 100 P_2O_5 and 300 K_2O kg ha^{-1} for cauliflower). Control treatment did not receive any fertiliser but was drip irrigated using the same amount of water as the rest of the treatments.

2.3. Plant and soil samplings

The watermelons and cauliflowers were harvested when the commercial size was obtained; shape criteria and the field evaluation (vigour, homogeneity and % coverage) were then determined. Marketable and non-marketable yield was determined based on fruit/curd quality parameters such as size, shape, colour, external appearance, damage, etc. A comparison of the production data was made among treatments and the macro- and micronutrients in plant leaves and marketable products were analysed. Representative plant material samples were taken randomly per plot, washed with distilled water, oven dried at 60 °C for 24 h, ground and stored for analysis.

In addition, the effect of the different fertilising treatments on soil enzyme activities, microbial biomass and physico-chemical properties was evaluated. For each plot, soil samples (0–20 cm depth) were taken in ten different, random sites and combined to obtain a representative sample. Special care was taken in order to sample the soil where plants were growing.

Each soil sample was divided into two fractions, one of which was immediately sieved to <2 mm and stored without drying at

Table 2
The fertilisation programme applied, as fertigation, for watermelon and cauliflower (for each crop, values are averages of the first and the second crop seasons).

Treatment	NH_4NO_3 (kg ha^{-1}) (34.5%)	H_3PO_4 (L ha^{-1}) (75%)	K_2SO_4 (kg ha^{-1}) (50%)
Watermelon			
Control	0	0	0
Cattle manure	0	42	127
Digestate	0	63	180
Mineral fertilisation	430	0	290
Cauliflower			
Control	0	0	0
Cattle manure	0	28	69
Digestate	0	81	260
Mineral fertilisation	450	0	305

<4 °C for biological and biochemical analyses, while the other fraction was air-dried. One aliquot of the air-dried soil sample was sieved to <2 mm for physico-chemical and chemical analyses and another aliquot was sieved to collect 0.25–4 mm aggregates for stability measurements.

The timing of the experiment for the first watermelon crop (2009) was: 27 February, first addition of organic amendments; 6 March, sowing and first soil sampling (S1, 7 d after the first addition of organic amendments); 17 April, transplanting (49 d after the first addition of organic amendments); 3 June–27 July, field assessment and harvesting; 29 July, second soil sampling (S2, 152 d after the first addition of organic amendments).

For the first cauliflower crop: 3 August 2009, second addition of organic amendments; 10 August 2009, sowing; 10 September, transplanting (38 d after the second addition of organic amendments); 20 October–13 December, field assessment and harvesting; and 4 February 2010, third soil sampling (S3, 185 d after the second addition of organic amendments).

For the second watermelon crop (2010): 12 March, sowing; 30 March, third addition of organic amendments; 30 April, transplanting (31 d after the third addition of the organic amendments); 11 June–26 July, field assessment and harvesting; 5 August, fourth soil sampling (S4, 128 d after the third addition of the organic amendments).

For the second cauliflower crop: 5 August 2010, sowing; 26 August 2010, fourth addition of organic amendments; 9 September 2010, transplanting (14 d after the fourth addition of organic amendments); 15 October–29 December 2010, field assessment and harvesting; and 10 February 2011, fifth soil sampling (S5, 168 d after the fourth addition of organic amendments).

The cropping period from transplanting to the end of harvesting was about 90 and 110 d for watermelon and cauliflower, respectively.

2.4. Analytical methods

The following parameters were determined in the digestate and the cattle manure samples: EC and pH (directly, after sample homogenisation, for digestate and in a 1:10 (w/v) cattle manure:water extract) and moisture content, after drying to constant weight at 105 °C. The total organic carbon (TOC) and total nitrogen (TN) were measured by automatic microanalysis (EuroVector elemental analyser, Milan, Italy). After nitric acid-perchloric acid digestion, P, K, Ca, Mg, Na, micronutrients and heavy metals were analysed by inductively coupled plasma-mass spectrometry (XSERIES 2 ICP-MS; Thermo Scientific, MA, USA). The digestate was analysed fresh and the manure after air drying, and the results were expressed on a fresh and dry weight basis, respectively.

In soil, the particle size distribution was determined by the hydrometer method; both TOC and TN were determined with a EuroVector automatic microanalyser, while water-soluble organic carbon (WSC) was determined using an automatic analyser for liquid samples (TOC-V CSN + TNM-1 Analyzer, Shimadzu); the $CaCO_3$ content was measured with a calcimeter; pH was determined for saturated soil pastes and EC was measured in a 1:5 (w/v) soil:water extract; NH_4–N was extracted with 2 M KCl and determined by a colorimetric method based on Berthelot's reaction (Sommer et al., 1992), adding sodium citrate to complex divalent cations, while NO_3–N was measured in 1:5 (w/v) soil:water extracts with a nitrate-selective electrode (USEPA, 2007); available-K was determined by flame photometry after extraction with 1 N ammonium acetate at pH 7 (Schollemberger and Simon, 1954); available-P was extracted with 0.5 M $NaHCO_3$ (1:10, w/v) for 30 min and measured colorimetrically (Watanabe and Olsen, 1965), and the percentage of water stable aggregates was determined by the method of Lax et al. (1994).

The soil microbial biomass C (B_C) and N (B_N) were obtained by the fumigation–extraction method (Vance et al., 1987) and determined with an automatic analyser for liquid samples (TOC-V CSN + TNM-1 Analyzer, Shimadzu), being calculated as $B_C = 2.22$ (fumigated soil C - unfumigated soil C) and $B_N = 2.22$ (fumigated soil N - unfumigated soil N), according to Jenkinson (1988) and Wu et al. (1990), respectively. Soil respiration was calculated as the amount of CO_2–C emitted during a 10-day incubation period: 10 g of dry soil were placed in a 250-mL incubation vessel, the moisture was adjusted to 50% of water-holding capacity and a vial containing 10 mL of 0.1 M NaOH was placed inside the incubation vessel for retention of the evolved CO_2. After 10 d the vials were titrated with 0.1 M HCl in an excess of $BaCl_2$, using empty vessels as blanks (in triplicate). The methods used for analysing the soil biochemical parameters (dehydrogenase, urease, protease, alkaline phosphatase and β-glucosidase activities) were described by Roldán et al. (2005).

The total concentrations of P, K, Ca, Mg, Na, S, Fe, Mn, Zn, Cu and B in the plant material were determined by inductively coupled plasma-mass spectrometry (XSERIES 2 ICP-MS; Thermo Scientific, MA, USA), after nitric acid–perchloric acid digestion. The TN concentration was determined by the Kjeldahl method.

All physico-chemical analyses were performed in duplicate and the soil microbial biomass and biochemical analyses in triplicate; the results are expressed on a dry weight basis (24 h at 105 °C).

2.5. Statistical analyses

Statistical analyses were carried out with the programme SPPS 18.0 for Windows. The normal distribution of the data was checked by the Shapiro–Wilk test; when data failed this test, the percentage of stable aggregates was arcsin-transformed and the other parameters were log-transformed to achieve normality. The data were subjected to ANOVA and differences between means were determined according to Tukey's test ($P < 0.05$).

Table 3
Physico-chemical characteristics of the soil according to the fertiliser treatments and samplings (dry weight basis). Sampling time S1: after the first application of organic materials but before watermelon planting, S2: after watermelon cropping in the first year and before cauliflower planting, S3: after cauliflower cropping in the first year, S4: after watermelon cultivation in the second year and S5: after cauliflower cropping in the second year.

Parameter	Sampling	Control	Cattle manure	Digestate	Mineral fertiliser	ANOVA
pH	S1	7.9a	7.8a	7.7b	nd	**
	S2	8.0	7.9	7.9	7.9	NS
	S3	8.1	8.0	8.0	8.1	NS
	S4	7.8	7.8	7.8	7.7	NS
	S5	8.1	8.0	8.0	8.0	NS
EC (dS m^{-1})	S1	0.12b	0.14ab	0.16a	nd	**
	S2	0.16	0.25	0.23	0.24	NS
	S3	0.13	0.14	0.14	0.13	NS
	S4	0.23b	0.24b	0.22b	0.31a	**
	S5	0.12	0.14	0.14	0.14	NS
TN (g kg^{-1})	S1	1.7b	2.1a	2.0a	nd	**
	S2	1.5ab	1.8a	1.3b	1.3b	**
	S3	1.5a	1.3a	1.2b	1.1b	*
	S4	1.6ab	1.8a	1.3c	1.5bc	**
	S5	1.3a	0.9b	1.0b	1.1ab	**
NH_4–N (mg kg^{-1})	S1	1.5	1.4	1.7	nd	NS
	S2	0.1b	3.0a	3.2a	0.2b	***
	S3	15.4a	14.0b	7.5c	14.0b	***
	S4	1.8	1.1	1.3	1.5	NS
	S5	5.0	4.3	3.4	3.8	NS
NO_3–N (mg kg^{-1})	S1	14.5b	25.1b	64.7a	nd	***
	S2	2.8	2.9	3.2	7.2	NS
	S3	1.4	1.6	1.5	1.7	NS
	S4	6.9	9.2	8.9	11.3	NS
	S5	2.4	2.8	4.4	3.8	NS
Available-P (mg kg^{-1})	S1	28.5	36.3	36.7	nd	NS
	S2	24.2b	37.4ab	48.2a	32.2ab	*
	S3	24.8b	42.4ab	54.8a	32.9b	*
	S4	39.7b	53.5ab	75.8a	59.4ab	*
	S5	38.3	27.8	34.6	46.4	NS

EC: electrical conductivity and TN: total nitrogen. NS: not significant.
Mean values denoted by the same letter in a row (sampling point/time) are not statistically different according to Tukey's test at the 5% probability level.
nd: not determined, as mineral fertiliser was applied after S1 (same as control soil).
* Significant at probability level $P < 0.05$.
** Significant at probability level $P < 0.01$.
*** Significant at probability level $P < 0.001$.

3. Results

3.1. Physico-chemical properties of soil

The soil pH was only affected slightly by the digestate in the short term (Table 3), probably due to the high buffering capacity of the calcareous soil used. Organically amended plots had higher EC values than the control at S1, while the mineral fertiliser treatment gave the highest EC value at S4. Soil EC values were clearly higher at S2 and S4 for all treatments, coinciding with the summer period characterised by high temperatures and low rainfall (Fig. 1), which favours salt accumulation in the soil. The values of pH and EC were in the appropriate range for plant growth and no accumulation of salts was observed with the successive applications of the fertilising treatments to the soil.

The soil treated with digestate or cattle manure had the highest concentration of TN after the first addition of the organic amendments. Later, only plots treated with cattle manure showed significantly higher TN concentrations than the digestate and the mineral fertiliser treatment (S2, S3 and S4). At the end of the experiment (S5), no statistically significant differences were found for TN content when comparing the digestate, mineral fertiliser and cattle manure treatments (being lower than in the control treatment).

The NH_4–N concentration in plots amended with digestate or cattle manure were higher than for the control and mineral fertiliser treatment after the first watermelon crop (S2), although values were very low in all samplings with the exception of S3. These results indicate that nitrification was only partial, as the nitrate concentration was the lowest of all the samplings (Table 3).

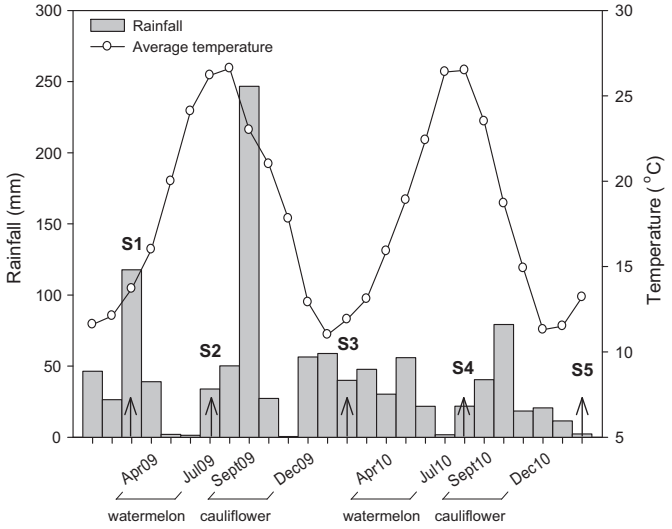

Fig. 1. Monthly rainfall and average temperature during the experiment (arrows indicate soil samplings, S1–S5).

The first addition of digestate (S1) caused a significant increase in the soil nitrate concentration (Table 3), but there were no significant differences among treatments in the other samplings.

Amended plots with digestate or cattle manure had higher concentrations of available-P than the control in all samplings, although the differences were not statistically significant for some

Table 4
Soil organic carbon fractions and microbial biomass parameters. TOC: total organic carbon, WSC: water-soluble organic carbon, B_C: soil microbial biomass carbon, B_N: soil microbial biomass nitrogen and qCO_2: biomass specific respiration rate.

Parameter	Sampling	Control	Cattle manure	Digestate	Mineral fertiliser	ANOVA
TOC ($g\,kg^{-1}$)	S1	9.0b	10.4a	9.4ab	nd	*
	S2	9.4	9.7	9.4	9.1	NS
	S3	9.2	9.8	9.6	9.3	NS
	S4	8.8b	10.4a	8.5b	9.1b	**
	S5	9.4	10.7	9.2	9.3	NS
WSC ($mg\,kg^{-1}$)	S1	51b	70a	55b	nd	**
	S2	44	49	46	45	NS
	S3	77b	93a	73b	76b	*
	S4	78	114	79	89	NS
	S5	40b	59a	49ab	41b	*
B_C ($\mu g\,g^{-1}$)	S1	109b	149a	116b	nd	**
	S2	98b	128a	122a	126a	*
	S3	80b	136a	107ab	91ab	*
	S4	141	176	157	161	NS
	S5	123b	179a	184a	175a	**
B_N ($\mu g\,g^{-1}$)	S1	20.8a	26.2a	12.3b	nd	**
	S2	18.7b	25.6a	22.1ab	24.4ab	*
	S3	16.0b	23.3a	19.0ab	16.7b	*
	S4	17.0	23.4	18.9	20.2	NS
	S5	19.8b	29.2a	30.4a	29.4a	**
CO_2–C ($\mu g\,C\,g^{-1}\,d^{-1}$)	S1	8.3b	13.4a	8.1b	nd	***
	S2	6.7b	9.9a	8.1ab	7.6b	**
	S3	7.7b	11.1a	8.0b	7.7b	*
	S4	7.6	8.8	8.5	9.3	NS
	S5	7.4c	11.3a	10.0ab	9.0b	***
B_C/TOC	S1	1.21	1.43	1.24	nd	NS
	S2	1.05	1.32	1.30	1.39	NS
	S3	0.88	1.41	1.11	0.99	NS
	S4	1.61	1.70	1.85	1.78	NS
	S5	1.34b	1.88a	1.68ab	2.00a	*
qCO_2 ($mg\,CO_2$–$C\,g^{-1}\,B_C\,d^{-1}$)	S1	76.7b	90.3a	70.0b	nd	*
	S2	73.0	77.6	64.5	60.3	NS
	S3	95.7	86.1	76.8	85.8	NS
	S4	53.8	50.4	54.1	60.2	NS
	S5	60.6	63.6	54.5	51.5	NS

NS: not significant.
Mean values denoted by the same letter in a row (sampling point/time) are not statistically different according to Tukey's test at the 5% probability level.
Sampling time S1: after the first application of organic materials but before watermelon planting, S2: after watermelon cropping in the first year and before cauliflower planting, S3: after cauliflower cropping in the first year, S4: after watermelon cultivation in the second year and S5: after cauliflower cropping in the second year.
nd: not determined, as mineral fertiliser was applied after S1 (same as control soil).
* Significant at probability level $P<0.05$.
** Significant at probability level $P<0.01$.
*** Significant at probability level $P<0.001$.

samplings. After the first watermelon cropping, the available-P concentration was significantly higher in plots amended with digestate than in the control (S2). This trend remained at S3 and S4, but not at the end of the experiment, when differences among treatments were not statistically significant.

3.2. Biological properties of soil

Digestate addition to soil did not provoke any significant effect on TOC with respect to the control and mineral fertiliser, while the addition of the cattle manure resulted in statistically significant increases in both TOC (S1 and S4) and WSC (S1, S3 and S5, Table 4). With respect to soil microbial biomass parameters, both organic amendments (cattle manure and digestate) as well as the mineral fertilisation caused increases in B_C and B_N compared to the control soil, particularly the cattle manure (Table 4). Thus, the addition of cattle manure to the soil led to significantly higher B_C (S1, S2, S3 and S5) and B_N (S2, S3 and S5) contents as well as to higher C–CO_2 production (S1, S2, S3 and S5), compared to the control. For B_C at samplings S2 and S5 and for B_N at S5, both the digestate and mineral fertiliser led to higher contents with respect to the control soil. The B_C/TOC was higher in amended plots, the differences with respect to control soil being statistically significant only at the end the experiment. The specific respiration activity or metabolic quotient (qCO_2)-calculated as the amount of CO_2–C evolved per unit of biomass C– was statistically higher in plots treated with the cattle manure with respect to the rest of the treatments only after its first application (S1, Table 4). Generally, the values of qCO_2 were not significantly affected by the applied treatments and they seemed to reach a steady-state in the last two soil samplings (S4 and S5).

The data from S1 show that the addition of cattle manure or digestate increased soil dehydrogenase activity by about 40% relative to the control soil (Fig. 2A), without significant differences between the two treatments. After watermelon cropping, the soils amended with cattle manure (S2 and S4) or digestate (S2) had higher dehydrogenase activity than control soil but similar values to the mineral fertiliser-treated soil. The differences between treated soils and control soil disappeared after the cauliflower crops (S3 and S5). In the first sampling, only the soil amended with the cattle manure had higher β-glucosidase activity than the control soil (Fig. 2B). Except for after the cauliflower crop (S3), no differences existed between the soils treated with the organic amendments (cattle manure or digestate) and the control soil.

At sampling S1, neither manure nor digestate had a significant effect on protease and urease activities (Fig. 2C and D). All soils reached similar levels of urease activity after each harvest,

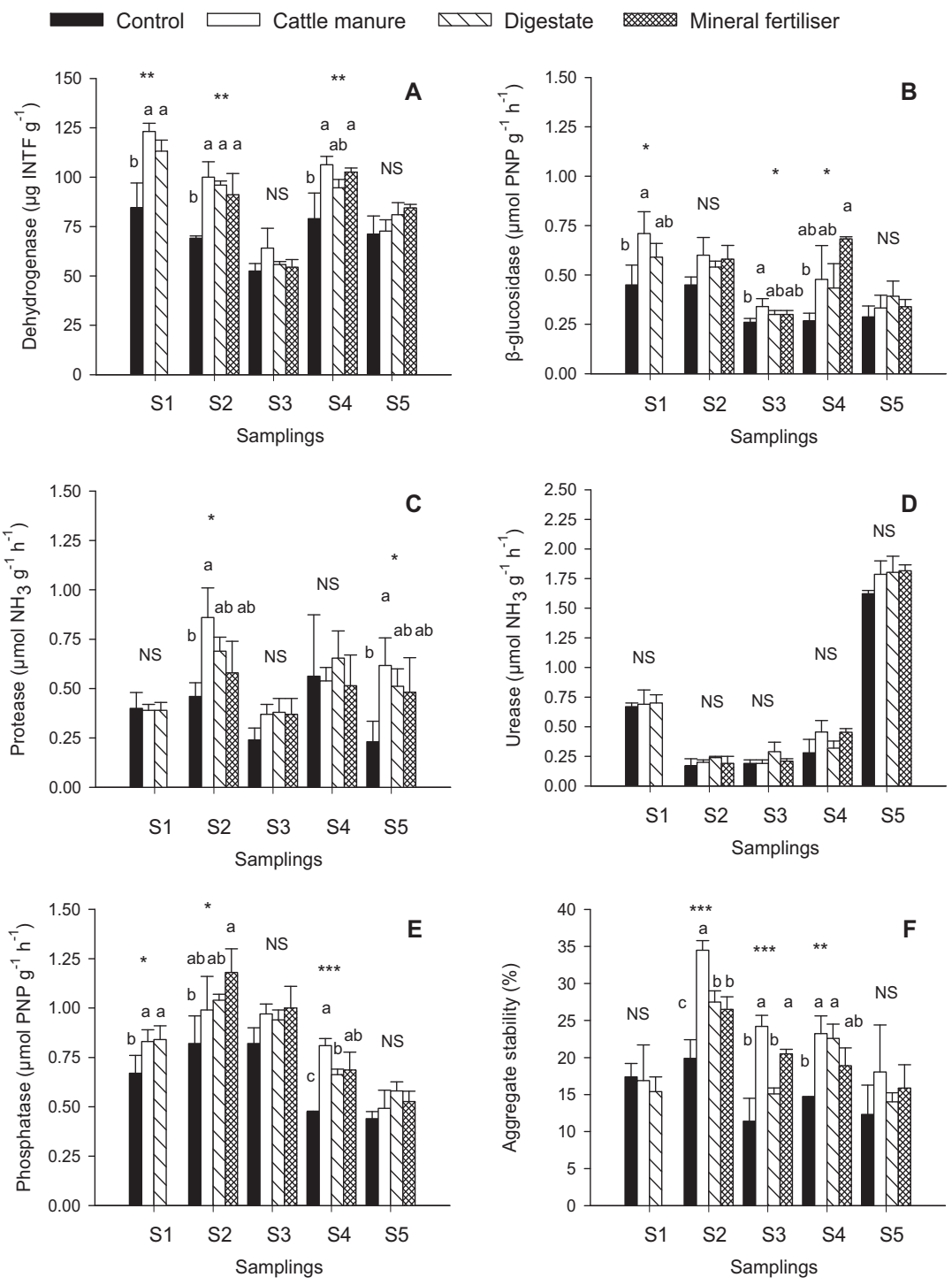

Fig. 2. Changes in enzyme activities and aggregate stability in the different soil samplings. For each sampling point, bars denoted by the same letter are not statistically different according to Tukey's test at the 5% probability level. NS: not significant, *, ** and ***: significant at probability level $P < 0.05$, 0.01 and 0.001, respectively. INTF: iodonitrotetrazolium formazan and PNP: p-nitrophenol. Sampling time S1: after the first application of organic materials but before watermelon planting, S2: after watermelon cropping, first year, S3: after cauliflower cropping, first year, S4: after watermelon cropping, second year and S5: after cauliflower cropping, second year. The mineral fertiliser treatment was not evaluated at the first sampling because the fertiliser was applied after soil sampling S1.

independent of the sampling date. After S2 and S5 samplings, the soil amended with cattle manure showed the highest protease activity (Fig. 2C). However, the digestate and mineral fertiliser did not increase it. Just before the first addition of the organic amendments (S1), the phosphatase activity was increased by both organic amendments (Fig. 2E), by about 25% with respect to the control soil. Only at S4, did all the treatments assayed significantly increase the phosphatase activity compared to the control soil, manure being the most effective.

Regarding the aggregate stability, the addition of manure or digestate did not have any effect on the percentage of stable aggregates at S1 (Fig. 2F). After the watermelon crop, the soil amended with manure presented the highest aggregate stability followed by the soil amended with digestate or mineral fertiliser. The

differences produced by the treatments assayed with respect to the control soil diminished throughout the experiment. At the end of experiment, there were no significant differences between treated soils and the control soil, associated with the ploughing of the soil between crops.

3.3. Watermelon and cauliflower crop production

The marketable yields of watermelon fruits obtained with the digestate and mineral fertiliser treatments were higher than for the cattle manure and control (Table 5); however, the differences were only statistically significant during the second crop season for the mineral fertiliser. The values of mean fruit weight were similar for all treatments tested (Table 5). In both growing seasons, watermelon plants in plots treated with the digestate or mineral fertiliser had significantly greater strength and uniformity of growth and a higher percentage of ground cover than in the cattle manure and control treatments (data not shown).

Differences for macro-(N, P, K, Ca and Mg) and micronutrient (Fe, Cu, Mn and Zn) concentrations in watermelon plant leaves were not statistically significant amongst treatments (data not shown). However, plants treated with the mineral fertiliser showed the highest nitrogen content in the marketable fruit in both cropping years (1.61 and 1.93% with respect to 1.28 and 1.30% in the rest of treatments for the first and second crop seasons, respectively, $P < 0.05$) as well as the highest potassium concentration (statistically different only for the second season, 3.21% with respect to 2.48% in the rest of the treatments, $P < 0.05$).

Production results for cauliflower were similar in both growing seasons (Table 6). Thus, digestate, cattle manure and the unamended soils led to a significantly lower marketable yield than the mineral fertiliser plots (the latter showing better plant development and vigour). With regard to the characteristics of the cauliflower plants during field evaluation, plants from the mineral treatment had good appearance and colour while those from the organic treatments had light-coloured leaves and were upright, with little foliage, while the controls showed a reddish colour – maybe due to low temperature – indicating the weakness of the plants.

With respect to the macro- and micronutrient contents of cauliflower, plants from the organic treatments had leaf nitrogen concentrations that were similar to the control values (1.77%), much lower than in the mineral fertiliser treatment (2.55%). However, statistically significant differences were found only for P (higher in the mineral fertiliser treatment – 0.42% – compared to 0.31% in the other treatments, $P < 0.01$). In the commercial part, no significant differences in nutrient content were found, with the exception of lower S levels in plants fertilised with cattle manure and higher Fe values for the control soil (data not shown).

4. Discussion

4.1. Effects on physico-chemical and biological soil properties

The changes in inorganic–N (NH_4–N and NO_3–N) in the soil after the first application of digestate suggest a rapid nitrification of the ammonium–N added by the digestate to the soil. Digestate contains a high proportion of NH_4–N, which can be nitrified quickly in the soil, and relatively low quantities in organic forms (Alburquerque et al., 2012). The initial high NO_3–N concentration in the digestate-treated soil decreased in the successive sampling periods, so there was no accumulation of NO_3–N in soil at the end of the experiment. Nitrate can be taken up directly by plants and incorporated into tissues, but it also has a high potential for entering groundwater through leaching. Limited nitrification could have occurred in the soil during winter, especially during the first year, when the autumn rainfall was high.

Digestate addition was more effective at increasing the soil available-P content during the crop cycles than cattle manure or mineral fertiliser, which could have contributed to the increase in watermelon production in comparison with the cattle manure. In fact, a significant, positive correlation was found between available-P in soil and the marketable yield of watermelon ($P < 0.05$). This fact must be noted since P deficiency is one of the main nutrient problems in calcareous soils, where the high pH and soil carbonate content make P less available to plants and addition of organic amendments is an adequate strategy to mitigate P deficiencies (Bustamante et al., 2011; Melero et al., 2006). However, the increased concentration of available-P in the soil amended with the digestate did not correspond with an increase in the yield of cauliflower grown in this soil.

Addition of digestate to soil provided easily available organic matter, mostly degradable in the short term, which did not contribute to increases in the soil organic carbon content as cattle manure did in some of the samplings. Cattle manure provided a much higher input of organic matter into the soil than did the digestate at the application rates tested. However, the soil microbial biomass carbon and nitrogen data revealed a significant stimulation of the soil microbial populations by the digestate, particularly in the summer crop (watermelon). The organic and mineral fertiliser treatments increased the values of the microbial biomass parameters (B_C, B_N and B_C/TOC), compared with the control. This must be related to the nutrient supply as well as to better crop development, derived from a positive effect on the microbial community associated with the crop rhizosphere. Fuchs et al. (2008) also obtained positive effects on soil biological activity after digestate amendment and Melero et al. (2006) noted that organic management had a positive effect on soil organic matter, leading to soil quality and productivity improvements, since the microbial biomass constitutes a nutrient reservoir which contributes to the maintenance of long-term agricultural sustainability. Increased microbiological activity was also revealed by the variations in dehydrogenase activity, which has been proposed as a valid biomarker to indicate changes in microbial activity due to changes in soil management under different agronomic practices and climates (García et al., 1997). It is worth noting that increased dehydrogenase activity as a consequence of the digestate or cattle manure addition was observed only in the soil cultivated with watermelon. These results denote a clear temperature limitation of soil respiration in the winter crops (cauliflower).

Organic matter transformation in soil depends on microbial and enzymatic activity, which determines the release and availability of soil nutrients (Tejada and González, 2006). Therefore, changes in enzyme activities (urease, protease-BAA, alkaline phosphatase and β-glucosidase) are clear indicators of changes in soil fertility, since they are related to the mineralisation process and the supply of nutrients. In soils treated with digestate, significant increases in alkaline phosphatase activity (linked to the phosphorus cycle) were generally found, which may have been responsible for the greater P availability in these soils, especially at the S4 sampling, when the differences in phosphatase activity and available P between digestate-treated and control soil were greatest. In contrast, no changes in β-glucosidase activity (linked to the carbon cycle) were measured, possibly due to the scarce input of easily available carbon sources added with the digestate. The values were similar to those detected for the cattle manure treatment. Likewise, the soil urease and protease activities (linked to the nitrogen cycle) were not affected by digestate addition, probably related to the negligible content of organic nitrogen in this material.

In addition to supplying nutrients, organic amendments create a favourable environment for plant growth by improving the

Table 5
The main effects of the tested fertiliser treatments on watermelon production: marketable and non-marketable yield (Mg ha^{-1}), and mean fruit weight (kg per fruit).

Treatment	Marketable production	Non-marketable production	Mean fruit weight
First year			
Control	32.1	0.6	2.27
Cattle manure	37.7	1.8	2.34
Digestate	47.9	2.7	2.56
Mineral fertilisation	42.0	2.4	2.10
ANOVA	NS	NS	NS
Second year			
Control	31.6b	0.6	2.29
Cattle manure	31.2b	1.4	1.90
Digestate	41.9ab	0.6	2.13
Mineral fertilisation	56.6a	0.0	2.20
ANOVA	*	NS	NS

NS: not significant.
Mean values denoted by the same letter in a column are not statistically different according to Tukey's test at the 5% probability level.
* Significant at probability level $P < 0.05$.

Table 6
The main effects of the tested treatments on cauliflower production: marketable and non-marketable yield (Mg ha^{-1}) and mean curd weight (kg per piece).

Treatment	Marketable production	Non-marketable production	Mean curd weight
First year			
Control	2.6b	10.0ab	1.04
Cattle manure	5.9b	16.0a	1.04
Digestate	4.8b	14.1a	1.19
Mineral fertilisation	25.6a	6.8b	1.17
ANOVA	**	**	NS
Second year			
Control	2.0b	10.3a	1.05
Cattle manure	2.8b	10.3a	1.36
Digestate	9.8b	13.1a	1.30
Mineral fertilisation	37.3a	2.6b	1.39
ANOVA	**	**	NS

NS: not significant.
Mean values denoted by the same letter in a column are not statistically different according to Tukey's test at the 5% probability level.
** Significant at probability level $P < 0.01$.

structural stability of soil. Organic material has a cementing effect, due to the polysaccharides present (Lax and García-Orenes, 1993), and reactivates microbial populations (Borken et al., 2002). In our particular case, the addition of cattle manure or digestate to the soil produced a very significant increase in the levels of stable aggregates after both watermelon crops. Reactivation of the microbial population leads to increased levels of bacteria, and particularly of fungal populations, which are principally responsible for the formation of aggregates larger than 0.2 mm (Andrade et al., 1998). Positive, statistically significant correlations between the levels of stable aggregates and protease ($P < 0.01$), β-glucosidase ($P < 0.05$) and phosphatase ($P < 0.001$) activities, soil respiration ($P < 0.05$) and soil microbial biomass nitrogen ($P < 0.001$) were found, which suggests that the reason for the increased aggregate stability after the addition of residue is fundamentally microbiological. On the other hand, the decrease of soil structural stability through the experiment and the absence of effects of cattle manure and digestate on such physical parameter at the end of the experiment could confirm the disruption of soil aggregates due to ploughing applied between crops. Ploughing continually exposes new soil to wet-dry cycles at the soil surface (Beare et al., 1994), thereby increasing the susceptibility of aggregates to further disruption.

4.2. Effects on crop production

Organic amendments can enhance soil fertility and productivity, improving the plant nutrient status for potentially limiting nutrients such as N, P and K as well as for several micronutrients (Liu et al., 2009). In this respect, positive correlations were found in our experiment between watermelon yield and fruit nutrient concentration (N and K at $P < 0.01$), and between the mean fruit weight and both fruit N concentration ($P < 0.05$) and fruit P concentration ($P < 0.01$). Likewise, the marketable yield of cauliflower was correlated significantly with both leaf N and P ($P < 0.01$) and K ($P < 0.05$); these results highlight the great impact of plant nutrient status on crop production.

As mentioned before, the digestate showed good fertilising properties in the summer watermelon crops. Both the digestate and mineral fertilisation produced significantly greater plant development, strength and homogeneity compared to the control and cattle manure treatments. Therefore, the digestate can be used in fertilisation regimes for watermelon, as a basal fertiliser. For such summer crops, the digestate provided enough plant available-N for crop production; so, it can substitute for mineral N by providing the appropriate balance of nutrients, with the shortcomings of the digestate being supplemented by mineral fertilisation. Neither deficiency nor toxicity symptoms were evident during the watermelon experiment. A simple economic comparison between the conventional mineral fertiliser and the digestate can be made for watermelon, taking into account only the costs of fertilisers (Table 7), since the remaining tasks (soil preparation, seeds, planting and harvesting, labour, water, etc.) can be considered the same for both treatments. The economic value of the digestate can be estimated as 7 €m^{-3} based on its N, P, K and OM concentrations (3.8, 0.5, 2.4 and 8.5 kg m^{-3} for N, P_2O_5, K_2O and OM respectively) and their prices per FU (fertilising unit): 0.90, 1.04, 1.17 and 0.03 € per FU (MARM, 2010a). Hence, the partial substitution of mineral fertiliser by digestate for the watermelon crop can achieve a saving of 390 €ha^{-1}, but the cost of digestate transportation and application should be considered.

Table 7
Costs associated with fertiliser application to the watermelon crop (average data for the two crop seasons).

	Application rate (kg ha^{-1})	Cost[a] (€kg^{-1})	Cost per ha (€)
Mineral fertiliser			
15–15–15	647	0.35	226.5
NH_4NO_3	430	0.30	129.0
K_2SO_4	290	0.58	168.2
		Total cost (€ha^{-1}):	523.7
Digestate			
Digestate	64,000	–	–
H_3PO_4	63	0.41	25.8
K_2SO_4	180	0.58	104.4
		Total cost (€ha^{-1}):	130.2

[a] MARM (2010a).

In contrast to watermelon, cauliflower plant growth was poorer in the plots treated with the organic materials (digestate or cattle manure) than in those receiving mineral fertiliser, leading to a low marketable production; normal production is 25–35 Mg ha^{-1} (MARM, 2010b), which was obtained with the mineral fertiliser. Thompson et al. (2000) noted how the marketable yield of cauliflower is very sensitive to N application, an adequate supply being necessary during the whole crop season. Therefore, the low cauliflower yields under organic fertilisation could have been related to N deficiency, according to the lower N concentrations of the plants grown with the organic treatments, with respect to the mineral fertiliser. This indicates limited N mineralisation and nitrification at the lower temperatures prevalent during the winter crop, with respect to the summer. Blatt (1991) related crop N deficiencies produced by organic treatments to slow mineralisation rates under moist, cool soil conditions, since soil N availability is conditioned greatly by microbial activity, which affects process such as mineralisation, immobilisation and nitrification. The organic amendments may not have satisfied the high N demand of the crop since the availability and release of nutrients through OM mineralisation (mainly in the manure treatment) can be reduced in winter due to the lower temperatures, in comparison with spring or summer conditions (Blatt, 1991; Bustamante et al., 2011). Nitrogen mineralisation seems not to be a limiting step for plant growth after digestate addition since most N in the digestate is in the form of ammonium (87% in the present study), which is easily nitrified under favourable conditions to become available in the soil. However, NO_3–N loss by leaching could have been relevant, particularly in the first year when a high-rain season followed digestate application, as could a slow or incomplete nitrification process. In this context, Rodhe et al. (2006), Kapuinen et al. (2007) and Bermejo and Ellmer (2010) underlined the importance of the timing of digestate application and its fractionation, when comparing crop yield from digestate treated soil to that obtained with conventional mineral fertiliser. It must be timed to avoid nitrogen losses (lost fertiliser value), considering that nutrients bound to organic forms in the digestate are released and hence available to plants after OM mineralisation. Thus, digestate addition to soil must be adapted to the plant requirements and climate conditions in order to reach the maximum nutrient efficiency.

5. Conclusions

The changes in the soil physico-chemical properties provoked by digestate tended to decrease with time, leading to a scarce residual effect. The digestate provided a significant amount of ammonium N, which is rapidly nitrified and thus directly available to crops in the short-term. Moreover, the addition of digestate led to an increased amount of available P in the soil; hence, its agronomic use should be based not only on the N but also on the P it supplies. The digestate increased soil microbial biomass and dehydrogenase, alkaline phosphatase and β-glucosidase activities, although this effect was always less significant than that of the cattle manure, which provided a greater amount of organic carbon to the soil.

Digestate addition to soil had a positive effect on the yield of watermelon, cultivated in the summer, but very little effect compared to mineral fertilisation for cauliflower, cultivated in the winter. This may be related to the winter conditions (rain favouring nutrient leaching and low temperature slowing microbially mediated processes such as nitrification) and a higher N demand together with the longer crop cycle of cauliflower. Thus, digestate application (rate and timing) must be optimised to satisfy the crop demand during the whole crop cycle, considering the abovementioned factors.

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