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ORIGINAL PAPER

Short-term effects of organic and inorganic fertilizers on soil microbial community structure and function

A field study with sweet corn

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Abstract A field study was carried out to analyze the short-term impacts of replacing mineral by organic fertilizers on the microbial and biochemical parameters relevant for soil fertility and crop yield. Three types of fertilization regimes were compared: (1) conventional fertilizer regime with inorganic fertilizer, and combined integrated fertilizer regimes in which 25 % of the nutrients were supplied by either (2) rabbit manure or (3) vermicompost. The effects on microbial community structure and function (phospholipid fatty acid [PLFA] profiles, bacterial growth, fungal growth, basal respiration, β-glucosidase, protease and phosphomonoesterase activities), soil biochemical properties (total C, dissolved organic carbon [DOC], N-NH_4^+, N-NO_3^-, PO_4, total K) and crop yield were investigated in the samples collected from the experimental soil at harvest, 3 months after addition of fertilizer. The integrated fertilizer regimes stimulated microbial growth, altered the structure of soil microbial community and increased enzyme activity relative to inorganic fertilization. Bacterial growth was particularly influenced by the type of fertilizer regime supplied, while fungal growth only responded to the amount of fertilizer provided. The use of manure produced a fast increase in the abundance of PLFA biomarkers for Gram-negative bacteria as compared to inorganic fertilizer. Nutrient supply and crop yield with organic fertilizers were maintained at similar levels to those obtained with inorganic fertilizer. The effects of the organic amendments were observed even when they involved a small portion of the total amount of nutrients supplied; thereby confirming that some of the beneficial effects of integrated fertilizer strategies may occur in the short term.

Keywords Vermicompost · Manure · Organic fertilizers · Sustainable agriculture · PLFAs · Soil enzymes

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Introduction

Increasing the sustainability of cropping systems involves the reduction of agrochemical and fertilizer inputs through the reliance in soil ecosystem processes and biological interactions for the provision of plant nutrients (Drinkwater and Snapp 2007). Of particular importance are soil microbial processes as they are crucial for plant nutrient supply given their central role in soil organic matter decomposition and nutrient dynamics (Paul 2007; Wardle 2002). Management of soil fertility through organic fertilizers has always been a pivotal principle of sustainable agriculture. Yet, the impacts of these fertilizers on soil microbial community structure and function as well as on nutrient availability can vary widely, having extremely different impacts on crop productivity (Chivenge et al. 2011; Herencia et al. 2008). Better understanding of the microbial processes that take place in soil under organic fertilization could help identify the main drivers determining nutrient availability in order to improve crop growth.

Organic fertilizers typically increase soil microbial biomass through the supply of C-rich organic compounds to the generally C-limited microbial communities in arable soils (see reviews by Diacono and Montemurro 2010; Knapp et al. 2010). Further, C addition seems to select for specific microbial groups that feed primarily on organic compounds, changing the composition of the microbial community (Marschner et al. 2003; Ros et al. 2006; Hu et al. 2010; Zhong et al. 2010). While many bacteria feed on easily available C compounds, fungi seem to prefer more complex C compounds (Meidute et al. 2008). Incorporation of organic fertilizers can also increase microbial activity in soils between 16 % and 20 % as compared to inorganic fertilizers (Dinesh et al. 2010; González et al. 2010). Increases in the enzyme activities involved in the release of the main plant macronutrients with organic fertilizers have also been signaled in several studies (Marinari et al. 2000; Dinesh et al. 2010). Consequently, organic fertilizers can stimulate soil microbial processes and increase crop yields as compared to inorganic fertilization—yet, this has often been associated to the increase in organic matter and soil fertility after long-term repeated application of organic fertilizers (Herencia et al. 2008; Diacono and Montemurro 2010). Nevertheless, some studies have shown that organic fertilizers can increase crop growth and yield even in a single growing season and when they are applied in small quantities (Arancon et al. 2004, 2005), suggesting the existence of some sort of short-term biological plant growth promoting mechanism. Soil microbial and biochemical parameters can react rapidly to changes in soil management (Gil-Sotres et al. 2005), and fast increases in soil microbial biomass and activity have been observed shortly after application of organic fertilizers (Dinesh et al. 2010). Significant changes in soil microbial community structure in the short term are not always observed (Stark et al. 2007). Moreover, a change in microbial community structure does not always involve a change in microbial community function or an increase in availability of plant nutrients and crop productivity (Yao et al. 2000; Marschner et al. 2003; Nannipieri et al. 2003; Franco-Otero et al. 2012). In fact, increases in soil microbial biomass can reduce short- term nutrient availability to the plants due to microbial immobilization (Geisseler et al. 2010; Inselsbacher et al. 2010). Consequently it has been often pointed out that the use of organic fertilizers may compromise crop yield as compared to inorganic fertilizers because of the reduced input of readily available plant nutrients and the absence of rapid and short-term beneficial effects on microbial and biochemical properties (Pimentel et al. 2005).

In this study, we carried out a field experiment with sweet corn in which the main objective was (1) to determine the short-term impacts of replacing inorganic fertilizers by small amounts of organic fertilizers on the structure of the soil microbial community as compared to full inorganic fertilization. In addition, (2) we aimed to explore whether the changes in microbial community structure are also accompanied by changes in microbial community function and (3) to determine how these changes influence nutrient availability in soil and crop yield. Furthermore, (4) we hypothesized that microbial properties would be differently influenced by inorganic and organic fertilizers, but also by two different types of organic fertilizers with different mineralization degree and C content such as manure and vermicompost.

Material and methods

Site description

A field trial was carried out in Pontevedra (NW Spain) (42° 24′20.44″N, 8°38′39.67″W, 20 m above sea level). The climate is mild and humid with an annual rainfall of≈ 1,600 mm. The soil is a Humic cambisol with a sandy loam texture (53 % sand, 28 % silt, 18 % clay) which had been subjected to conventional corn cropping, but with a high organic matter content, as it is characteristic from the Galician region (NW Spain) (Leirós et al. 1999). The main physicochemical characteristics of the soil are summarized in Table 1.

Materials

Two types of organic fertilizers, vermicompost and rabbit manure, which are mineralized to different degrees, and have different microbial and biochemical properties (Domínguez et al. 2010), were studied. Rabbit manure was obtained from the facilities of the Todoverde vermicomposting company (Ourense, NW Spain), and consisted of a mixture of rabbit faeces and urine deposited on the floors of rabbit hutches. The vermicompost was produced commercially (by the same company) from rabbit manure (in

Table 1 Main physicochemical properties of the bulk soil at the experimental site, the vermicompost and the manure used in the experiment

	Bulk soil	Vermicompost	Manure
Moisture (%)	12.3±0.05	55.5±0.15	63.3±4.88
pH	6.6±0.02	7.2±0.11	7.7±0.08
EC (mS cm^{-2})[a]	0.02±0.00	0.31±0.02	0.27±0.01
DOC[b] (mg kg dw^{-1})	187±67	2404±367	8817±763
Total C (g kg dw^{-1})	31±0.8	234±4.8	304±2.7
Total N (g kg dw^{-1})	3.4±0.1	21±0.3	19±0.1
P_2O_5 (g kg dw^{-1})	22±0.3	132±1.5	81±4
K_2O (g kg dw^{-1})	51±0.1	46±0.2	28±8.2

[a] Electrical conductivity

[b] Dissolved organic C

1 m^3 continuous feed vermireactors with the earthworm species *Eisenia fetida*) and had a homogeneous earthy appearance. Five samples (500 ml) each of manure and of vermicompost were collected from the stockpiled materials and analyzed for their physicochemical properties (Table 1). In addition, commercial N (10.5 %N-NO_3^-, 10.2 %N-NH_4^+, 6.5 % MgO), P (28 % P_2O_5) and K (50 % K_2O) mineral fertilizers were used.

Experimental design

Three types of fertilizer regimes were compared: (1) a conventional fertilizer regime with NPK inorganic fertilizer, and two integrated fertilizer regimes in which 75 % of the nutrients were supplied as NPK inorganic fertilizer and the remaining 25 % of the nutrients were supplied by either (2) rabbit manure or (3) vermicompost. All three fertilizer treatments were supplied at two different doses, a *standard* dose (80:24:20 $kg\,ha^{-1}$ of N/P.K) for an expected final crop yield of 4 $t\,ha^{-1}$ of dry weight grain, and a *high* dose of 120:36:30 $kg\,ha^{-1}$ of N/P/K for an expected final yield of 6 t dry grain ha^{-1}, according to the dry grain yield and fertilization recommendations reported for sweet corn. Combination of the abovementioned fertilizer regimes and doses resulted in the following six fertilizer treatments:

- Standard dose of inorganic fertilizer: 80:24:20 $kg\,ha^{-1}$ of N/P/K
- Standard dose of vermicompost: 80:24:20 $kg\,ha^{-1}$ of N/P/K, 25 % supplied as vermicompost (4.2 $t\,ha^{-1}$)
- Standard dose of manure: 80:24:20 $kg\,ha^{-1}$ of N/P/K, 25 % supplied as manure (5.4 $t\,ha^{-1}$)
- High dose of inorganic fertilizer: 120:36:30 $kg\,ha^{-1}$ of N/P/K
- High dose of vermicompost: 120:36:30 $kg\,ha^{-1}$ of N/P/K, 25 % supplied as vermicompost (6.3 $t\,ha^{-1}$)
- High dose of manure: 120:36:30 $kg\,ha^{-1}$ of N/P/K, 25 % supplied as manure (8.2 $t\,ha^{-1}$)

Prior to application of the fertilizers, five soil samples were taken randomly from the top 15 cm for determination of soil properties. The amounts of vermicompost, manure and mineral fertilizers required to supply these nutrient requirements were calculated taking into account the nutrient content (NPK) of the soil and the fertilizers. As a consequence of the nutrient content of the substrates, the plots treated with organic fertilizers received surplus of organic C, relative to the plots treated with inorganic fertilizers (0.24 and 1.6 kg C m^{-2} in the manure treatments and 0.9 and 1.4 kg C m^{-2} in the vermicompost treatments, for standard and high doses of fertilizer, respectively).

Experimental units consisted of 10 m^2 plots each with one combination of fertilizer regime and dose, resulting in a total of 60 plots (3 regimes×2 doses×10 replicates). Plots were arranged in the field following a randomized complete block design. In each plot, the fertilizers were applied manually and incorporated by mixing into the top 20 cm of soil. For all treatments, 40 % of the total nitrogen was supplied before sowing, and the remaining 60 % was provided as top dressing during stalk formation, 2 months after sowing. Plots were sown in May, 1 week after application of the fertilizers. Sweet corn (*Zea mays* L.) was sown in each experimental plot containing two central and two border rows spaced 0.80 m apart, with 25 two-plant hills spaced 0.21 m apart; plots were overplanted and thinned to obtain a final density of approximately 60,000 plants ha^{-1}. Plots were hand-weeded throughout the experiment. No pesticides or fungicides were applied and no supplementary water was supplied to the plants. The mean temperature during the growing season was 18.9±0.8 °C, and the mean precipitation was 70.1±20.7 mm.

At harvest (late August), one soil sample (0–15 cm depth) was taken from between the two central rows in each plot in order to evaluate the effects of the fertilizer treatments on soil biochemical and microbial properties. Samples from each plot comprised three sub-samples collected at three random spots between the two central rows and were subsequently pooled, homogenized and placed in labeled plastic bags. Soil samples were immediately transported to the laboratory where they were sieved (2 mm) and processed for subsequent analysis. Soil samples were either: (1) dried at 60 °C until constant weight for determination of total C, N, P and K, (2) freeze-dried for phospholipid fatty acid (PLFA) analysis, or (3) stored at 4 °C for the other biochemical and microbial analyses. Processing and analysis of fresh samples was carried out within the first 2 weeks of sample collection.

Following collection of soil samples, five plants were selected at random from the two central rows of each plot. The ears were removed from each plant, and dried for determination of crop yield. Leaf samples were also collected, rinsed in distilled water, and dried at 60 °C for determination of N, P and K contents.

Analytical methods

Moisture and organic matter contents of the soil, vermicompost and manure, were calculated gravimetrically after drying at 105 °C for 24 h and ashing at 450 °C for 6 h, respectively. Total C and N contents were determined in a Carlo Erba 176 1500C/N analyzer, with dried (60 °C) soil and leaf samples. Total P and K were determined by X-ray fluorescence after drying (60 °C), grinding and pelleting the samples. Total C, N, P and K analyses were performed in the central laboratory facilities (CACTI) at the University of Vigo. Dissolved organic C (DOC) in the soil, manure and vermicompost samples was determined colorimetrically at

590 nm after moist digestion ($K_2Cr_2O_7$ and H_2SO_4) of aliquots of 0.5 M K_2SO_4 extracts (1:10 w/v) of the samples. Inorganic N (NH_4^+ and NO_3^-) was determined in 0.5 M K_2SO_4 extracts (1:10 w/v) of the soil, manure and vermicompost samples by the modified indophenol blue technique (Sims et al. 1995), in a microplate reader (Bio-Rad Model 550). Available inorganic P (PO_4^-) was determined colorimetrically in 2.5 % CH_3COOH extracts of the samples (Allen et al. 1986).

The microbial community structure was assessed by PLFA analysis (Gómez-Brandón et al. 2010). The retention times and the mass spectra of each of the FAMEs identified in the present study were compared with those obtained from known standard mixtures or pure PLFAs (i.e., 37-component FAME mix #47885-U (1,000 $\mu g ml^{-1}$; Supelco, Bellefonte, USA); BR1 #90–1051 (1,000 $\mu g ml^{-1}$); methyl 13-methyltetradecanoate #21- 1413 (250 $\mu g ml^{-1}$); methyl 15-methylhexadecanoate #21-1615 (250 $\mu g ml^{-1}$); methyl *cis*-9,10-methylenehexadecanoate #48-23-1709-7 (250 $\mu g ml^{-1}$); methyl *cis*-11,12-methyleneoctadecanoate #48-23-1911-7 (250 $\mu g ml^{-1}$)). These latter standards were supplied by Larodan Lipids (Malmö, Sweden). This permitted us to build a calibration curve for each FAME, including those from the *cis* and *trans* forms of PLFA 18:2ω6 and subsequently, to identify them by gas chromatography-mass spectrometry (GC-MS) analysis. For each sample, PLFA data are expressed as $\mu g g^{-1}$ dry weight. The viable microbial biomass was determined as the sum of all identified PLFAs (total PLFAs) expressed as $\mu g g^{-1}$ dry weight. Certain PLFAs were used as biomarkers to determine the presence and abundance of specific microbial groups (Zelles 1997, 1999). The PLFAs considered to be predominantly of bacterial origin were further classified as Gram-positive bacterial PLFAs (i14:0, i15:0, a15:0, i16:0, i17:0 and a17:0) and Gram-negative bacterial PLFAs (16:1ω7c, cy17:0, 17:1ω7c, 18:1ω7c and cy19:0). The PLFA 10 Me18:0 was used as biomarker for actinobacteria, and the PLFAs 18:2ω6c and 18:1ω9c were used as fungal biomarkers.

The overall microbial activity was estimated from the basal respiration. Samples (2 g fresh weight) were placed in respiration vials, sealed and incubated at room temperature for 19 h. The amount of CO_2 produced was then determined by gas chromatography. Bacterial growth was estimated by the leucine incorporation technique (Bååth 1994), as modified by Bååth et al. (2001). The fungal growth rate was assessed by the acetate in ergosterol method (Newell and Fallon 1991) adapted for soil (Bååth 2001). The amount of ergosterol in the soil samples was used to estimate fungal biomass-C. β-Glucosidase activity was assessed by determination of the *p*-nitrophenol (PNP) released, after incubation of the samples with β-D-glucopyranoside (0.025 M) for 1 h at 37 °C. Absorbance of the samples was measured at 400 nm (Eivazi and Tabatabai 1988). Protease activity was measured by determining the amino acids released after incubation of the samples with sodium caseinate (2 %) for 2 h at 50 °C, and with Folin–Ciocalteu reagent. Absorbance was measured in a microplate reader (Bio-Rad Model 550) at 700 nm (Ladd and Butler 1972). Alkaline phosphomonoesterase activity was estimated by determining the PNP released after incubation of the samples with *p*-nitrophenyl phosphate for 1 h at 37 °C; absorbance was determined at 400 nm in a Bio-Rad 550 microplate reader (Eivazi and Tabatabai 1972).

Statistical analyses

The influence of the fertilizer regime, the dose and their interaction, on the measured plant, soil and microbial variables was assessed through linear mixed models (PROC MIXED SAS version 9.1; SAS Inc., Cary, NC) (Littell et al. 2006). The block was introduced as a random factor in the mixed model in order to account for any possible spatial variability. Significant differences were further analyzed using the Tukey HSD post-hoc test, at $p \leq 0.05$. A discriminant analysis (DA) of the whole PLFA profile of the soil samples was used in order to explore the underlying effect of the fertilizer regimes on the structure of the soil microbial community. DA is an eigenanalysis multivariate technique that maximizes the among-group variation relative to the within-group variation, thereby highlighting the differences between previously established groups. The central concept of the DA is to find an equation that classifies new samples into defined groups. The number of dimensions needed to distinguish among the treatments is identified by the number of significant canonical discriminant functions (McCune and Grace 2002). In the present study, each function is a linear combination of PLFA variables, and the first function has the most power to discriminate among the treatments. DA was performed using SPSS (SPSS v.20) software program.

Results

Effects on composition of the soil microbial community

The total PLFA content of the soil, indicative of viable microbial biomass, ranged between 18 and 22 $\mu g g^{-1}$ dw soil. Total PLFA content of the soil samples was significantly affected by the type of fertilizer regime ($P=0.04$), as it was significantly higher in response to application of manure as compared to inorganic fertilizer (Table 2) while no differences were observed with application of vermicompost. The dose of fertilizer provided had no significant influence on the viable microbial biomass of the soil (dose: *P=0.77)*.

A major proportion of the identified PLFAs were bacterial biomarkers while no actinobacterial biomarkers were detected

Table 2 Abundance of the main microbial groups analyzed in the initial and in the final soil samples with three different fertilizer regimes (inorganic, vermicompost and manure) at crop harvest

	Initial soil	Inorganic	Vermicompost	Manure
∑PLFA	16.6±1.4	18.5±1.2^{b}	19.4±1.3^{ab}	22.6±1.0^{a}
Bacterial PLFAs	13.7±1.2	15.7±1.1^{b}	17.2±1.3^{ab}	19.9±0.8^{a}
Gram-positive bacterial PLFAs	0.73±0.3	1.8±0.21	1.7±0.17	2.1±0.06
Gram-negative bacterial PLFAs	12.93±1.1	13.8±1.04^{b}	15.5±1.20^{ab}	17.8±0.8^{a}
Fungal PLFAs	0.207±0.01	0.176±0.006	0.175±0.007	0.170±0.003
Fungal biomass-C[1]	n.d.	81.9±5.02	93.04±3.78	91.10±5.04

Values represent least square means of the two doses assayed ± standard error. Different letters within the same row indicate significant differences at $P<0.05$. The initial soil was not included in the statistical comparisons. All the variables were expressed in μg g dw^{-1}

[1] Fungal biomass C was estimated through the ergosterol content of the soil samples

(data not shown), and the amount of fungal PLFA biomarkers was remarkably low (Table 2). Bacterial biomass significantly increased with the addition of manure, as compared to inorganic fertilizer (fertilizer regime: $P=0.02$), whereas increasing the fertilizer dose have no significant effect on this parameter (dose: $P=0.76$). Among the different bacterial groups studied, the type of fertilizer regime only affected Gram-negative bacterial biomass (fertilizer regime: $P=0.03$), as the biomass was highest in the integrated treatment with manure (Table 2). The dose of fertilizer supplied did not influence biomass of either Gram-positive or Gram-negative bacteria (data not shown), but significantly increased fungal biomass-C, from 79±3.3 μg g^{-1} dw with the standard dose to 98±3.3 μg g^{-1} dw with the high dose (dose: $P=0.0002$). Fungal biomass-C was otherwise not affected by the fertilizer regimes ($P=0.14$).

In addition to the observed differences between treatments in specific PLFAs, the overall PLFA profile of the soil samples was different between the applied fertilizer treatments. DA of the identified PLFAs showed that the fertilizer treatment determined the structure of the microbial community in the soil samples (Fig. 1a). The discriminant functions associated with the first and second eigenvalues, DF1 and DF2, accounted for 44.2 % and 28.7 % of the variance for a total explained variance of 72.9 %. The first discriminant function differentiated soils treated with standard doses of manure and high doses of vermicompost from soils treated with high doses of inorganic fertilizers or manure. Vermicompost and standard manure samples were grouped within the negative values of the DF1, which was related to higher abundances of C16:0 (ubiquitous) and C18:2ω6t, respectively (Fig. 1). High dose of inorganic fertilizer was associated with the positive side of DF1 related to a high concentration of 18:1ω7c (Gram-negative bacterial biomarker) and C15:0 PLFAs. The second discriminant function differentiated the plots treated with manure from plots treated with inorganic fertilizer or vermicompost. This was related to an increase in C16:0 and C18:2ω6t PLFAs in plots treated with manure, and a decrease in C10:0 PLFA strongly associated with the negative side of DF2 (Fig. 1b).

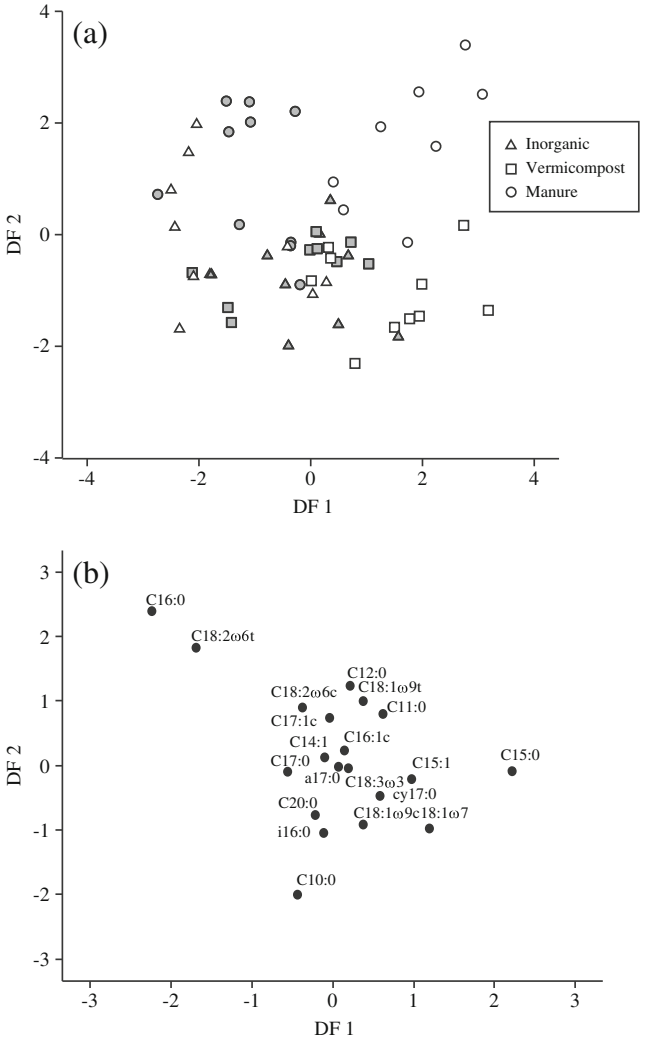

Fig. 1 Changes in the microbial community structure of the soil samples with inorganic fertilization, and integrated fertilizer regimes (including vermicompost and manure) at standard (*gray symbols*) and high doses (*white symbols*): **a** discriminant analysis performed on the 26 PLFAs identified in soil samples; **b** standardized discriminant scores of the PLFAs analyzed

Effects on soil microbial community function

Microbial activity, determined as basal respiration, was significantly affected by the different fertilizer regimes assayed (P=0.003). Respiration was significantly higher in the plots receiving manure than in those treated with vermicompost or inorganic fertilizer, regardless of the dose applied (Fig. 2). Bacterial growth was higher in plots treated with the high dose of vermicompost and manure than in those treated with high dose of inorganic fertilizer (Fig. 3a) (fertilizer regime × dose: P=0.0006), whereas the differences between regimes were negligible at the standard dose of fertilizer. Fungal growth did not differ between the fertilizer regimes, although in the vermicompost-amended plots this parameter increased significantly in response to the high dose (Fig. 3b) (fertilizer regime × dose: P=0.02).

The β-glucosidase activity was significantly higher when manure or vermicompost were added to the soil than when inorganic fertilizers were used alone (Fig. 4) (fertilizer regime: P=0.01), regardless of the dose applied. The trends were similar for protease (fertilizer regime: P=0.0008) and phosphomonoesterase activities (fertilizer regime: P=0.003), with higher values in plots treated with organic fertilizers (Fig. 4).

Effects on soil biochemical properties

The total C content of the soil samples varied significantly with the different fertilizer treatments applied (Table 3), although this depended on the dose considered (fertilizer regime × dose: P=0.028). No differences were found between the different fertilizer treatments in the total C content of the soil at harvest when the standard fertilization doses were applied. Significant increases in soil C content at harvest time were only observed with the high dose of vermicompost as compared to inorganic fertilizer. No differences were observed in DOC content of the soil subjected to the different regimes and doses at harvest (Table 3; fertilizer regime: P=0.832; dose: P=0.259).

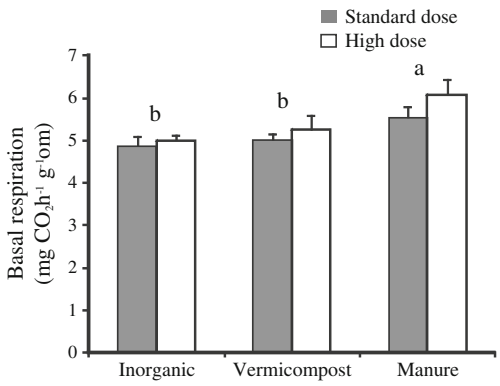

Fig. 2 Microbial activity measured as basal respiration in the soil amended with inorganic fertilizer, and integrated fertilizer regimes (including vermicompost and manure) at standard (*gray bars*) and high doses (*white bars*). Bars are means ± standard error. Different letters indicate significant differences at P<0.05. Posthoc comparisons were only made between fertilizer regimes

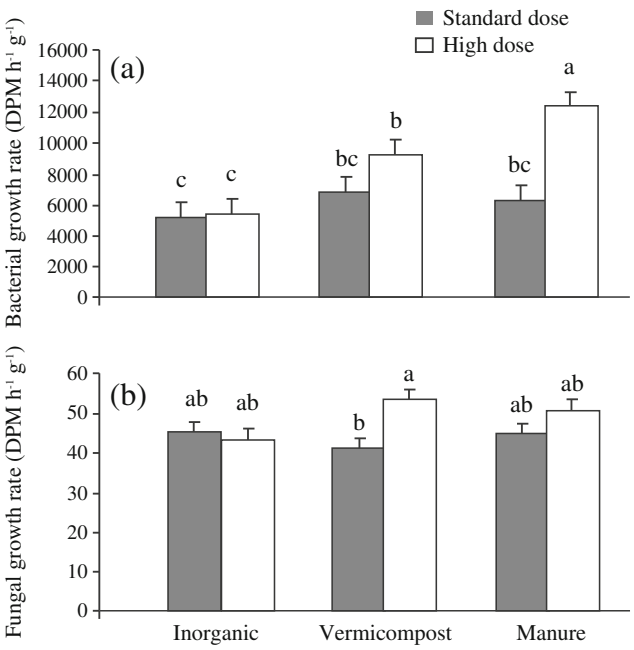

Fig. 3 Bacterial growth rate (**a**) and fungal growth rate (**b**) of soils amended with inorganic fertilizer, and integrated fertilizer regimes (including vermicompost and manure), at standard (*gray bars*) and high doses (*white bars*). Bars are means ± standard error. Different letters indicate significant differences at P<0.05

There were no differences in the concentration of N-NH_4^+ of the soil samples at harvest (Table 3; fertilizer regime: P=0.604; dose: P=0.443). Similarly, there were no significant differences in the N-NO_3^- content of the soil samples between the different regimes and doses (Table 3; fertilizer regime: P=0.538; dose: P=0.263). Nevertheless, the available P content (measured as PO_4^-) was significantly higher in the plots to which vermicompost was added than in the plots that received only inorganic fertilizer or manure (Table 3). However, this only occurred when high fertilizer doses were applied, while no differences were observed between the regimes at the standard dose (fertilizer regime × dose: P=0.02). Total K content, measured as K_2O, was not affected by the different fertilizer regimes at either of the doses (fertilizer regime: P=0.877; dose: P=0.267).

Effects on crop yield

The different fertilizer regimes being tested produced similar crop yields (Table 3), and no differences between integrated or inorganic fertilization were observed (P=0.86). Higher doses of fertilizer produced slight increases in crop yields, yet this was not statistically significant (P=0.07). The foliar N content of the sweet corn plants did not differ significantly between the

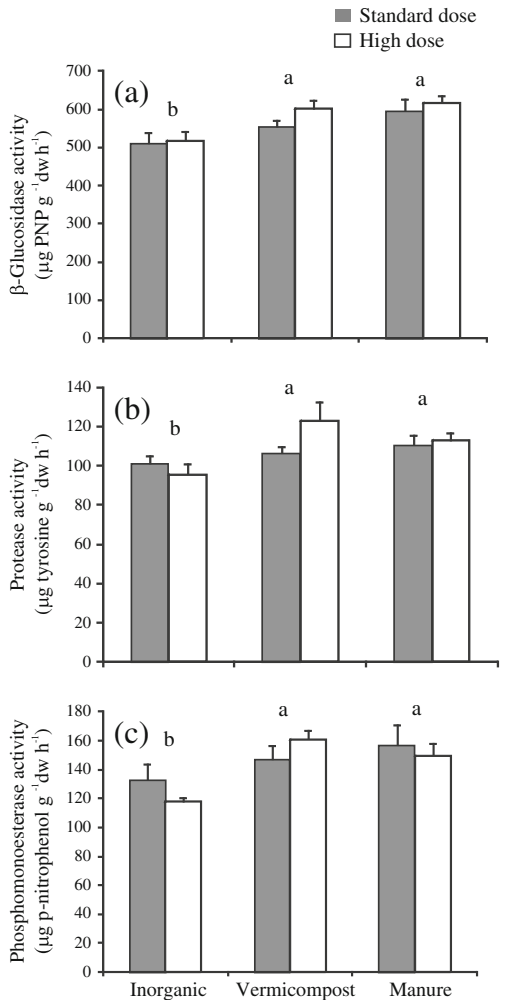

Fig. 4 β-Glucosidase (**a**), protease (**b**), and phosphomonoesterase (**c**) enzyme activity of soils amended with inorganic fertilizer, and integrated fertilizer regimes (including vermicompost and manure) at standard (*gray bars*) and high doses (*white bars*). Bars are means of the two doses assayed ± standard error. Different letters indicate significant differences at $P<0.05$. Posthoc comparisons have only been made between fertilizer regimes

different regimes or doses (Table 4) (fertilizer regime: $P=0.81$; dose: $P=0.56$). Nitrogen content on the grain was not different between the plants grown with the different fertilizers ($P=0.90$) but increased significantly from 25.9±0.4 gkg^{-1} (standard fertilizer dose) to 27.3±0.4 gkg^{-1} at the high dose ($P=0.04$). The foliar P content was higher in the plants grown with the integrated fertilizer regimes (Table 4) than in those grown with inorganic fertilizer ($P=0.02$). There were no significant differences in the foliar P content between the two doses assayed (dose: $P=0.20$). The different regimes or doses assayed did not produce significant differences in the foliar K content of the sweet corn plants (fertilizer regimes: $P=0.47$; dose: $P=0.72$).

Discussion

Results show that the partial replacement of inorganic by organic fertilizers has a significant short-term impact on the structure and function of the soil microbial community and on soil fertility. Surprisingly, the impacts of organic fertilizers were strong even when initial content of soil organic matter was high and organic fertilizers constituted a small portion of the total amount of fertilizer supplied to the soil (25 %). While the type of fertilizer regime used (inorganic, vermicompost or manure) had a strong influence on the soil microbial community structure and function, the effects of the dose of fertilizer were smaller and restricted to an increase in fungal biomass with the highest dose.

At harvest, soil microbial biomass, determined as total PLFA content, was relatively higher than previously reported figures for arable soils (2–12 μgg dw $soil^{-1}$) (Aciego Pietri and Brookes 2009; Dungait et al. 2011). In addition, microbial biomass was higher in the plots where organic fertilizers were incorporated than in those without organic fertilizers. Similar short-term increases in microbial biomass have been reported previously (Arancon et al.

Table 3 Effects of fertilizer treatments on soil biochemical properties and crop yield at harvest

	Standard N/P/K dose			High N/P/K dose		
	Inorganic	Vermicompost	Manure	Inorganic	Vermicompost	Manure
Total C (g kg dw^{-1})	30.6±0.13^{b}	31.5±0.13^{b}	33.8±0.27^{b}	33.1±0.18^{b}	39.3±0.22^{a}	31.3±0.15^{b}
DOC (g kg dw^{-1})	6.4±1.6	7.7±1.2	7.1±2.0	5.1±1.2	5.25±1.4	6.4±2.2
K_2O (g kg dw^{-1})	4.6±0.03	4.7±0.03	4.6±0.03	46.5±0.03	46.3±0.01	4.6±0.02
$N\text{-}NH_4^+$ (mg kg dw^{-1})	4.4±0.3	5.1±0.3	4.6±0.3	6.1±2	5.1±0.4	4.5±0.3
$N\text{-}NO_3^-$ (mg kg dw^{-1})	27.4±6.4	28.8±5.3	24.3±6.3	36.9±6.4	28.8±3.9	28.8±4.9
PO_4^- (mg kg dw^{-1})	161±4.7^{bc}	164±4.2^{b}	162±4.4^{bc}	152±2.5^{c}	177±2.4^{a}	166±4.0^{ab}
Crop Yield (kg ha^{-1})	1051±116	1075±115	1159±115	1284±118	1239±116	1276±119

Values represent least square means ± standard error. Different letters within the same row indicate significant differences at $P<0.05$

DOC dissolved organic C

Table 4 Effects of the fertilizer treatments on leaf NPK contents and grain N of sweet corn plants at crop harvest

	Inorganic	Vermicompost	Manure
N_{leaf} (g kg dw^{-1})	29.2±0.1	28.9±0.1	28.3±0.1
P_{leaf} (g kg dw^{-1})	2.4±0.1[b]	2.8±0.1[a]	2.9±0.1[a]
K_{leaf} (g kg dw^{-1})	16.7±1.7	18.1±1.7	18.5±1.7
N_{grain} (g kg dw^{-1})	26.7±0.5	26.7±0.5	26.5±0.5

Values represent least square means of the two doses assayed ± standard error. Different letters within the same row indicate significant differences at $P<0.05$

2006; Dinesh et al. 2010) and attributed to the supply of organic C substrates. The microbial community composition at the experimental site was dominated by bacteria, while fungal biomass was lower, as it is typical of intensively cultivated agricultural soils (Bardgett 2005). Bacteria were also the most sensitive microbial group to the different fertilizers; this seems reasonable, as bacteria have a much shorter turnover time than fungi and can react faster to the environmental changes in soil. Bacterial growth is often limited by the lack of readily available C substrates, even in soils with a high C/N ratio, and comprised the first group of microorganisms to assimilate most of the readily available organic substrates after they are added to the soil (Demoling et al. 2007; Kuzyakov 2010). Scarcity of labile C compounds in the soil would explain the bacterial proliferation after addition of manure and vermicompost. Both substrates promoted the growth of bacteria showing that the organic substrates added were used by the soil microorganisms not only for maintenance, but also for production of new biomass, which therefore reinforces the hypothesis of the C limitation on the soil (Demoling et al. 2007). Among bacteria, only Gram-negative were significantly influenced by the addition of organic fertilizers. Increased abundance of soil Gram-negative bacteria in relation to the use of organic fertilizers has previously been reported (Peacock 2001; Zhong et al. 2010). It has been observed that fast-growing Gram-negative bacteria proliferate soon after the addition of organic materials to the soil and decrease later, to the benefit of other more slowly growing microorganisms such as Gram-positive bacteria or fungi (Bossio and Scow 1998; Peacock 2001; Marschner et al. 2003; Esperschütz et al. 2007; Feng and Simpson 2009).

Fungal biomass in soil at harvest was low as compared to bacterial biomass and was not affected by the type of fertilizer used. Lower fungal biomass is typical of intensively cultivated agricultural soils and it has been attributed to different factors such as physical disturbance, and altered amount and complexity of the nutrient inputs and decrease in soil organic matter as compared to undisturbed soils. The absence of response in fungal growth and biomass to the different fertilizer treatments indicates that other factors, different from the fertilizer type, limited fungal growth. In fact, fungal biomass was slightly increased with the amount of fertilizer added to the soil. Yevdokimov et al. (2008) found that the addition of increasing amounts of N fertilizer increased soil fungal biomass significantly. A high sensitivity of fungi to other nutrients, such as N, than to C could therefore explain the increase in fungal biomass observed with the increase dose of fertilizer (Zhong et al. 2010). Alternatively, it is possible that fungi did not react as fast as bacteria to the addition of C substrates with the organic fertilizers. It has also been observed that bacterial proliferation after the addition of labile organic substrates had antagonistic effects on fungal growth (Meidute et al. 2008) which could explain the absence of response to organic fertilizers in our experiment. Therefore the surplus of organic C provided with the organic fertilizers not only increased the soil microbial biomass but also selected for specific microbial groups (i.e., Gram-negative bacteria), thus altering the composition of the soil microbial community, as confirmed by the DA of the whole PLFA profile of the soil samples.

Manure had a stronger effect than vermicompost on viable microbial biomass, bacterial growth and Gram-negative bacteria. This is presumably related to the higher input of labile organic matter and readily available organic compounds via manure (i.e., higher DOC content) than via vermicompost, since the type of C input has been found to be the main factor determining the predominant type of microorganisms in soil (Meidute et al. 2008). Processing of manure by earthworms during vermicomposting produces strong mineralization and promotes humification of the organic substrates resulting in a lower content of readily metabolizable compounds in vermicompost than in manure (Domínguez et al. 2010). Therefore, the higher presence of readily metabolizable C in manure appears to be responsible for the stronger short-term effects on the structure of the soil microbial community, whereas the effects of the more resistant C forms in vermicompost may be more important for long-term positive effects on soil processes (Bastida et al. 2008). In fact, the higher C content observed at harvest in the vermicompost-treated soils indicates that the amount of C lost was lower than that of added C, and could therefore indicate the presence of more resistant forms of C in vermicompost than in manure.

The relationship between soil microbial community structure and function is not straightforward due to the complexity of the soil system and the existence of several microbial groups that carry out similar functions (Nannipieri et al. 2003). Consequently, previous studies did not find a direct relation between changes in the composition and the function of the soil microbial community, even in long-term experiments (Marschner et al. 2003). Nevertheless, in our experiment the observed changes in the structure of the soil microbial community with organic fertilizer treatments were

also accompanied by changes on the function of the microbial community. Manure-amended soils exhibited higher microbial activity than the inorganic fertilizer treatment at harvest time. Similar to microbial biomass, microbial activity is a reliable indicator of the amount of easily decomposable organic C, and it has shown to be significantly higher in fresh than in humified organic materials (Monaco et al. 2008). Both manure and vermicompost increased the potential activity of the soil enzymes that degrade organic C, N and P compounds between 12 % and 22 % as compared to soils amended with inorganic fertilizers. Similar increases in enzyme activities (19–38 %) have been reported by Dinesh et al. (2010) in a short-term study comparing inorganic with integrated fertilizer regimes. Enzyme activity typically increases shortly after the addition of organic amendments to the soil (Gianfreda and Ruggiero 2006). Soil enzyme activity is known to be positively correlated with the organic matter content of the soil, and with the water soluble soil organic C (Chang et al. 2007; Gilani and Bahmanyar 2008). Soil microbial and enzyme activity are considered as indicators of soil quality as they are responsible for the degradation of organic substrates and release of plant nutrients (Gil-Sotres et al. 2005).

Presumably, the higher enzyme activity in the soils where manure and vermicompost were added contributed to maintain the availability of inorganic N and P in the soil as compared to inorganic fertilizers. Interestingly, although the same amount of P was added with the different types of fertilizers, soils in which high doses of vermicompost and manure were added had a higher concentration of available P at harvest time. Furthermore, the foliar P content in plants grown with vermicompost and manure was significantly higher than in plants grown in inorganically fertilized soils, thus suggesting greater P mineralization in soils and higher plant P uptake with these fertilizers. This is consistent with the higher phosphomonoesterase activity observed in the fertilizer regimes that included manure and vermicompost. Similar increases in soil phosphomonoesterase activity in response to manure and vermicompost treatments were observed by Saha et al. (2008) in a 3-year field study, even if the organic fertilizers were supplied at lower rates than inorganic nutrients. It has been observed that the mixed input of organic and inorganic substrates increases the synthesis of soil hydrolytic enzymes (Guo et al. 2010). Nannipieri (1994) reported that soil phosphomonoesterase activity increased with the addition of C and N sources to the soil rather than inorganic P. Further, phosphomonoesterase activity seemed to be repressed in some cases by high soil P concentrations, yet the relationship between both is not simple (Nannipieri 1994). Phosphomonoesterase activity is highly correlated with soil microbial biomass (Nannipieri et al. 1983; Saha et al. 2008) and in turn, the provision of organic C and stimulation of microbial growth under the combined treatments could have promoted the synthesis of phosphatase enzymes. Therefore, the application of organic fertilizers did not result in the immobilization of plant available nutrients but increased nutrient turnover through both increased microbial biomass and activity. Altogether, the changes in microbial community function with organic fertilizers seemed to be enough to maintain and even increase the uptake of plant nutrients by the sweet corn crop as compared to inorganic fertilization.

Changes in the composition, and particularly the function, of soil microbial community due to agricultural management practices can have large impacts on crop health and productivity (Bossio et al. 1998; Chaparro et al. 2012; Franco-Otero et al. 2012). Even though organic fertilizers have been traditionally used as sources of plant nutrients, the understanding of their role on plant growth and soil fertility has not always been well understood (Manlay et al. 2007). The results of the present study show that partial replacement of inorganic fertilizers by vermicompost or manure had a significant short-term impact on the soil microbial community, which was directly related to an increase in P uptake of the sweet corn crop. The supply of a fertilizer with a more varied chemical composition than the simple inorganic fertilizers, and that included C compounds of different complexity seemed to be responsible for the observed effects on soil microbial community structure, function and crop yield. Beneficial effects occurred even when the organic amendments were only a small portion of the total amount of fertilizers supplied to the soil and in 3 months, suggesting that a shift to more sustainable production systems, could significantly improve soil fertility in just one growing season while maintaining crop yield at levels comparable of those of inorganically fertilized corn.

Future research should be directed to increase the understanding of the impacts of organic fertilizers on soil microbial processes and nutrient cycling and disentangling the influence of different factors such as crop species, soil type, and compost properties, in order to increase crop yields under sustainable production systems. The combination of modern microbiological techniques with the knowledge of soil ecological processes would provide a unique opportunity to improve agronomical practices. The goal is to use and optimize the biological resources already existent in the soil and optimize fertilizer management to maximize yields while reducing environmental impacts.

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