The earthworm species Metaphire posthuma modulates the effect of organic amendments (compost vs. vermicompost from buffalo manure) on soil microbial properties. A laboratory experiment

Thuy Thu Doan, My Dung Jusselme, Jean-Christophe Lata, Bo van Nguyen, Pascal Jouquet

Doan Thu et al. published in *European Journal of Soil Biology*

The earthworm species *Metaphire posthuma* modulates the effect of organic amendments (compost vs. vermicompost from buffalo manure) on soil microbial properties. A laboratory experiment.

Thuy Doan Thu [a,b], Dung My Jusselme [c], Jean-Christophe Lata [d], Bo Van Nguyen [e], Pascal Jouquet [b,*]

[a] *Soil and Fertilizer Institute, Dong Ngac, Tu Liem, Ha Noi, Vietnam*

[b] *IRD - BIOEMCO, UMR 211, Bondy, France, UE*

[c] *CNRS – ECOBIO UMR 6553, Rennes I Université, Rennes Cedex, France, UE*

[d] *UPMC - BIOEMCO, UMR 7618, ENS, Paris, France, UE*

[e] *Vietnamese Academy of Agricultural Science (VAAS), Ha Noi, Viet Nam*

Corresponding author.

e-mail : pascal.jouquet@ird.fr (Pascal Jouquet)

ABSTRACT

The aim of this study was to determine the influence of compost and vermicompost produced from buffalo manure on soil bacterial diversity and activity in the presence and absence of the endogeic earthworm *Metaphire posthuma*. This experiment was carried out for 15 months with a maize-tomato-maize cycle under greenhouse conditions in Northern Vietnam. It showed a positive influence of compost and vermicompost on soil microbial properties, with higher cultivable bacteria, higher bacterial and catabolic diversity (Shannon diversity 'H' and Richness 'S') indices and higher enzymatic activities than control soils which only received mineral fertilizers. Differences also occurred between compost and vermicompost with lower activity and diversity in the soil amended with vermicompost, probably because of its higher molecular stability. The presence of *M. posthuma* led to divergent dynamics of bacterial community in soils amended with compost and vermicompost. Earthworms negatively influenced soil microbial properties in composted soil (lower Average Well Color Development 'AWCD'), probably because of competition between bacteria and earthworms for organic resources and/or because of the consumption of microbes by earthworms. Conversely, the presence of earthworms increased bacterial diversity and activity with higher AWCD, and H and S indices for the vermicompost treatment, probably as a result of a stimulation of microorganisms that allow the degradation of stable organic matter and its further consumption by earthworms. In conclusion, this study clearly confirmed the different impacts of compost and vermicompost on bacterial activity and diversity and highlighted the importance considering the interaction of these organic substrates with local endogeic earthworms.

Keywords

Biolog analysis; DGGE; Enzymatic activities; *Metaphire posthuma*; organic fertilization; Tropical soil.

1. Introduction

Increasing soil organic matter (SOM) stocks and stability by addition of organic amendment offers a good way to substantially improve soil quality and therefore agricultural sustainability [17, 28, 45]. Most of the studies aimed at comparing the influence of animal waste amendment on soil quality have been done using swine, poultry, cow or horse manure as original substrates [6, 51]. However, comparatively less research had been focused on buffalo manure, although it is often the main source of organic amendment for many farmers in Asia, especially in Vietnam [62] where it is usually applied on the fields several times per year as compost to improve soil fertility [18, 49, 50].

Understanding microbial and biochemical processes in soils are important for the management of farming systems [54]. The utilization of organic amendment usually leads to important modifications of both soil microbiological and biochemical properties [31, 42]. Several studies have indeed pointed out the higher microbial biomass and diversity in soils amended with compost [13]. Microbial respiration, enzymatic activities such as dehydrogenase and phosphatase activities, and N mineralization rates are also usually enhanced after the amendment of organic substrates [26, 41, 44].

Soil macrofauna has also been considered as an important component of soil quality [53]. Amongst soil organisms, earthworms are considered as key soil engineers due to their influences on soil structural properties [32, 39]. As a consequence, earthworms regulate microbial populations with the elimination of some microorganisms and the proliferation of others [14, 19, 43, 47]. Earthworms have also

been used for the management of organic wastes and the production of high-quality compost called 'vermicompost' [21]. Vermicompost is especially interesting as an organic fertilizer because of its high contents of plant available nutrients (N, P, K) [20, 21, 34]. The formation of vermicompost, which involves the activity of epigeic earthworms in controlled environments, appears to be a useful indirect avenue for improving physico-chemical properties of soils [21, 29]. Several studies also pointed out the specific structure and higher activity of bacterial communities in vermicompost [2, 51, 56, 63, 64, 65].

Recent studies have compared the influence of compost and vermicompost from buffalo manure and purely chemical fertilizers on tropical soil quality and plant growth [4, 18, 33, 34, 49, 50]. These studies suggest that the application of composted and vermicomposted buffalo manure reduces significantly the amount of chemical fertilizers needed, as well as improving soil quality and reducing off-site effects such as nutrient leaching and water pollution. Recently, Amossé et al. [4] and Doan et al. [18] also showed that endogeic earthworms are more active when compost is used as fertilizer in comparison with vermicompost with a better development of earthworm abundance and biomass and cast production but variable effects on plant growth. Therefore, the aim of this study was to determine the influence of the interaction between organic fertilization (compost vs. vermicompost produced from buffalo manure) and endogeic earthworm activity on soil microbial diversity, activity and some associated biochemical properties. We used soil samples from the experiment carried out by Doan et al. [18] to test our hypotheses: (1) that organic fertilization should increase microbial activities and diversity but that this effect should be more

pronounced when vermicompost was used, and (2) that these effects should be increased in the presence of endogeic earthworms.

2. Materials and Methods

2.1. Soil properties and organic amendments

The soil was sampled in the 0-10 cm layer of a fallow in the red river delta (Dong Ngac commune, Ha Noi, Vietnam, 21°5'28"N, 105°47'2"E), air-dried and sieved at 2 mm to discard stones and litter residues. This soil is described as Eutric Fluvial [23, 30] with low organic carbon (OC = 9.7 mg g^{-1}) and nutrient content (total nitrogen = 0.9 mg g^{-1}; total phosphorus, P_2O_5 = 1.8 mg g^{-1}; available phosphorous = 0.46 mg g^{-1}; and total potassium, K_2O = 1.1 mg g^{-1}). Soil was alkaline (pH_{H2O} = 8.0, pH_{KCl} = 7.7) and mainly sandy (61.0% sand, 28.7% silt and 10.3% clay, mainly kaolinite). Compost and vermicompost were produced in a farm in Dong Cao village from domestic buffalo manure after 3 months of maturation in two different and separated units, as reported by Jouquet et al. [33]. The procedure adopted was based on local farmer's knowledge. Briefly, buffalo manure was placed in 500 L bags and covered by a lid to prevent evapotranspiration and moisture addition due to rainfall, thereby conserving compost humidity and preventing anaerobic conditions due to rainwater addition. Every 1–2 weeks the compost was mixed thereby increasing aeration and favouring aerobic conditions. Vermicomposting was carried out in a similar way, the only difference being the addition of earthworms. The compost was significantly more alkaline and with higher C but lower N contents than the vermicompost substrate (pH_{H2O} = 8.5 (SE: 0.2) and 7.5 (SE: 0.1), OC = 178 (SE: 3) and 164 mg g^{-1} (SE: 8), and N = 14 (SE: 1)

and 16 mg g^{-1} (SE: 1) respectively for compost and vermicompost). Concentration in P and K were similar between compost and vermicompost with 14 (SE: 2) and 11 mg g^{-1} (SE: 1), respectively for total P and K. More information on the chemical properties of these substrates and those of the buffalo manure that was used to produce compost and vermicompost can be found in Ngo et al. [50]. The earthworm species used to produce vermicompost was *Eisenia andrei*.

2.2. Greenhouse experiment

The experiment was carried out during 15 months in a greenhouse at the Soil and Fertilizer Research Institute (SFRI), in Hanoi, Vietnam. The air humidity was always high, between 75 and 100% and the average daily temperature varied from 15 to 25°C during the year. The experiment was set up in 10 L baked clay pots filled with 5 kg of soil mixed thoroughly with compost, vermicompost, or the equivalent amount of inorganic nutrients, applied as mineral fertilizers (n = 3 replicates). These amendments were repeated after each vegetation period, in total 3 times during the experiment. The amount of compost and vermicompost was calculated to be equivalent to applying 20 t ha^{-1} (dry weight) of organic substrate (90.5 g pot^{-1}). Mineral fertilizers were urea (CH_4N_2O, %N = 46.3%), potash (K_2O, %K = 60%) and phosphate (P_2O_5, %P = 16%). The equivalent amount of N, P and K found in compost and vermicompost was applied in the control soil as mineral fertilizers. When necessary, additional nutrients were also applied to the compost and vermicompost substrates (more information on the experiment is available in Doan et al. [18]). This protocol allowed us to compare the influence of substrates which had exactly the same N, P and K content. Thereafter we

planted common agricultural crops: maize (June to September 2008, variety LNS 222), tomato (November 2008 to April 2009, variety HT14), and maize again (June to September 2009, variety LNS 222). For the three dates, only one plant was grown per pot. The influence of the treatments on plant growth and yield, and SOM quality can be found in Doan et al. [18] and Ngo et al. [50], respectively. Briefly, this study showed that plant yield was always higher for the mineral treatment and that the beneficial positive effect of vermicompost decreased over time. Overall plant yields were always the highest for the mineral and lowest for compost treatments. Although the application of vermicompost led to a similar yield as the control treatment for the first maize planting, its beneficial influence decreased during the experiment until its effect was similar to that of the compost treatment.

The influence of earthworms on soil microbial properties was investigated by adding two adults of the endogeic *Metaphire posthuma* per pot (n = 3 replicates) before each cultivation (before the 2 maize and tomato plantings), then reaching a density close to the one observed in the field. This species was found in the garden of the SFRI institute. *Metaphire posthuma* is a medium size endogeic geophagous earthworm ~10 cm in length and 5 mm in diameter on average at the adult stage which produces approximately five to 10 fold its own weight in casts per day [11, 34, 35, 36]. At the end of the experiment, the number of earthworms reached 0, 1.2 (±0.2) and 3.5 (±0.8), in the mineral, vermicompost and control treatments respectively [18]. Earthworms only had an impact on plant growth and yield in the compost treatment. The effect of earthworms was initially null (i.e. for the first maize crop), positive (i.e. for tomato planting) and finally negative (i.e. for the second maize planting).

In total, 18 pots were set up (3 fertilization treatments x 2 earthworm treatments x 3 replicates). Soil samples were collected at the end of the experiment.

2.3. Soil bacterial diversity

Total DNA samples were extracted from 1 g of dry soil from three randomly selected soil cores for each plot. The DNA extracts were purified using a Spin Column S-400 HR (Amersham) and quantified with a spectrophotometer at 260 and 280 nm (NanoDrop® ND-1000 UV-Vis Spectrophotometer).

Amplifications were attempted using universal primers 338F/518R [38, 48] with a GC-clamp [55] attached to the 5' extremity to the forward primer for DGGE optimization [48]. Taq polymerase Ready-To-Go (Amersham Pharmacia, USA) was added to 0.25 µM of each primer and 50 ng of template DNA. Amplifications were performed in 25 µl reaction mixtures with a thermo cycler (Eppendorf). PCR cycles were: initial denaturation 95°C for 5 min, and 30 cycles of denaturation 94°C for 1 min, annealing 65°C for 1 min and elongation 72°C for 3 min, and a final extension step at 72°C for 10 min. After amplification, PCR products were applied to 8% (w/v) polyacrylamide gels, except that denaturing gradient was made from 40 to 70% (100% denaturant contains 7M urea and 40% formamide). The electrophoresis was run for 16 h at 60°C and 75 V in a DcodeTM Universal Mutation Detection System (Biorad, USA) containing 1 x TAE buffer. The gels were after stained with ethidium bromide for 15 min, washed with MilliQ water and visualized by UV transillumination with the GelDoc 2000 system (BioRad, Richmond, California, USA). Scanned images of the DGGE gels were analyzed with TotalLab Quant software (Nonlinear Dynamics Ltd.,

Newcastle upon Tyne, UK) and the density and mobility of the bands (OTU: Operation Taxonomy Units) were calculated.

2.4. Sole carbon source utilization profile

Bacterial cells were extracted from soil for inoculation in Ecoplates™ (AES Laboratory France). The Ecoplates™ contain 3 replicates of 31 different environmentally relevant carbon sources and one control well per replicate [15]. A volume of 150 µl supernatant was inoculated in each well, and the plates were incubated in darkness on a shaker (150 rpm) at 25°C for 7 days. The color development was measured as absorbance at 595 nm using a Microplate spectrophotometer (EL800-PC, Biotek Instruments, Winooski, USA) every 24h for one week. All kinetic curves suggested that after 72h incubation time, the wells with the most active microbial communities reached the asymptote of color development. Therefore, this point was considered as the optimal incubation time for further statistical analyses. Data were collected using Microlog 4.01 software. They were normalized by average well color development (AWCD), as described by Garland and Mills [25]: AWCD = $\sum$ (C-R)/n, where C is color production within each well, R is the value of the no-substrate control well of each plate, and n is the number of substrates (n = 31). The sole carbon source utilization profiles were determined on three replicate samples of each soil.

2.5. Enzyme activity

Enzyme activities were used as a broad-spectrum indicator of soil biological activity [46]. Enzyme activity measurements were made on samples with 75% of their water

holding capacity and incubated at 25°C for 48 h to reactivate the microflora. These assays were carried out in triplicate to measure activities. All enzyme activities were determined at optimum conditions of pH, temperature and substrate concentration in order to get an assessment of their maximum potential activity in soil. *N*-acetyl-glucosamidase, *β*-Glucosidase, amylase and xylanase activities were determined according to Mora et al. [46]. Urease activity was determined according to Tabatabai [58] and alkaline and acid phosphatase activities were measured following Kandeler et al. [37]. These enzymes were chosen based on their importance in the nutrient cycles of C (β-glucosidase, amylase and xylanase), N (urease and *N*-acetyl-glucosamidase) and P (alkaline and acid phosphatase).

2.6. Statistical analyses

Data from DGGE analysis and catabolic potential using Biolog EcoPlate™ were used to evaluate the structural and functional diversity of bacterial community as suggested by Eicherner et al. [22] and Zak et al. [66], respectively for the DGGE and Biolog analyses. The Shannon indices (H) were obtained as follows: $H = - \sum P_i \ln (P_i)$, where $P_i = n_i / N$, and n_i corresponded to the height of a DGGE band peak, or OTU (Operation Taxonomy Unit), and N the sum of all the peak heights. For the Biolog analysis, P_i corresponded to the ratio of the consumption of a particular substrate to the sums of consumption of all substrates after 72 h of incubation. Richness indices (S) were either the number of OTUs detected from each lane or the number of wells with a corrected absorbance greater than 0.25 in Biolog EcoPlate™.

Results were analysed by analysis of variance (two-way ANOVA) with treatments (i.e., type of fertilizer and presence or absence of earthworms) as independent variables. Comparisons between means were tested with LSD test. A Principal Component Analysis (PCA) was carried out to describe enzyme activity analyses. All statistical calculations were carried out using R [51]. Differences among treatments were declared significant at the <0.05 probability level.

3. Results

3.1. Genetic structure of bacterial community

A dendrogram of similarity showed a similarity of 64% between the initial soil substrate and those sampled after 15 months of cultivation (Figure 1). The percentage of similarity was also very high between organically and chemically fertilized soils (77% of similarity) on one hand and composted and vermicomposted soils on the other hand (85% of similarity). Earthworms had no effect on the structure of bacterial communities in soils amended with mineral fertilization (95% of similarity) and their effects were low in soils amended with vermicompost and compost (90% and 85% of similarity, respectively).

The influence of the different treatments on the genetic diversity of bacterial populations is shown in Table 1a. Composted soils were characterized by highest H and S values and mineral soils by lowest values. The utilization of vermicompost led to intermediate results. Earthworms had no influence on the bacterial community structure, except for the vermicompost treatment where they increased H and S indices in soil.

3.2. Functional diversity by Biolog analysis and enzyme activity

Microbial metabolic activity was assessed through the measurement of AWCD in Biolog EcoPlate™ (Figure 2), and through substrate H and S indices (Table1b). The amendment of compost and vermicompost led to increase AWCD, H and S with higher values for composted soils when compared to vermicomposted and mineral fertilized soils with and without earthworms. The inoculation of earthworms did not influence these indices for the mineral treatment. Conversely, an interaction between organic fertilization and earthworm presence was observed for the compost and vermicompost treatments. Earthworms increased AWCD, H and S indices for vermicompost treatment but the opposite effect was observed for the compost treatment.

A principal component analysis was carried out from the enzyme metabolic properties of soils (Figure 3, details are shown in Table 2). Treatments were mainly separated along the first axis which explained 68.3% of the total variability. Chemical fertilization led to lowest enzymatic activities, with no influence of earthworms. The amendment of vermicompost led to higher xylanase, amylase and β-Glucosidase activities and lower N-acetyl-glucosamidase and alkaline phosphatase activities than for the compost treatment. The presence of earthworms entailed a reduction in xylanase, β-glucosidase and alkaline phosphatase for the vermicompost treatment. Conversely, the presence of earthworms had negative effect on N-acetyl-glucosamidase, alkaline and acid phosphatase, and urease activities for the compost treatment.

4. Discussion

4.1. Effect of organic amendments on soil microbial properties

Maintenance of SOM is important for the long-term productivity of agroecosystems. The amendment of organic substrates, such as crop residues and compost, aims to counteract the progressive loss of OM in agricultural soils [44, 59]. As a consequence, soil microbiological properties are usually improved after the amendment of OM [8, 24, 67, 57]. Our study confirmed this general trend and showed the positive influence of buffalo manure amendment, either in form of compost or vermicompost, on soil microbial properties. Indeed, our study showed that soils amended with compost and vermicompost were characterized by higher bacterial and catabolic diversity (H and S) indices and higher enzymatic activities than control soils which only received mineral fertilizers.

Organic amendments are sources of available energy for soil microorganisms [27]. However, the quality of the organic amendments is of major importance in the regulation of microbiological properties. Several studies related the quality and stability of composted and vermicomposted organic substrates to their effects on microbiological properties [1, 7, 56, 64]. These studies indicate that the bacterial community structure in vermicompost considerably differs from that in analogous composts. Vermicomposts are usually more stable than composts, with higher availability of nutrients and improved microbiological properties [5, 51, 61, 63]. In our study, vermicompost was characterized by lower C and higher N content in comparison with the compost substrate (see Material and Method). This suggests that vermicomposting led to higher microbial activity and higher rate of OM decomposition

than composting. After 15 months of experiment, soils amended with vermicompost were characterized lower metabolic activity (Figure 3) and lower genetic and functional diversity and richness (Table 1). Therefore, these results do not confirm the general assumption that vermicompost enhances microbial and enzymatic activities in soils [51, 63]. They are however in line with those of Aira and Dominguez [3] who found higher microbial biomass but lower microbial activity in vermicompost in comparison with compost. As suggested by Ngo et al. [49], the epigeic *E. andrei* accelerates the decomposition of the buffalo manure during vermicomposting probably through an enhancement of the microbial activity. The end-product is a more decomposed and stabilized organic substrate with lower forms of C and more N available to microorganisms. As a consequence, microbial populations were more active and diversified in the soil amended with compost, as shown by higher AWCD, and H and S indices which reflects the oxidative capacity of soil microorganisms, but also H and S indices analyzed by DGGE which represent the number and diversity of bacterial community.

4.2. Earthworms modulate the effect of organic fertilizers on soil microbial properties

Despite the high diversity of tropical earthworms, only a small number of species have been intensively studied in relation to soil processes and ecosystem functioning. The fast turnover of OM in tropical environments and the low amount of litter and fresh OM debris in tropical agro-ecosystems lead to a predominance of endogeic earthworm species [40]. As endogeic earthworms are known to affect soil physical, chemical and microbial properties [40], we hypothesized that endogeic earthworm activity can

modulate the effect of organic fertilizers on soil microbial properties. Whether the positive influence of compost on soil microbial properties is maintained in the presence of endogeic earthworms remains however unexplored. As shown with the dendrogram of similarity, the bacterial community structure was mainly determined by the fertilization treatment (mineral, compost vs. vermicompost amendment), since its effect exceeded the effect of earthworms. From the same experiment, Doan et al. [18] showed that *M. posthuma* development was the highest in presence of compost, low in presence of vermicompost while earthworms did not survive in the mineral treatment. These results can easily be explained by the higher availability of C and N in compost than in vermicompost, and by the absence of easily available C resources in the mineral treatment [50]. These results also confirm the study of the Bisht et al. [10] who showed that *M. posthuma* develops very well in presence of cow dung and they suggest that this species is belonging to the polyhumic endogeic functional group [18]. Consequently, *M. posthuma* did not influence soil microbiological properties in the mineral treatment but it led to significant changes in compost and vermicompost treatments. Interestingly, its effects in compost treatment were very different, if not the opposite, from those observed for the vermicompost treatment. Indeed, although the diversity of bacteria was not influenced by *M. posthuma* in the compost treatments, significant increases in CFU and H and S diversity indices were observed for vermicompost treatment. Earthworms also differently influenced catabolic activities with higher AWCD and H and S diversity indices for vermicompost and lower AWCD and H diversity index in the compost treatment. Although increased enzyme activities are usually observed in presence of earthworms [56], our study showed decreasing soil enzyme activities for

both compost and vermicompost treatments. However, all the enzyme activities were not similarly affected, therefore reflecting an interaction between the nature of the organic amendment and *M. posthuma*. Despite a high degree of DGGE band similarity (OTU) between treatments, changes in the activity of enzymes of C, N and P cycles reflect the activity of specific microbial populations [56] and support the hypothesis that *M. posthuma* led to divergent dynamics of bacterial communities in soils amended with compost and vermicompost. These findings could partially be explained by the nature of the organic substrates. The negative effect of earthworms on soil microbial properties in composted soil could result from a competition between bacteria and earthworms for organic resources and/or from the consumption of microbes by earthworms [60]. Conversely, the higher metabolic activity of microbes in vermicomposted soil suggests that earthworm activity led to a stimulation of microbes in the presence of vermicompost. Litter and OM decomposition rates are often stimulated in experiments comparing soils with and without earthworms [12]. It is therefore likely that earthworms stimulate microbial activity through the fragmentation and alteration of vermicompost in their gut and/or through the production of more easily degradable organic substances (i.e., the mucus) leading to priming effect processes [9]. These results can be seen in line with the niche construction, or feedback loop hypothesis applied to soil engineers [32]. The stimulation of bacterial activity in vermicomposted soil led to increased palatability of the OM from highly polymerised organic substances to more simple molecules and/or microbial biomass which can afterwards be consumed by earthworms [16]. This hypothesis is supported by the fact that earthworm development was difficult at the beginning of the experiment in

vermicomposted soil, probably because of the low palatability of vermicompost for earthworms, but increased over time [18].

The presence of endogeic earthworms in soil is more a generality than an exception. This study therefore highlights the importance of the quality of organic resources applied as amendments and the importance of endogeic earthworms in evaluating the relevance of compost and vermicompost in the field.

Acknowledgment

This project was financially supported by CNRS (SystéMO project) and IRD (unit research UMR-211-BIOEMCO) French institutes. We acknowledge with gratitude Dr. Tran Duc Toan and the SFRI (Soils and Fertilizers Research Institute) Vietnamese institute for providing access to the greenhouse and laboratories and to Emma Rochelle-Newalle, Corinne Rouland-Lefevre and Didier Orange for valuable discussions.

References

[1] M. Aira, F. Monroy, J. Domínguez, *Eisenia fetida* (Oligochaeta: Lumbricidae) modifies the structure and physiological capabilities of microbial communities improving carbon mineralization during vermicomposting of pig manure. Microb. Ecol. 54 (2007) 662–671.

[2] M. Aira, J. Domínguez, Optimizing vermicomposting of animal wastes: Effects of rate of manure application on carbon loss and microbial stabilization. Environ. Manage. 88 (2008) 1525-1529.

[3] M. Aira, J. Domíınguez, Microbial and nutrient stabilization of two animal manures after the transit through the gut of the earthworm *Eisenia fetida* (Savigny, 1826). Hazardous Materials 161 (2009) 1234–1238.

[4] J. Amossé, Y. Bettarel, C. Bouvier, T. Bouvier, T.D. Tran, T.T. Doan, P. Jouquet, The flows of nitrogen, bacteria and viruses from the soil to water compartments are influenced by earthworm activity and organic fertilization (compost vs. vermicompost). Soil Biol. Biochem. (2013) in press.

[5] A. Anastasi, G.C. Varese, V.F. Marchisio, Isolation and identification of fungal communities in compost and vermicompost. Mycologia 97 (2005) 33–44.

[6] N.Q. Arancon, C.A. Edwards, R. Dick, L. Dick, Vermicompost tea production and plant growth impacts. Bio Cycle (2007) 51–52.

[7] R.M. Atiyeh, C.A. Edwards, S. Subler, J.D. Metzger, Pig manure vermicomposts as a component of a horticultural bedding plant medium: effects on

physicochemical properties and plant growth. Biores. Technol. 78 (2001) 11–20.

[8] KL Bailey, G. Lazarovits G, Suppressing soil-borne diseases with residue management and organic amendments. Soil Till. Res. 72 (2003) 169-180.

[9] L. Bernard, L. Chapuis-Lardy, T. Razafimbelo, M. Razafindrakoto, E.L. Pablo, E. Legname, J. Poulain, T. Brüls, M; O'Donohue, A. Brauman, J.L. Chotte, E. Blanchart, Endogeic earthworms shape bacterial functional communities and affect organic matter mineralization in a tropical soil. The ISME J. 6 (2012) 213–222.

[10] R. Bisht, H. Pandey, D. Bharti, S.P.S. Bisht, B.R. Kaushal, Reproductive potential of the earthworm *Metaphire posthuma* (Oligochaeta) in different food substrate. Trop. Ecol. 48 (2007) 107-114.

[11] N. Bottinelli, T. Henry-des-Tureaux, V. Hallaire, Y. Benard, J. Mathieu, T.T. Tran, P. Jouquet, How earthworms accelerate soil porosity under watering events. Geoderma 156 (2010) 43–47.

[12] G. Brown, B. Pashanasi, C. Villenave, J.C. Patron, B.K. Senapati, S. Giri, I. Barois, P. Lavelle, E. Blanchart, R.J. Blakemore, A.V. Spain, J. Boyer, Effects of earthworms on plant production in the tropics, in: Lavelle, P., Brussaard, L., Hendrix, P., (Eds). Earthworm Management in Tropical Agroecosystems, Wallingford, 1999, 87-137.

[13] L.R. Bulluck, M. Brosius, G.K. Evanylo, J.B. Ristaino, Organic and synthetic fertility amendments influence soil microbial, physical and chemical properties on organic and conventional farms. Appl. Soil Ecol. 19 (2002) 147–160.

[14] B.A. Byzov, N.V. Khomyakov, S.A. Kharin, A.V. Kurakov, Fate of soil bacteria and fungi in the gut of earthworms. Eur. J. Soil Biol. 43 (2007) 149-156.

[15] K.H. Choi, F.C. Dobbs, Comparison of two kinds of Biolog microplates (GN and ECO) in their ability to distinguish among aquatic microbial communities. J. Microbiol. Methods 36 (1999) 203– 213.

[16] J.P. Curry, O. Schmidt, The feeding ecology of earthworms - A review. Pedobiol 50 (2007) 463–477.

[17] M. Diacono, F. Montemurro, Long-term effects of organic amendments on soil fertility. A review Agro. Sustain. Dev. 30 (2010) 401-422.

[18] T.T. Doan, T.P. Ngo, C. Rumpel, V.B. Nguyen, P. Jouquet, Interaction between compost, vermicompost and earthworm activity. A one year greenhouse experiment. Sci. Hort. 160 (2012) 148-154.

[19] H.L. Drake, M.A. Horn, As the worm turns: the earthworm gut as a transient habitat for soil microbial biomes. Ann. Rev. Microbiol. 61 (2007) 169–189.

[20] C.A. Edwards, N.Q. Arancon, The use of earthworms in the breakdown of organic wastes to produce vermicomposts and animal feed protein, in: Edwards, C.A. (Ed.), Earthworm Ecology. CRC Press, Boca Raton, FL, 2004, 345–438.

[21] C.A. Edwards, J. Dominguez, Q.A. Norman, The influence of vermicomposts on plant growth and pest incidence, in: Shakir, S.H., Mikhail, W.Z.A. (Eds.), Soil Zoology for Sustainable Development in the 21st Century. Self-Publisher. Cairo, 2004, 397–420.

[22] C.A. Eicherner, R.W. Erb, K.N. Timmis, I. Wagner-Dobler, Thermal gradient gel electrophoresis analysis of bioprotection from pollutant shock in the activated sludge microbial community. Appl. Environ. Microbiol. 65 (1999) 102-109.

[23] FAO. Soil map of the world revised legend. World Soil Resources Rept. 60, FAO, Rome 1988.

[24] R. García-Ruiz, V. Ochoa, M.B. Hinojosa, J.A. Carreira, Suitability of enzyme activities for the monitoring of soil quality improvement in organic agricultural systems. Soil Biol. Biochem. 40 (2008) 2137-2145.

[25] J.L. Garland, A.L. Mills, Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level-sole carbon-source-utilization. Appl. Environ. Microb. 57 (1991) 2351–2359.

[26] F. Gil-Sotres, C. Trasar-Cepeda, M.C. Leirós, S. Seoane, Different approaches to evaluating soil quality using biochemical properties. Soil Biol. Biochem. 37 (2005) 877–887.

[27] E. Gomez, L. Ferreras, S. Toresani, Soil bacterial functional diversity as influenced by organic amendment application. Biores. Technol. 97 (2006) 1484–1489.

[28] T. Gomiero, M.G. Paoletti, D. Pimentel, Energy and environmental issues in organic and conventional agriculture. Critical Rev. Plant Sci. 27 (2008) 239-254.

[29] M. Gonzalez, E. Gomez, R. Comese, M. Quesada, M. Conti, Influence of organic amendments on soil quality potential indicators in an urban horticultural system. Biores. Technol. 101 (2010) 8897-8901.

[30] S. Haruyama, H.T. Dinh, V.T. Le, Problem Soils of the Paddy Fields in the Eastern Coastal Plain of the Red River Delta, VietNam. Journal of Geography 115 (2006) 737-749.

[31] S. Hueso, C. García, T. Hernández, Severe drought conditions modify the microbial community structure, size and activity in amended and unamended soils. Soil Biol. Biochem. 50 (2012) 167–173.

[32] P. Jouquet, J. Dauber, J. Lagerlöf, P. Lavelle, M. Lepage, Soil invertebrates as ecosystem engineers: intended and accidental effects on soil and feedback loops. Appl. Soil Ecol. 32 (2006) 153–164.

[33] P. Jouquet, T. Plumere, T.D. Thu, C. Rumpel, T.T. Duc, D. Orange, The rehabilitation of tropical soils using compost and vermicompost is affected by the presence of endogeic earthworms. Appl. Soil Biol. 46 (2010) 125–133.

[34] P. Jouquet, E. Bloquel, T.T. Doan, M. Ricoy, D. Orange, C. Rumpel, T.T. Duc, Does compost and vermicompost improve macronutrient retention and plant growth in degraded tropical soils? Compost Sci . Util. 19 (2011) 15-24.

[35] P. Jouquet, G. Huchet, N. Bottinelli, D.T. Thu, What are the limits of the drilosphere? An incubation experiment using *Metaphire posthuma*. Pedobiol. 54 (2011) 113–117.

[36] P. Jouquet, G. Huchet, N. Bottinelli, D.T. Thu, T.D. Tran, Does the influence of earthworms on water infiltration, nitrogen leaching and soil respiration depend on the initial soil bulk density? A mesocosm experiment with the endogeic species *Metaphire posthuma*. Biol. Fert. Soils 48 (2012) 561-567.

[37] E.P. Kandeler, M. Stemmer, M. Gerzabek, Tillage changes microbial biomass and enzyme activities in particle-size fractions of a Haplic Chernozem. Soil Biol. Biochem. 31 (1999) 1253–1264.

[38] D.J. Lane, 16S/23S rRNA Sequencing, in: Stackebrandt E, Goodfellow M, (Eds). Nucleic acid techniques in bacterial systematics. New York: John Wiley & Sons, 1991, 115–175.

[39] P. Lavelle, D. Bignell, M. Lepage, Soil function in a changing world: the role of invertebrate ecosystem engineers. Eur. Soil Biol. 33 (1997) 159–193.

[40] P. Lavelle, A.V. Spain, Soil Ecology. Kluwer Academic Publishers, Dordrecht, the Netherlands, 2001, 654 pp.

[41] Y.J. Liu, A. Zaprasis, S.J. Liu, H.L. Drake, M.A. Horn, The earthworm *Aporrectodea caliginosa* stimulates abundance and activity of phenoxyalkanoic acid herbicide degraders. ISME Journal 5 (2011) 473–485.

[42] C. Macci, S. Doni, E. Peruzzi, G. Masciandaro, C. Mennone, B. Ceccanti, Almond tree and organic fertilization for soil quality improvement in southern Italy. Environ. Manage. 95 (2012) 215–222.

[43] S. Marhan, E. Kandeler, S. Scheu, Phospholipid fatty acid profiles and xylanase activity in particle size fractions of forest soil and casts of *Lumbricus terrestris* L. (Oligochaeta, Lumbricidae). Appl. Soil Ecol. 35 (2007) 412–422.

[44] S. Marinari, G. Masciandaro, B. Ceccanti, S. Grego, Influence of organic and mineral fertilisers on soil biological and physical properties. Biores. Technol. 72 (2000) 9-17.

[45] B. Moeskops, D. Buchan, S. Van Beneden, V. Fievez, S. Sleutel, M.S. Gasper, T. D'Hose, S. De Neve, The impact of exogenous organic matter on SOM contents and microbial soil quality. Pedobiol. 55 (2012) 175-184.

[46] P. Mora, E. Miambi, J.J. Jiménez, T. Decaëns, C. Rouland, Functional complement of biogenic structures produced by earthworms, termites and ants in the neotropical savannas. Soil Biol. Biochem. 37 (2005) 1043–1048.

[47] D.L. Mummey, M.C. Rillig, J. Six, Endogeic earthworms differentially influence bacterial communities associated with different soil aggregate size fractions. Soil Biol. Biochem. 38 (2006) 1608–1614.

[48] G. Muyzer, E.C. de Waal, A.G. Uitterlinden, Profiling of complex microbial population by denaturing gradient gel electrophoresis analysis of polymerase chain reaction amplified genes coding for 16S rRNA. Appl. Environ. Microb. 59 (1993) 695–700.

[49] P.T. Ngo, C. Rumpel, M.F. Dignac, D. Billou, T.T. Duc, P. Jouquet, Transformation of buffalo manure by composting or vermicomposting to rehabilitate degraded tropical soils. Ecol. Eng. 37 (2011) 269–276.

[50] P.T. Ngo, C. Rumpel, T.T. Doan., P. Jouquet, The effect of earthworms on carbon storage and soil organic matter composition in tropical soil amended with compost and vermicompost. Soil Biol. Biochem. 50 (2012) 214-220.

[51] K.P. Pramanik, G.K. Ghosh, P.K. Ghosal, K.P. Bani, Changes in Organic - C, N, P and K and Enzyme Activities in Vermicompost of Biodegradable Organic Wastes under Liming and Microbial Inoculants. Biores. Technol. 13 (2007) 2485–2494.

[52] R Development Core Team, R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0. (2008) Accessed at www.R-project.org

[53] N. Ruiz, J. Mathieu, L. Célini, C. Rollard, G. Hommay, E. Iorio, P. Lavelle, IBQS: A synthetic index of soil quality based on soil macro-invertebrate communities. Soil Biol. Biochem. 43 (2011) 2032-2045.

[54] M. Sebastiana, C. Juan, P. Ruiz, F.H. Juan, M. Engracia, Chemical and biochemical properties in a silty loam soil under conventional and organic management. Soil Till. Res. 1 (2006) 162–170.

[55] V.C. Sheffield, J.S. Beck, E.M. Stone, R.M. Myers, A simple and efficient method for 632 attachment of a 40base pair, GC rich sequence to PCR amplified DNA. BioTechn. 12 (1992) 386–387.

[56] B. Sen, T.S. Chandra, Do earthworms affect dynamics of functional response and genetic structure of microbial community in a lab-scale composting system? Biores. Technol. 100 (2009) 804–811.

[57] C. Stark, L.M. Condron, A. Stewart, H.J. Di, M. O'Callaghan, Influence of organic and mineral amendments on soil microbial properties and processes. Appl. Soil Ecol. 35 (2007) 79-93.

[58] M.A. Tabatabai, Soil Enzymes, in: Methods of Soil Analysis, Page, A.L., Millar, E.M., Keeney, D.R (Eds.), ASA and SSSA, Madison, WI., 1982, 775-833.

[59] M. Tejada, J.L González, A.M. García-Martínez, J. Parrado, Application of a green manure and green manure composted with beet vinasse on soil restoration: effects on soil properties. Biores. Technol. 99 (2008) 4949–4957.

[60] A.V. Tiunov, S. Scheu, Carbon availability controls the growth of detritivores (Lumbricidae) and their effect on nitrogen mineralization. Oecologia 138 (2004) 83-90.

[61] A. Vivas, B. Moreno, S. Garcia-Rodriguez, E. Benitez, Assessing the Impact of Composting and Vermicomposting on Bacterial Community Size and Structure, and Microbial Functional Diversity of an Olive-Mill Waste. Biores. Technol. 100 (2009) 1319–1326.

[62] A. Yajima, P. Jouquet, D. Do Trung, T.D. Thi Cam, D. Orange, A. Montresor, High latrine coverage is not reducing the prevalence of soil transmitted helminthiasis in Hoa Binh province, Vietnam. Trans. R. Soc. Trop. Med. Hyg. 103 (2009) 237-241.

[63] A.V. Yakushev, S.A. Blagodatsky, B.A. Byzov, The effect of earthworms on the physiological state of the microbial community at vermicomposting. Microbiol. 78 (2009) 510–519.

[64] A.V. Yakushev, I.A. Bubnov, A.M. Semenov, Estimation of the effects of earthworms and initial substrates on the bacterial community in vermicomposts. Eurasian Soil Sci. 44 (2011) 1117–1124.

[65] M. Yasir, Z. Aslam, S.W. Kim, S.W. Lee, C.O. Jeon, Y.R. Chung, Bacterial community composition and chitinase gene diversity of vermicompost with antifungal activity. Biores. Technol. 100 (2009) 4396–4403.

[66] J.C. Zak, MR. Willig, D.L. Moorhead, H.G. Wildman, Functional diversity of Microbial communities: a quantitative approach. Soil Biol. Biochem. 26 (1994) 1101-1108.

[67] M. Zaman, K.C. Cameron, H.J. Di, K. Inubushi, Changes in mineral N, microbial and enzyme activities in different soil depths after applications of dairy shed effluent and chemical fertilizer. Nutr. Cycl. Agroecosyst. 63 (2002) 275–290.

Table 1. Shannon (H) and Richness (S) diversity indices calculated from (a) OTUs* of DGGE gel and (b) individual substrate consumption in Biolog EcoPlate™ in the initial soil (T_0) and in soil after 15 months of cultivation from a greenhouse experiment. Treatments are mineral, compost and vermicompost amendments incubated with (EW+) and without (EW-) earthworms. Standard errors are given in parenthesis. Values with the same letter are not different at $P = 0.05$ (LSD test, n=3).

Soil amendment			
(a) Structural diversity		Shannon diversity (H)	Richness (S)
Initial soil T_0		2.49 (0.03)	17.0 (0.0)
Mineral			
	EW-	2.78 (0.65)^{c}	22.3 (0.6)^{d}
	EW+	2.78 (0.05)^{c}	22.0 (0.0)^{d}
Compost			
	EW-	2.93 (0.07)^{a}	25.7 (0.58)^{a}
	EW+	2.92 (0.06)^{a}	25.3 (0.58)^{ab}
Vermicompost			
	EW-	2.80 (0.04)^{b}	23.0 (0.0)^{c}
	EW+	2.93 (0.03)^{a}	24.7 (0.6)^{b}
(b)	Functional diversity		
Initial soil T_0		1.40 (0.2)	16.7 (0.6)
Mineral			
	EW-	2.17 (0.3)^{e}	21.0 (2.6)^{c}
	EW+	2.18 (0.2)^{e}	21.0 (1.7)^{c}
Compost			

	EW-	4.60 (0.6)[a]	30.3 (1.1)[a]
	EW+	3.43 (0.2)[c]	30.0 (0.0)[a]
Vermicompost			
	EW-	2.68 (0.0)[d]	24.3 (1.5)[b]
	EW+	3.85 (0.3)[b]	28.0 (1.0)[a]

*Bands, which were best predicted by the variation in clusters, are identified as OTUs (Operation Taxonomy Units).

Table 2. Enzyme activities in soil (U g^{-1}) before the experiment (initial soil T_0) and after 15 months of cultivation with (EW+) or without (EW-) earthworms. Standard errors are given in parenthesis. For each variable, values with the same letter are not different at $P = 0.05$ (LSD test, n=3). nd: not detected.

Soil amendment	*N*-acetyl -glucosamidase	*β*-Glucosidase	alkaline phosphatase	acid phosphatase	Urease	Amylase	Xylanase
Initial soil To	0.73 (0.04)	0.68 (0.04)	13.81 (0.56)	4.42 (0.15)	0.19 (0.04)	nd	nd
Mineral							
EW-	0.12 (0.03)^{c}	0.83 (0.06)^{d}	17.95 (0.82)^{e}	6.95 (0.24)^{c}	1.00 (0.19)^{c}	nd	nd
EW+	0.11 (0.02)^{c}	1.04 (0.21)^{d}	17.45 (0.62)^{e}	7.87 (0.5)^{c}	0.80 (0.07)^{c}	nd	nd
Compost							
EW-	2.13 (0.16)^{a}	2.46 (0.08)^{c}	42.41 (2.72)^{a}	14.68 (1.44)^{a}	2.11 (0.14)^{a}	2.51 (0.18)^{b}	nd
EW+	1.83 (0.16)^{b}	2.43 (0.08)^{c}	33.07 (2.72)^{c}	10.39 (1.44)^{b}	1.74 (0.14)^{b}	2.4 (0.18)^{b}	nd
Vermicompost							
EW-	1.86 (0.07)^{b}	3.16 (0.77)^{a}	34.61 (1.89)^{b}	13.55 (0.71)^{a}	2.12 (0.08)^{a}	5.32 (0.70)^{a}	0.50 (0.10)^{a}
EW+	1.82 (0.05)^{b}	2.82 (0.67)^{b}	30.06 (0.42)^{d}	13.49 (1.03)^{a}	2.19 (0.02)^{a}	4.87 (0.61)^{a}	0.33 (0.00)^{b}

Figure 1. Dendrogram of similarity derived from DGGE analyses of 16S rRNA gene fragment amplified from initial soil (soil T_0) and soils after 15 months of experiment (n = 3). Treatments are Mineral, Compost or Vermicompost with (EW+) and without (EW-) earthworms

Figure 1

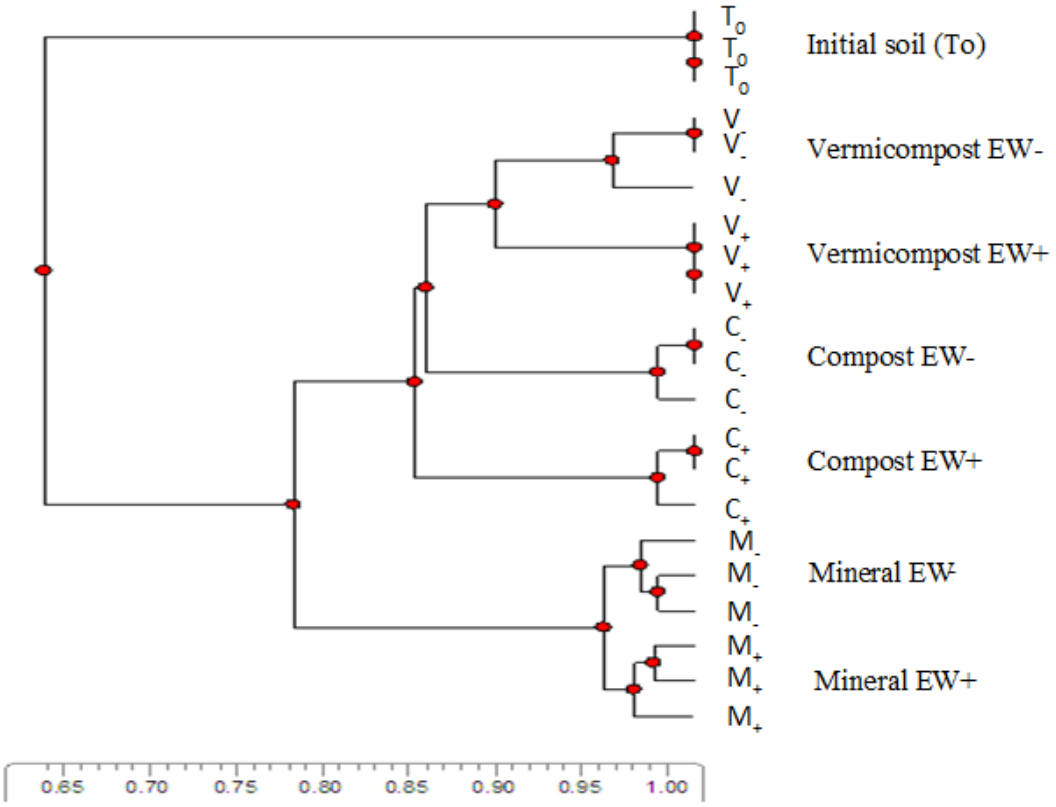


Figure 2. Average well color development (AWCD) calculated from Biolog EcoPlate™ absorbance data after 72 h of incubation (n = 3) in the soil samples at the end of the experiment and amendment of chemical nutrients (mineral), compost or vermicompost, and in presence (EW+, in grey) or absence (EW-, in white) of earthworms. For each variable,

values with same letter are not significantly different (bars are SE, $P < 0.05$, LSD test).

Figure 2

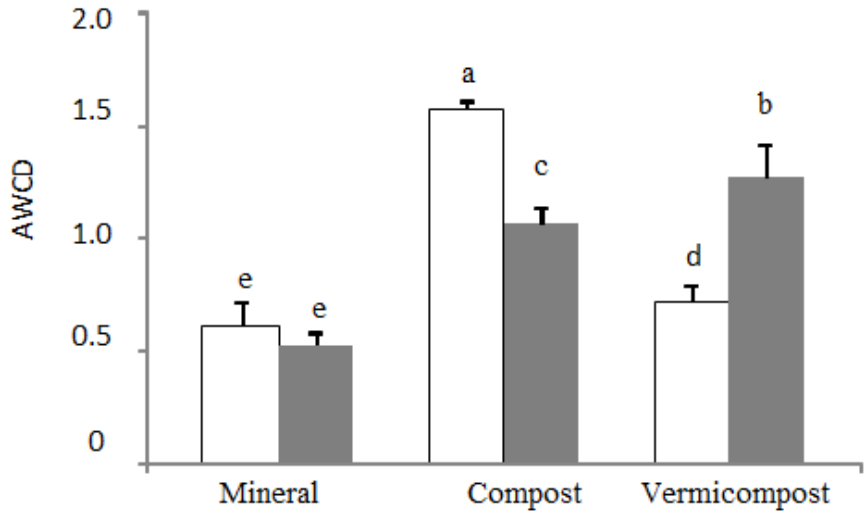
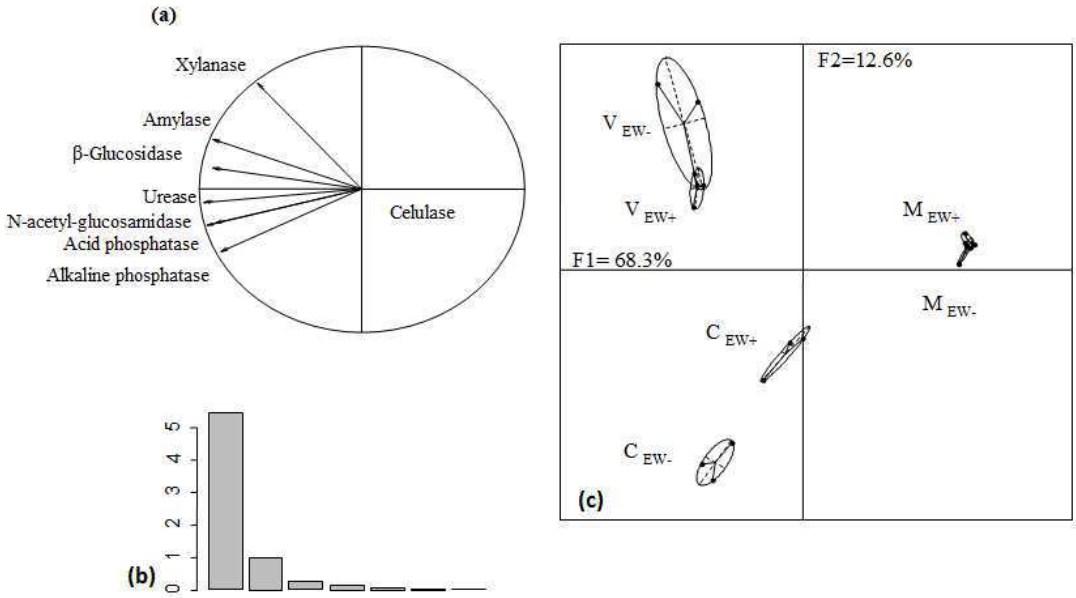


Figure 3. Principal components analysis (PCA) from soil enzyme activities at the end of the experiment. Treatments are mineral (M), compost (C) or vermicompost (V) treatments with (EW+) or without (EW-) earthworms. (a) Correlation circle on F1-F2 plane. Variables are: Beta-glucosidase, N-acetyl- glucosamine, amylase, xylanase, cellulase, urease, alkaline phosphatase, acid phosphatase; (b) Eigenvalue diagram and (c) Ordination in the plane defined by factors 1 and 2.

Figure 3


(a)
Xylanase
Amylase
β-Glucosidase
Urease
N-acetyl-glucosamidase
Acid phosphatase
Alkaline phosphatase
Celulase
(b)
0 1 2 3 4 5
F2=12.6%
V EW-
V EW+
M EW+
F1= 68.3%
M EW-
C EW+
C EW-
(c)