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The effects of earthworm functional diversity on microbial biomass and the microbial community level physiological profile of soils

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ABSTRACT

A novel experiment design based on the axial simplex design was used to study the effects of earthworm functional diversity on microbial biomass and microbial community level physiological profiles in soils. This mesocosm experiment was carried out at two densities of earthworm and at two levels of food supply. Soil mesocosms were maintained for 7 months and watered each week with 300 ml of distilled water. At the end of this period the soil columns were divided into upper and lower halves and microbial biomass carbon and functional diversity assessed. Greater microbial biomass and activity was found in the upper layer of soil. Food supply was the major driver leading to increased microbial activity and biomass. Increased earthworm biomass had the opposite effect. In the lower soil layer, the presence of anecic worms had the greatest effect on microbial biomass and activity. This effect was enhanced with increased food supply. Overall, the relationship between earthworm functional diversity and microbial community level physiological profile appeared to be idiosyncratic and the effect of the earthworms varied with soil layer, initial biomass and food supply. The loss of any earthworm functional group might lead to significant alterations in the microbial biomass, distribution and physiological profile.

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1. Introduction

Soil microbial communities drive nutrient transformation and hence play important roles in nutrient cycling in the soil. Earthworms facilitate soil C and N transformation through their influence on these microorganisms [2,17,22]. However, they have a variable influence on microbial biomass and activity [23], increasing the amounts of microbial biomass in some circumstances and decreasing it in others [3]. For example, the presence of endogeic species alone can reduce microbial biomass but when these worms are present with epigeic species no reduction occurs [23]. Occasionally they cause a change to a smaller more metabolically active microbial community [19,29].

Casts and burrows, which are enriched in available nutrients harbour greater microbial populations and biomass than surrounding soil [10,11,16,24] and actively feeding earthworms have an influence on the spatial distribution of microorganisms. However, the relationship between earthworm community structure and the structure and role of

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soil microbial communities in providing ecosystem functions is poorly understood [23]. As indicated above the types of functional groups of earthworms present have the potential to change the functioning of the soil microbial biomass.

The relationships between diversity and the functioning of ecosystem are central to both community and ecosystem ecology and need to be understood in order to predict how communities and ecosystems respond to environmental change [1]. In this study, we relate earthworm functional diversity to ecosystem function specifically through examining the effects of earthworm diversity on microbial biomass and the microbial community level physiological profile. Because the intensity of interaction between earthworm functional groups will be affected by the total earthworm biomass present and by the amount of resource available, we hypothesise that this could influence the effects on microbial populations.

2. Materials and methods

2.1. Experimental design

Because of the difficulties identified with many previous experimental designs [4,6,7,28] a novel design, using earthworm communities and based on the axial simplex design was used [8]. In this design, communities were set up as either monocultures (endogeic, epigeic or anecic), centroids, consisting of equal amounts of each functional group, binary mixtures, consisting of equal biomass of two functional groups or complete mixtures consisting of all functional groups, where the proportional contributions of the groups sum to one (Fig. 1). Therefore both species richness and evenness were varied systematically through the earthworm assemblages used.

In order to assess the effects of altered earthworm biomass and resource availability, the experiment was carried out at two levels of earthworm biomass and two levels of food availability. The densities of earthworm were 1.7 g and 3.4 g per mesocosm and at two levels of food supply were 0.32 g and 0.64 g of ground dried grass added to the soil surface each week, giving four combinations of biomass and food supply and a total of 52 communities. The biomass of earthworms used was decided upon biomass estimates from an Irish pasture [9]. The low level of biomass (1.7 g) was set to be equivalent to 100 g of earthworms per metre squared. The high level of biomass was set to be equivalent to 200 g of earthworms per metre squared. The amount of food to be added to each column was based on an average consumption rate of litter of 27 mg dry weight of litter g^{-1} live weight of worm day^{-1} [21]. This value was used to calculate the food supply on a weekly basis.

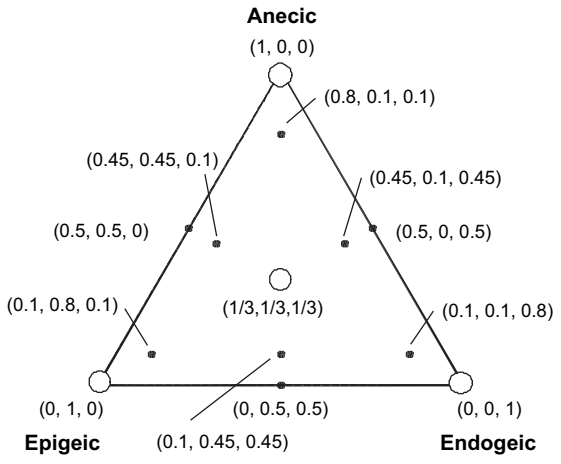

Fig. 1 – Simplex design proportions. For example, (0.1, 0.1, 0.8) indicates am earthworm community consisting of 10% anecic, 10% epigeic individuals and 80% endogeic individuals.

The experiment ran for 30 weeks. It was housed in a potting shed where temperature was never extreme and would certainly never reach values which would affect the viability of worms in soil.

Adult earthworms were obtained from a meadow in University College Dublin and sorted into three distinct functional groups based on Bouché [5]. The epigeic worms were *Lumbricus rubellus* (Hoffmeister), *Lumbricus castaneus* (Savigny) and *Satchellius mammalis* (Savigny), the anecic worms *Lumbricus friendi* Cognetti and *Aporrectodea longa* (Ude) and the endogeic worms *Aporrectodea caliginosa* (Savigny), *Octolasion cyaneum* (Savigny), *Allolobophora chlorotica* (Savigny) and *Aporrectodea rosea* (Savigny). The earthworms were allocated randomly to experimental units. Over the period of the experiment, the structure of the earthworm assemblage changed somewhat. These changes and the responses of nitrogen dynamics are reported in Sheehan et al. [25,26].

2.2. Experimental unit

The experimental units consisted of Plexiglas cylinders (15 cm diameter × 30 cm depth) containing 2.65 l of a slightly alkaline loam–clay loam soil. The soil had been stored for 10 months in the laboratory, broken down and sieved through 4 mm and then 2 mm mesh. The base of each soil column was fitted with 2 mm mesh to prevent earthworms escaping. Units were watered with distilled water once per week.

A soil suspension, derived from soil from the same location as the soil used in the mesocosms, was added to the mesocosms to encourage the microbial populations.

2.3. Microbial biomass carbon

The mesocosms were maintained for 7 months and were watered each week with 300 ml of distilled water. At the end of this period the soil was divided into upper and lower halves in each column (0–7 cm and 7–14 cm) and microbial biomass carbon (C_{mic}) estimated using the chloroform-fumigation method [15] and the microbial community level physiological profile [13] was assessed using the Biolog EcoPlate™ [14]. Although this method of assessing functional diversity of microorganisms is not without problems, especially in terms of interpretation, it has been used extensively and has proved to be a rapid easy method of characterising microbial communities [18].

2.4. Statistical analysis

We related C_{mic} to the proportion of each functional group in the initial earthworm assemblage composition, initial overall earthworm biomass (Bio), the amount of food supplied (Food) and the soil horizon (LU, lower or upper). The model used was based on those developed for the simplex design [8].

$$\text{Microbial biomass } C = \sum_{i=1}^{3} \beta_i P_i + \sum_{i=1}^{3} \gamma_i \text{Bio} \times P_i + \sum_{i=1}^{3} \delta_i \text{Food} \times P_i + \sum_{i=1}^{3} \lambda_i \text{Bio} \times \text{LU} \times P_i + \sum_{i=1}^{3} \eta_i \text{Food} \times \text{LU} \times P_i + \varepsilon$$

where P_i is the proportion of ith functional group in the initial earthworm community. Diversity effects occur as a result of interactions among functional groups. All functional group interactions (pairwise $= P_i \times P_j$ and among three functional groups $= P_1P_2P_3$) were added to the model (and interactions of these with all factors) and tested for significance. They indicate the additional performance of a mixture over the performance that would have been expected from the component monocultures. Of 20 possible functional group interaction terms, only two were significant at the 5% level. In view of the multiple comparisons involved, these were not pursued further. This model is a function of the initial community structure and so C_{mic} can be predicted for all possible communities and presented as a surface over the simplex. As the model was fitted to the data assuming a split-plot structure (mesocosm, mainplot; horizon within mesocosm, subplot) it required the use of software (Proc Mixed in SAS V8 [20]) to deal with this complexity in error structure. High order interactions were omitted if not significant and the final model is presented in Table 1. There was one replicate per treatment. This is part of the simplex design methodology—to give a range of communities from monocultures of each species to the centroid, a community in which each species is equally represented by initial biomass. The analysis is by multiple regression with the proportions of the species and food and biomass variables as the driving variables [8]. The design has the same efficiency for estimating a simple diversity effect as would 16 replications for a standard two-treatment design.

Colour development in the Biolog plates was fitted to Gompertz growth curves [30] and the slopes and asymptotes of the curves for the 31 substrates analysed using redundancy analysis (RDA) with forward selection in the CANOCO package [27]. The slopes of these curves indicate the rate at which colour develops on the plates while the asymptotes give the highest level of colour development.

Table 1 – Parameter estimates of fitted model for microbial biomass C (μg C_{mic} g^{-1} dry soil)

	t (df = 47)	P-value
A	6.90	**<.0001**
Epi	6.15	**<.0001**
Endo	3.85	**<.0001**
A × Bio	0.40	0.693
Epi × Bio	0.00	0.990
Endo × Bio	0.66	0.517
A × Food	1.62	0.111
Epi × Food	0.71	0.485
Endo × Food	0.32	0.759
A × Layer	2.27	**0.028**
Epi × Layer	5.54	**<.0001**
Endo × Layer	1.15	0.254
A × Bio × Layer	3.00	**0.004**
A × Food × Layer	3.09	**0.003**

A, Epi and Endo are the initial biomass proportions of anecic, endogeic and epigeic worms respectively. Bio represents the overall earthworm biomass and Food the amount of food added to the mesocosms. Layer refers to the distinction between the upper (0–7 cm) and lower (7–14 cm) soil layers. Coefficients in bold type are significant at the 5% level.

3. Results

The functional groups of earthworms differentially affected the amount of microbial biomass but there were no significant interactions between the groups. The biomass was always low when endogeic species were present (Fig. 2). However, the effects of the other two functional groups were contingent on other factors (Table 1). For example, the biomass was always higher in the surface layer and for the anecics this was further affected by the amount of food provided and the biomass of the worms. The amount of microbial biomass was highest at low earthworm biomass in the upper horizon but it was higher with greater worm biomass in the lower horizon (Fig. 2a). This obviously reflects the role of this functional group in burying organic matter and mixing the soil horizons. The microbial biomass was always higher in the upper soil layer when greater amounts of food were added with anecic species but this was not so marked in the lower horizon (Fig. 2a).

Both the slopes and asymptotes of the Gompertz growth curves varied significantly between the upper and lower layers of soil with greater activity occurring in the upper layer. Therefore, in order to facilitate presentation and analysis of the data, the slopes and asymptotes were analysed separately for the upper and lower layers.

Earthworm biomass and food supply proved to be the only two significant factors associated with the variation in asymptotes in the upper layer of soil. The values of the asymptotes for all substrates increased as the food supply increased ($P = 0.01$) while increased earthworm biomass had the opposite effect (Fig. 3a). These two factors also influenced the slopes of the Gompertz curves but there was an additional significant interaction effect of anecic × food indicating that as the proportion of anecic worms increased the effects of food were diminished (Fig. 3b).

The effects were more complex in the lower layers of soil and while food was the only significant factor for the slopes there were two significant interactions for the asymptotes. A significant anecic worms × food interaction indicated a mitigating effect of this group on the effect of food. There was also a significant epigeic × endogeic interaction and the ordination diagram suggests that the combination of the two functional groups has a diametrically opposed effect to the groups in isolation (Fig. 3c). The slopes for the lower layers

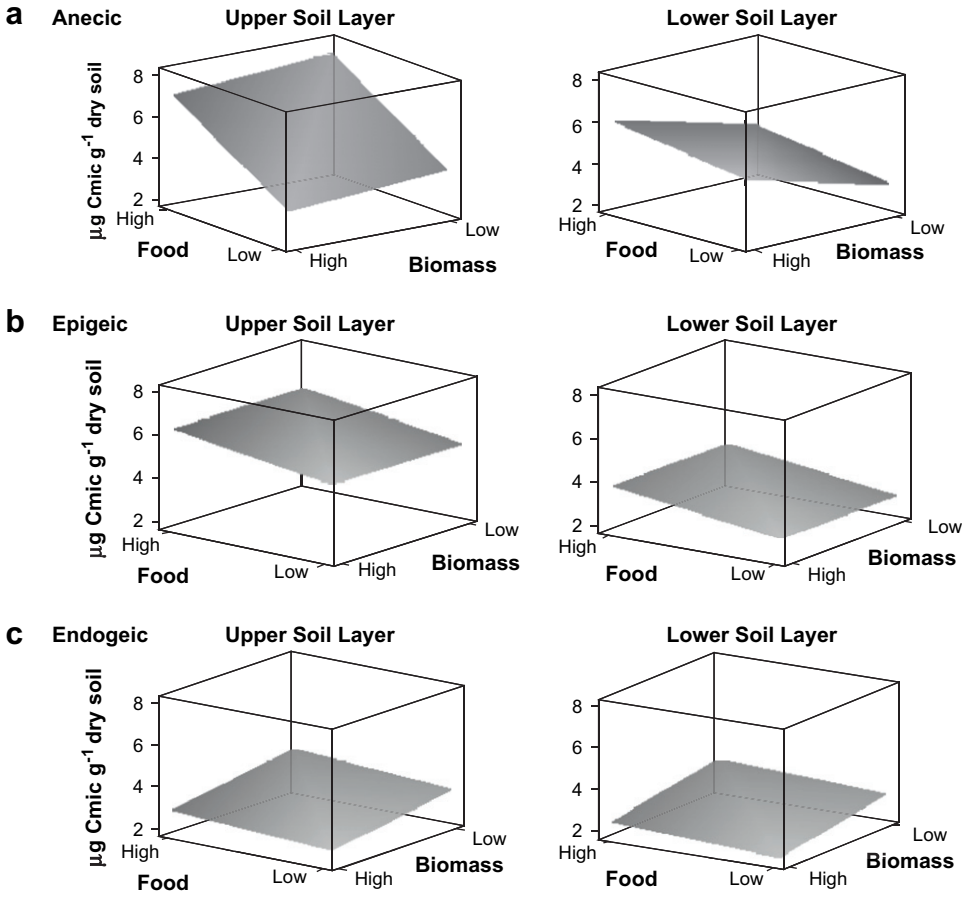

Fig. 2 – Effects of overall initial earthworm biomass (Biomass) and quantity of food (Food) on microbial biomass C (μg C_{mic} g^{-1} dry soil) predicted for monocultures of (a) anecic, (b) epigeic and (c) endogeic earthworm functional groups at upper (0–7 cm) and lower (7–14 cm) soil layers.

of soil suggest that the potential utilization of all substrates is reduced in the presence of endogeics (Fig. 3d).

It can be seen from the four ordinations that anecic groups have a diametrically opposite effect on the slopes and asymptotes of the Gompertz curves to the other two functional groups. In the upper layer, the epigeic and endogeic earthworms are associated with increased microbial activity while in the lower layer it is the anecic worms which have this effect.

4. Discussion

Functional groups had a varied effect on C_{mic} and the microbial community level physiological profile as indicted by carbon source utilisation. As expected, for the epigeics and anecic species the effects varied with soil layer. The activity of epigeic species is largely confined to the soil surface and therefore, in isolation, they would not be expected to have a significant impact on lower horizons. On the other hand, anecic species mix soil layers and have a significant effect in terms of the burial of organic matter both of which would be expected to affect the microbial biomass in the lower horizons. Food supply was identified as the major driver leading to increased microbial activity in the upper horizons where the greater C_{mic} and activity would be expected given that food was added directly to the soil surface. On the other hand increased earthworm biomass had the opposite effect, presumably due to either increased utilisation of microorganisms by the earthworms or competition for food resources.

In the lower soil layer, the presence of anecic worms had the greatest effect on microbial community and activity. This effect was enhanced with increased food supply. This is probably due to the vertical burrowing behaviour of this functional group. The distribution of microorganisms and nutrients by these earthworms along burrow walls, together with improved aeration and moisture content, appear to form the basis of a strong stimulation of microbial biomass and activity along gallery walls [12]. These results are largely as would be expected and are similar to those seen in other studies (e.g. [23]). However, Scheu et al. [23] showed that while microbial biomass was reduced in the presence of endogeic species, the effect was altered when epigeic species were present. This was not seen in the present study perhaps because of the reduction in epigeic biomass as the study progressed [26].

EcoPlates™ have proved to be a rapid easy method of characterising microbial communities and of assessing the functional diversity of microorganisms. However, the method is

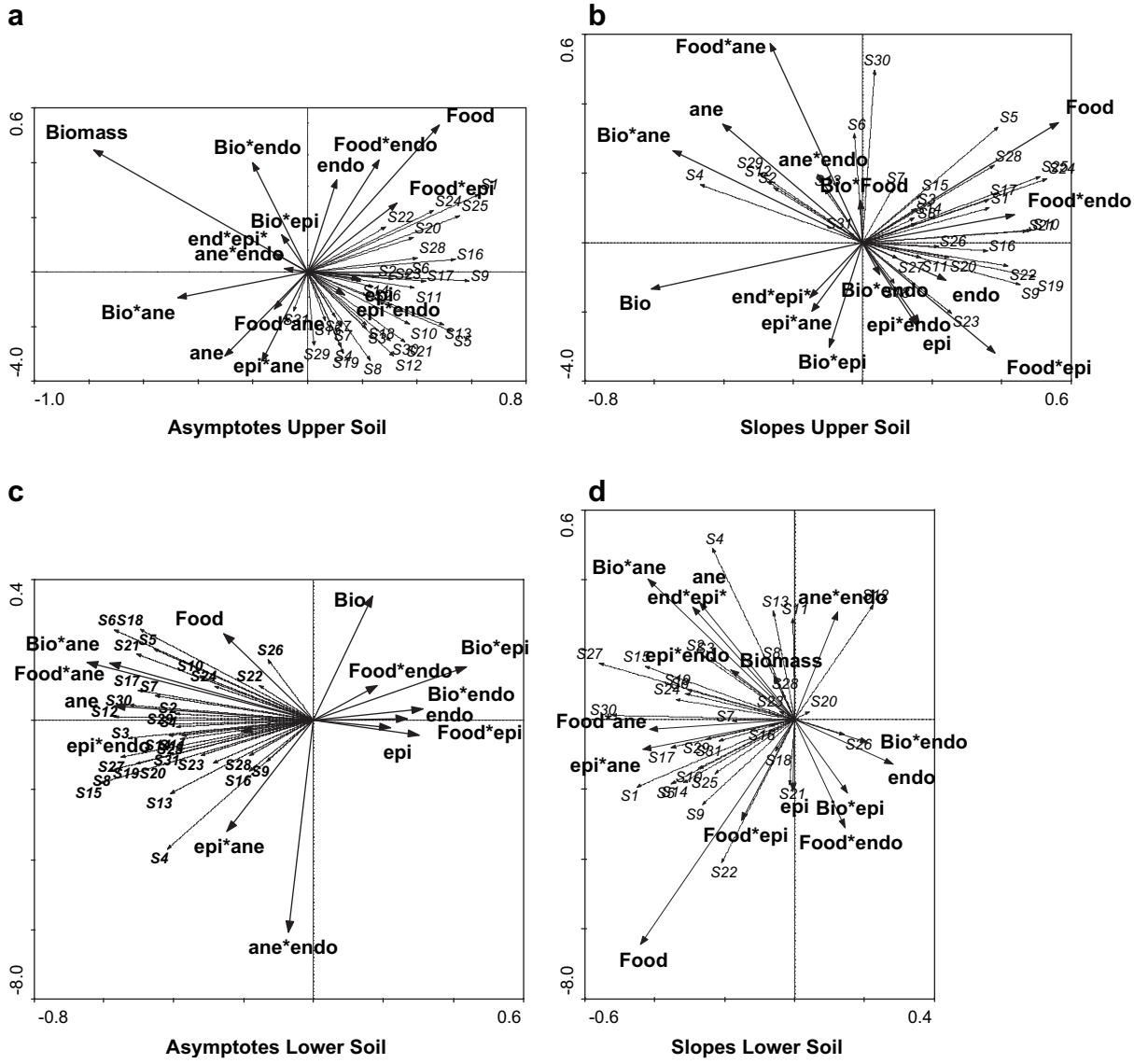

Fig. 3 – Redundancy analysis (RDA) of Gompertz slopes and asymptotes from carbon source utilisation analysis. (Upper layer 0–7 cm, lower layer 7–14 cm). The proportional contribution of anecic, endogeic and epigeic earthworms to the earthworm communities are indicated by ane, endo and epi respectively. Bio represents the overall earthworm biomass and Food represents the amount of food added to the mesocosms.

not without problems, especially in terms of interpretation. For example, not all species of bacteria and no fungi can reduce the dye used in the plates, and there are potential problems with inoculum density and time of observation [18]. We believe that these difficulties do not affect our interpretations because we were interested in detecting changes, rather than characterising the microbial communities, and the kinetic and ordination analyses used overcome other problems. Had no changes been detected we would have been less confident in the validity of the result because that might have arisen as a result of the difficulties mentioned above.

The three functional groups affected the patterns of carbon utilisation by the C_{mic} in different ways. In the absence of anecic worms, activity would be concentrated in the upper soil horizons while the other two groups had the opposite effect. The combinations of earthworm functional groups present also markedly affected the substrate utilisation as indicated by Biolog plates. For example, in the surface soil mixtures of epigeic and endogeic worms were associated with increased slopes and asymptotic values for the Gompertz curves for virtually all substrates but the presence of anecic worms, either on their own or in mixtures, had the opposite effect. In the lower soil horizons, mixtures of epigeics and endogeics promoted quite different community level physiological profiles to either of these groups occurring on their own. It is therefore clear that the relationship between earthworm functional group diversity and microbial community level physiological profile is dependent on the identity of the earthworm functional groups present and is thus idiosyncratic. It is also clear that the loss of any functional group

would lead to significant alterations in the microflora and in its ability to provide the ecosystem services associated with it. In conclusion, it is apparent that the three functional groups of earthworms, both alone and in various combinations, have different effects on the microbial community level physiological profiles and thus that the ecosystem services supplied by the microbes is dependent on the diversity and identity of the earthworm functional groups present.

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REFERENCES

[1] J. Bengtsson, Which species? What kind of diversity? Which ecosystem function? Some problems in studies of relations between biodiversity and ecosystem function, Appl. Soil Ecol. 10 (1998) 191–199.
[2] J.M. Blair, R.W. Parmelee, P. Lavelle, Influences of earthworms on biogeochemistry, in: P.F. Hendrix (Ed.), Earthworm Ecology and Biogeography in North America, Lewis Publishers, Boca Raton, FL, 1995, pp. 127–158.
[3] J.B. Bohlen, C.A. Edwards, Earthworm effects on N dynamics and soil respiration in microcosms receiving organic and inorganic nutrients, Soil Biol. Biochem. 27 (1995) 341–348.
[4] T. Bolger, The functional value of species biodiversity – a review. Biology and Environment: Proc. R. Irish Acad. 101B (2001) 199–224.
[5] M.B. Bouché, Stratégies lombriciennes, in: U. Lohm, T. Persson (Eds.), Soil Organisms as Components of Ecosystems, Ecol. Bull. 25 (1977) 122–132.
[6] J. Connolly, On difficulties with replacement-series methodology in mixture experiments, J. Appl. Ecol. 23 (1986) 125–137.
[7] J. Connolly, P. Wayne, F.A. Bazzaz, Interspecific competition in plants: how well do current methods answer fundamental questions, Am. Nat. 157 (2001) 107–125.
[8] J.A. Cornell, Experiments with Mixtures, second ed. John Wiley & Son, New York, 1990.
[9] D.C.F. Cotton, J.P. Curry, The effects of cattle and pig slurry fertilisers on earthworms (Oligochaeta, Lumbricidae) in grassland managed for silage production, Pedobiologia 21 (1980) 181–188.
[10] W. Devliegher, W. Verstraete, *Lumbricus terrestris* in a soil core experiment: nutrient-enrichment processes (NEP) and gut-associated processes (GAP) and their effect on microbial biomass and microbial activity, Soil Biol. Biochem. 27 (1995) 573–580.
[11] W. Devliegher, W. Verstraete, *Lumbricus terrestris* in a soil core experiment: the effect of nutrient-enrichment processes (NEP) and gut-associated processes (GAP) on plant nutrient and heavy metal availability, Soil Biol. Biochem. 28 (1996) 489–496.
[12] W. Devliegher, W. Verstraete, Microorganisms and soil physio-chemical conditions in the drilosphere of *Lumbricus terrestris*, Soil Biol. Biochem. 29 (1997) 1721–1729.
[13] J.I. 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. Microbiol. 57 (1991) 2351–2359.
[14] H. Insam, A new set of substrates proposed for community characterization in environmental samples, in: H. Insam, A. Rangger (Eds.), Microbial Communities. Functional versus Structural Approaches, Springer, 1997, pp. 260–261.
[15] D.S. Jenkinson, D.S. Powlson, The effects of biocidal treatments on metabolism in soil V. A method for measuring soil biomass, Soil Biol. Biochem. 8 (1976) 209–213.
[16] M. Lavelle, A. Martin, Small-scale and large-scale effects of endogeic earthworms on soil organic matter dynamics in soil of the humid tropics, Soil Biol. Biochem. 24 (1992) 1491–1498.
[17] A. Martin, A. Mariotti, J. Balesdent, P. Lavelle, Soil organic matter assimilation by a geophagous tropical earthworm based on ^{13}C measurements, Ecology 73 (1992) 118–128.
[18] J. Preston-Mafham, L. Boddy, P.F. Randerson, Analysis of microbial community functional diversity using sole-carbon-source utilisation profiles – a critique, FEMS Microb. Ecol. 42 (2002) 1–14.
[19] B.E. Ruz-Jerez, P.R. Ball, R.W. Tilman, Laboratory assessment of nutrient release from a pasture soil receiving grass or clover residues, in the presence or absence of *Lumbricus rubellus* or *Eisenia fetida*, Soil Biol. Biochem. 24 (1992) 1529–1534.
[20] SAS, SAS User's Guide: Statistics, Version 8 ed. SAS Inst. Inc, Cary, NC, 1998.
[21] J.E. Satchell, Lumbricidae, in: A. Burgess, F. Raw (Eds.), Soil Biology, Academic Press, London, 1967, pp. 259–322.
[22] S. Scheu, Microbial activity and nutrient dynamics in earthworm casts (Lumbricidae), Biol. Fertil. Soils 5 (1987) 230–240.
[23] S. Scheu, N. Schlitt, A. Tiunov, J.E. Newington, T. Hefin Jones, Effects of the presence and community composition of earthworms on microbial community functioning, Oecologia 133 (2002) 254–260.
[24] C. Shaw, S. Pawluk, Faecal microbiology of *Octolasion tyrtaeum, Aporrectodea turgida* and *Lumbricus terrestris* and its relation to carbon budgets of three artificial soils, Pedobiologia 29 (1986) 377–389.
[25] C. Sheehan, L. Kirwan, J. Connolly, T. Bolger, The effects of earthworm functional group diversity on nitrogen dynamics in soils, Soil Biol. Biochem. 38 (2006) 2629–2636.
[26] C. Sheehan, L. Kirwan, J. Connolly, T. Bolger, The effects of earthworm functional group diversity on earthworm community structure, Pedobiologia 50 (2007) 479–487.
[27] C.J.F. Ter Braak, P. Šmilauer, CANOCO Reference Manual and User's Guide to Canoco for Windows. Software for Canonical Community Ordination (version 4), Centre for Biometry, Wageningen, Netherlands, 1998.
[28] D.A. Wardle, Is ''Sampling Effect'' a problem for experiments investigating biodiversity–ecosystem function relationship? Oikos 87 (1999) 403–407.
[29] V. Wolters, R.G. Joergensen, Microbial carbon turnover in beech forest soils worked by *Aporrectodea caliginosa* (Savigny) (Oligochaeta: Lumbricidae), Soil Biol. Biochem. 24 (1992) 171–177.
[30] M.H. Zwietering, I. Jongenburger, F.M. Rombouts, K. Van't Riet, Modelling of the bacterial growth curve, Appl. Environ. Microbiol. 56 (1990) 1875–1881.