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Interaction of earthworms and enchytraeids in organically amended soil

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Abstract. A laboratory study was conducted with soil microcosms to deepen our understanding on earthworm and enchytraeid decomposition activity regarding soil fertility improvement in sustainable agricultural management systems. We investigated the effects of different earthworm and enchytraeid species and their interaction on the consumption and decomposition of barley straw and cattle manure, commonly used as organic amendments in agriculture. In total, 55 microcosms were set up covering the following treatments: straw of three length classes (2.5 cm, 5 cm, 10 cm), two length classes (2.5 cm, 10 cm) and cattle manure which were offered to earthworms (*Lumbricus terrestris*, *Aporrectodea caliginosa)*; enchytraeids (*Enchytraeus crypticus*, *Enchytraeus christenseni*); mixed earthworms and enchytraeids (*L. terrestris*, *A. caliginosa*, *E. crypticus*, *E. christenseni*); no animals (control). After 45 days of dark incubation at 15°C the consumption rate, soil microbial biomass, pH, nitrogen (N-NH_4^+, N-NO_3^-, Nt), and organic carbon were measured. The results indicated that mixed faunal treatments revealed the highest consumption rate. Short straw was consumed preferentially in all treatments. Earthworms increased microbial biomass and contents of N-NO_3^- and N-NH_4^+. In the enchytraeid treatments soil microbial biomass and organic carbon were lower compared with control, while soil nitrate was lowest when straw was used as organic source. Soil fauna in the mixed treatment reduced soil microbial biomass, N-NO_3^- and soil organic carbon content. It is concluded that earthworm-enchytraeid-interactions might have important impacts on soil fertility.

Key words: Earthworms, enchytraeids, soil fertility, barley straw, cattle manure, decomposition.

Introduction

Conventional agricultural practices have been faced during last decades with critics due to their dependency on external inputs and the associated environmental problems (Holland 2004, Stoate et al. 2009). Consequently, in order to achieve the sustainability of agricultural systems, new management strategies like the reduction of the amount of mineral fertilizers used and the increase of organic matter added to the soil were adopted worldwide. It is well documented (Eltun et al. 2002, Forge et al. 2003, Robertson & Grandy 2006, Kassam et al. 2009) that the use of organic amendments can improve soil fertility with minimum economical costs. However, these practices are requiring a better understanding of their efficiency and of decomposition and nutrients release processes.

Organic matter decomposition and nutrient cycling processes are mediated by soil biota (Lavelle & Spain 2005). Fungi and bacteria are considered as primary decomposers but the whole range of soil fauna has a major contribution to the decomposition process as well (Vetter et al. 2004).

In temperate agroecosystems under conservation tillage, earthworms and enchytraeids can reach high densities (Overstreet et al. 2010, Röhrig et al. 1998) having direct influence on decomposition by feeding, enhancing microbial activity and changing soil properties (Lavelle & Spain 2005). The importance of earthworms on organic matter turnover is well documented. While several studies (Cortez et al. 1989, Marinissen & de Ruiter 1993, Subler et al. 1998, Bohlen et al. 1999, Sandor & Schrader, 2007) showed that earthworms are key organisms in nutrient cycling, less data are available on enchytraeids affecting organic matter decomposition and nutrient cycling (Maraldo et al. 2011). Parmelee et al. (1990) pointed out that the main influence of enchytraeids in decomposition and nutrient cycling processes may be indirect, through their interaction with soil microflora, while other studies (Marinissen & Didden 1997, Briones et al. 1998, Koutika et al. 2001, van Vliet 2004) proved that enchytraeid activity leads to a higher decomposition rate. Maraldo et al. (2011) estimated an input of 0.3 g N m^{-2} $year^{-1}$ through NH_4^+ excretion by an enchytraeid community in a nutrient-poor sandy soil. Most studies concerning involvement of earthworms and enchytraeids on decomposition deal with only one or a combination of species from one of the families *Lumbricidae* or *Enchytraeidae*. However, the interaction of both groups on the mineralization of organic matter is a less studied aspect (Parmelee et al. 1990, Koutika et al. 2001, Räty & Huhta 2003). It is an open question in which way the relationship between these groups affects decomposition processes.

The aim of this study was to investigate earthworm-enchytraeid-interactions during decomposition of two organic sources, i.e. barley straw and cattle manure, commonly used as amendments in agriculture. Different combinations of species were used in microcosm experiments in order to evaluate the effects of species combinations on decomposition and mineralization of organic matter. Synergic, antagonistic or neutralistic relationships among earthworms, enchytraeids and soil microflora were investigated with respect to their impact on soil proprieties.

Material and methods

Experimental set-up

A total number of 55 microcosms were set up using two types of polypropylene columns filled with arable soil from the Ap horizon. Large columns (30 cm in height, 12 cm in diameter) were used for earthworm (E1, E2, E3), mixed earthworm and enchytraeid (EEn1, EEn2, EEn3) and non-faunal control experiments (C1, C2, C3). Small columns (5 cm in height, 11 cm in diameter) were used for enchytraeid (En1, En2) experiments. The soil was a luvisol derived from loess (clay 12%, silt 85%, sand 3%) with a pH ($CaCl_2$) of 6.3. The soil contained 1.4% organic carbon (C_{org}) and 0.14% total nitrogen (N_t) resulting in a C-to-N ratio of 10:1. The soil was sieved through a 5 mm mesh sieve to remove larger fragments of organic matter. After sieving, the soil was defaunated by two cycles of freezing at -20°C for seven days and thawing at room temperature for three days. These freezing-thawing cycles killed the soil fauna except for some nematodes and protozoa which may have survived (Huhta et al. 1989). After freezing-thawing procedure the columns were filled with soil to a bulk density of 1.25 g cm^{-3}. The water content of the soil was adjusted to 20% and the bottom part of the columns was closed with a 20 µm mesh.

Cattle manure (C:N ratio 12:1) or barley straw (C:N ratio 30:1) of different lengths (2.5 cm, 5 cm, 10 cm) were used as organic material and spread uniformly on the soil surface of the large columns (Table 1). For the small column experiments straw of only one length (i.e. 2.5 cm) or cattle manure was used (Table 1). In this case, half of the organic material was mixed with soil and the other half was spread on the soil surface. After adding organic materials, the microcosms were placed for five days at 15°C for microfloral development. Then, microcosms were inoculated with adult clitellate individuals of *Lumbricus terrestris, Aporrectodea caliginosa, Enchytreus christenseni* and *Enchytreus crypticus* as described in Table 1. Non-faunal treatments served as control.

The earthworms used for experiments were collected from arable fields and kept for two weeks in the same soil like that used in microcosms. The enchytraeids were obtained from a laboratory culture. Prior to inoculation, the earthworm biomass was recorded. The mean initial individual biomass of *L. terrestris* and *A. caliginosa* was 6.3 g (1SD: 1.4) and 0.75 g (1SD: 0.2), respectively.

The microcosms were set up in an experimental design (Table 1) with three different organic treatments (two lengths of barley straw, three lengths of barley straw and cattle manure) and four animal treatments (only earthworms, earthworms and enchytraeids, only enchytraeids and no animals). Each treatment had 5 replicates resulting in a total number of 55 microcosms. The microcosms were incubated for 45 days in dark at 15°C. During incubation period soil water content was kept at 20%. In general, the experiments followed the recommendations proposed by Fründ et al. (2010).

Sampling and sample processing

After 45 days, remaining organic material from the surface of large columns was collected while for the small columns we collected also the undecomposed organic material present within the soil. The collected organic material was weighed and the community consumption rate was recorded as percentage of the initial amount. From each microcosm we sampled the first 10 cm of soil using a small tube-shaped corer (10 cm long, 2 cm diameter). To estimate the enchytraeid density, three soil samples were taken from each column followed by wet funnel extraction according to O'Connor (1962). Thereafter, we sampled 70 g of soil from each column and processed the soil as follows: 10 g of soil was kept at 4°C for microbial biomass analysis, 30 g was frozen (-20°C) for N-NO_3^- and N-NH_4^+ analysis, 15 g was air dried for pH measurements and 15 g was dried at 110°C overnight for C_{org}, and N_t analyses.

The columns were then carefully destructured to collect and weigh the earthworms. Microbial biomass was estimated by using the substrate induced respiration method (Anderson & Domsch 1978). N-NO_3^- and N-NH_4^+ measurements were done with a Skalar-photometer after extraction with 250 ml 0.0125M $CaCl_2$. The soil pH was measured electrochemically in $CaCl_2$ solution with a Sartorius Professional Meter PP-25. C_{org} and N_t were measured by dry combustion with LECO TruSpec CN.

Data evaluation

The Kolmogorov-Smirnov test confirmed that all data were normally distributed. For this reason, an analysis of variance (ANOVA) was carried out to compare treatment effects of type of organic matter and faunal diversity as

Table 1. Type and quantity of organic matter applied and combination of earthworm and enchytraeid species in experimental treatments (n = 5).

Treatment code	Type of organic matter	Quantity of organic matter (g dry matter)	Species combination and number of individuals
E1	2.5 cm straw	4.65	1 ind. *Lumbricus terrestris*
	10 cm straw	4.65	2 ind. *Aporrectodea caliginosa*
E2	2.5 cm straw	3.1	1 ind. *L. terrestris*
	5 cm straw	3.1	2 ind. *A. caliginosa*
	10 cm straw	3.1	
E3	cattle manure	1.5	1 ind. *L. terrestris*
			2 ind. *A. caliginosa*
EEn1	2.5 cm straw	4.65	1 ind. *L. terrestris*
	10 cm straw	4.65	2 ind. *A. caliginosa*
			25 ind. *Enchytraeus christenseni*
			75 ind. *Enchytraeus crypticus*
EEn2	2.5 cm straw	3.1	1 ind. *L. terrestris*
	5 cm straw	3.1	2 ind. *A. caliginosa*
	10 cm straw	3.1	25 ind. *E. christenseni*
			75 ind. *E. crypticus*
EEn3	cattle manure	1.5	1 ind *L. terrestris*
			2 ind. *A. caliginosa*
			25 ind. *E. christenseni*
			75 ind. *E. crypticus*
En1	2.5 cm straw	3.1	25 ind. *E. christenseni*
			75 ind. *E. crypticus*
En2	cattle manure	1.5	25 ind. *E. christenseni*
			75 ind. *E. crypticus*
C1	2.5 cm straw	4.65	non-faunal control
	10 cm straw	4.65	
C2	2.5 cm straw	3.1	non-faunal control
	5 cm straw	3.1	
	10 cm straw	3.1	
C3	cattle manure	1.5	non-faunal control

independent variables and soil parameters as dependent variables. ANOVA was followed by Tukey means comparison test ($p<0.05$). The mean differences between consumption rates of different organic material were tested using *t*-test. All statistical analyses were performed with SPSS 13.0.

Results

Dynamic of earthworms and enchytraeids

At the end of the experiment all earthworm individuals were alive. During the incubation period a high amount of casts was deposited at the soil surface indicating that the earthworms were active. During incubation the total earthworm biomass decreased in all treatments but the differences were not significant (Table 2). The biomass decrease was more evident in mixed earthworm-enchytraeid treatments, being 8% lower in EEn1 compared with E1, 11% lower in EEn2 compared with E2, respectively with 18% lower in EEn3 compared with E3. In addition, the losses in biomass were more evident for *L. terrestis* than *A. caliginosa*.

Enchytraeid populations thrived well in mixed earthworm-enchytraeid treatments reaching a maximum estimated density of 772 (1SD: 442) individuals in EEn1, 328 (1SD: 242) in EEn2, respectively of 218 (1SD: 82) in EEn3 (Table 1). In the small microcosm experiment, where only enchytraeids were used, the estimated density decreased to 92 (1SD: 92) individuals in the straw treatment (En1) while in the manure treatment (En2) the density increased to 200 (1SD: 65).

Food consumption rate

Community consumption rate expressed as percent of organic matter consumed from the initial amount was significantly higher in earthworm and mixed earthworm and enchytraeid treatments compared to control treatments (Table 3).

Regarding faunal food preferences, a significant difference was found for consumption rate of small straw (2.5 cm length) comparing to other

Table 2. Changes of earthworm biomass during experiments (means ± SD) (for meaning of treatment codes see table 1).

Treatment code	Earthworm biomass at the beginning of the experiment (g)		
	A. caliginosa	*L. terrestris*	Total
E1	1.7 (1SD: 0.4)	6.2 (1SD:1.3)	7.9 (1SD: 1.5)
E2	1.1 (1SD: 0.09)	5.8 (1SD:1.1)	6.9 (1SD: 1.1)
E3	1.6 (1SD: 1.2)	6.9 (1SD: 1.8)	8.5 (1SD: 1.7)
EEn1	1.4 (1SD: 0.1)	4.4 (1SD: 0.7)	5.8 (1SD: 0.6)
EEn2	1.3 (1SD: 0.07)	4.8 (1SD: 0.8)	6.1 (1SD: 0.8)
EEn3	1.3 (1SD: 0.2)	4.8 (1SD: 1.2)	6.1 (1SD: 1.4)

Treatment code	Earthworm biomass at the end of the experiment (g)		
	A. caliginosa	*L. terrestris*	Total
E1	1.4 (1SD: 0.3)	5.0 (1SD: 0.9)	6.4 (1SD: 1.0)
E2	1.0(1SD: 0.06)	5.1 (1SD: 1.2)	6.1 (1SD: 1.2)
E3	1.6 (1SD: 0.2)	5.9 (1SD: 0.7)	7.5 (1SD: 0.8)
EEn1	1.2(1SD: 0.08)	3.1 (1SD: 0.7)	4.3 (1SD: 0.7)
EEn2	1.0 (1SD: 0.2)	3.7 (1SD: 0.6)	4.7 (1SD: 0.8)
EEn3	1.05(1SD: 0.1)	3.3 (1SD: 0.9)	4.3(1SD: 0.07)

Table 3. Community consumption rates of different organic sources (barley straw, cattle manure) for earthworms, enchytraeids and mixed treatments (for meaning of treatment codes see table 1). *t* test values and significance of consumption rates of different organic matter types between faunal and corresponding non-faunal control treatments.

Treatment code	Type of organic matter	Initial amount of different types of organic matter (g dry matter)	Community consumption rate in percent of the initial amount (%)	*t* test values and level of significance
E1	2.5 cm straw	4.65	23.8	t = 13.6 (P<0.01)
	10 cm straw	4.65	12.7	ns
E2	2.5 cm straw	3.1	28.6	t = 9.5 (P<0.01)
	5 cm straw	3.1	16.5	ns
	10 cm straw	3.1	15.5	ns
E3	Manure	1.5	63	t = 9.06 (P=0.001)
EEn1	2.5 cm straw	4.65	30.3	t = 5.6 (P<0.01)
	10 cm straw	4.65	22.5	ns
EEn2	2.5 cm straw	3.1	40.3	t = 9.7 (P<0.01)
	5 cm straw	3.1	24.4	t = 5.8 (P<0.01)
	10 cm straw	3.1	19.6	ns
EEn3	Manure	1.5	100	t = 32.3 (P<0.01)
En1	2,5 cm straw	3,1	14	ns
En2	Manure	1,5	38	t = 3.9 (P=0.016)
C1	2.5 cm straw	4.65	11.6	-
	10 cm straw	4.65	13.9	-
C2	2.5 cm straw	3.1	12.9	-
	5 cm straw	3.1	13.8	-
	10 cm straw	3.1	17.4	-
C3	Manure	1.5	25.6	-

sizes (5 cm and 10 cm), indicating a preference for the smaller sized straw. In the mixed earthworm-enchytraeid treatment (EEn1), the consumption rate of small sized straw was 6.5% higher compared to the corresponding earthworm treatment (E1), while in EEn2 the rate was 11.7% higher compared to E2 for the same straw size. The consumption rate of 5 cm straw was significantly higher in mixed animal treatments than in non-faunal control (Table 3). Although the consumed amounts of 10 cm straw were not significantly different between treatments the recorded values are higher in animal treatments (E1, E2, EEn1, EEn2) comparing to the control (C1, C2).

The consumption rate of manure was directly influenced by the structure of the biotic community. Thus, the rate reached a value of 63% in earthworm treatments, 100% in mixed earthworm-enchytraeid treatments, 38% in enchytraeid treatments and 25.6% in control treatments, respectively. The highest consumption rate was recorded in treatments where both faunal groups were present.

Soil parameters

Microbial biomass

Generally, the type of organic matter, the faunal diversity in the microcosms as well as the interaction of both factors affected the microbial biomass significantly (Table 4). The microbial biomass measured at the end of the experiment showed the highest values in earthworm treatments (E1, E2, E3) indicating that the presence of earthworms had a positive effect on soil microbial biomass (Fig. 1). In mixed earthworm-enchytraeid treatments (EEn1, EEn2, EEn3) and in enchytraeid treatments (En1, En2), the microbial biomass showed a significant decrease compared to control treatments (Fig. 1). For all treatments the microbial biomass was higher when straw was used as organic material compared to the manure treatments.

pH

According to ANOVA, the type of organic matter and the species combination (faunal diversity) influenced the soil pH (Table 4). As a general trend, the recorded pH values were higher at the end of the experiments compared to the initial start value (Fig. 2). The pH did not vary significantly in earthworm (E1, E2, E3) and enchytraeid (En1, En2) treatments compared to control (C1, C2, C3), while in mixed earthworm-enchytraeid treatments (EEn1, EEn2, EEn3) pH values were significantly higher in EEn2 and EEn3 compared to the corresponding control treatments (Fig. 2). pH values of straw treatments were always higher than those of manure treatments.

Table 4. F values and levels of significance ($p<0.05$) from 2 – ANOVA of each factor (organic matter type, faunal diversity) and each dependent variable (soil parameters).

	Microbial biomass	pH	NO_3^-	NH_4^+	Corg	Nt
OM type	32.72*	8.15*	140.97*	11.51*	3.02	0.55
Diversity	99.09*	8.78*	66.72*	1.31	78.2*	35.55*
OM type x Diversity	2.42*	1.89	14.74*	0.105	8.11*	8.58*
R^2	0.901	0.56	0.92	0.78	0.83	0.73

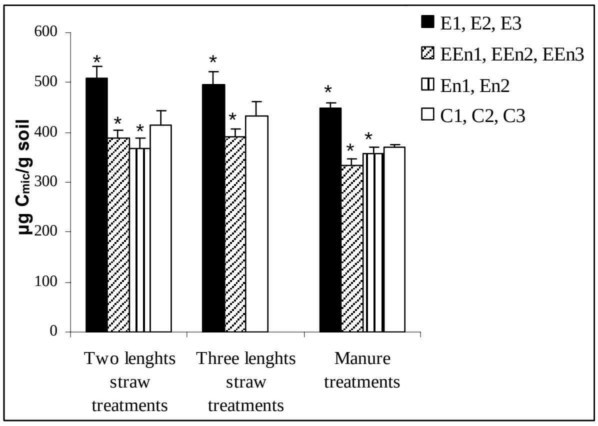

Figure 1. Microbial biomass carbon (C_{mic}) in all treatments at the end of experiments (for meaning of treatment codes see table 1); *indicates significant differences ($p<0.05$) between faunal and corresponding non-faunal control treatments.

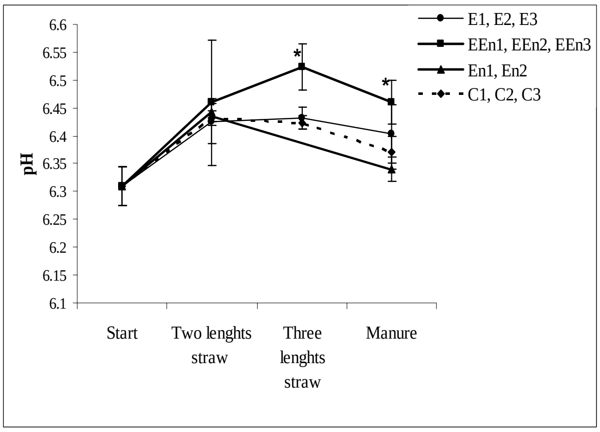

Figure 2. pH values of soil in all treatments at the beginning and at the end of experiments (for meaning of treatment codes see table 1); *indicates significant differences ($p<0.05$) between faunal and corresponding non-faunal control treatments.

Nitrate and ammonium

The amount of soil nitrate ($N\text{-}NO_3^-$) changed according to the type of organic matter applied and faunal species combination (Fig. 3, Table 4). In straw experiments, the amount of nitrate recorded in earthworm treatments (E1, E2) were significantly higher compared to that in the control treatments (C1, C2). The recorded differences were 11.4% and 17.8% higher in E1 and E2 compared to C1 and C2, respectively (Fig. 3). On the other hand, in mixed earthworm-enchytraeid treatments (EEn1, EEn2), the amount of nitrates recorded at the end of the experiment was significantly lower compared to those in the corresponding control treatments (C1, C2). The recorded values were 22.8% lower in EEn1 compared with C1, respectively, 17.9% lower in EEn2 compared with C2. A significantly lower value of nitrate compared to the control level was also recorded for the enchytraeid treatment (En1) (Fig. 3).

The combination of species also influenced the soil nitrate amounts in manure treatments. Similar to the situation found in the straw treatments, the highest value of nitrate was recorded in earthworm treatment (E3) which was 30.7% higher than the control (C3). Likewise, the nitrate values measured in enchytraeid treatment (En2) were higher than the control level, while in mixed earthworm-enchytraeid treatment (EEn3) the values were marginally lower but not statistically significant.

Comparing manure to straw treatments the amount of extractable nitrate was significantly higher when manure was used as organic matter.

No significant differences were observed between the values of $N\text{-}NH_4^+$ at the end of the experiment compared to the start value for straw treatments. The mean values were highest in manure treatments compared to straw (Table 3).

Total nitrogen and organic carbon

Faunal diversity had a significant effect on total nitrogen (N_t) measured at the end of the experiment and there was also a significant interaction between organic matter type and faunal diversity (Table 4). For both organic sources (straw and manure), the highest N_t values were recorded in earthworm treatments while the lowest values were recorded in enchytraeid treatments.

Regarding soil C_{org} in earthworm treatments similar values to the initial amount were found in the straw treatments at the end of the experiment, while only a slight increase in C_{org} values was observed when manure was applied (Fig. 4). In mixed earthworm-enchytraeid treatments (EEn1, EEn2, EEn3), as well as for enchytraeid treatments (En1, En2), a significant decrease of soil organic carbon was observed at the end of the experiment. ANOVA revealed a significant effect for faunal diversity and its interaction with the type of organic matter (Table 4).

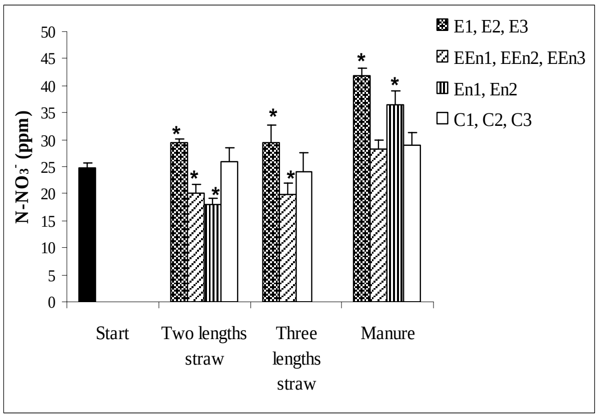

Figure 3. Soil N-NO_3^- values in all treatments at the beginning and at the end of experiments (for meaning of treatment codes see table 1); *indicates significant differences ($p<0.05$) between faunal and corresponding non-faunal control treatments.

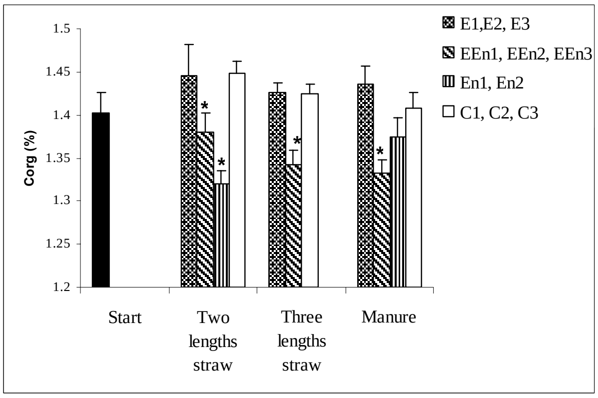

Figure 4. C_{org} values in all treatments at the beginning and at the end of experiments (for meaning of treatment codes see table 1); *indicates significant differences ($p<0.05$) between faunal and corresponding non-faunal control treatments.

Discussion

Dynamic of earthworms and enchytraeids

At the end of our experiment a slight decrease of the total earthworm biomass was observed in all treatments. Authors of similar studies (Shipitalo et al. 1988, Bohlen & Edwards 1995, Eriksen-Hamel & Whalen 2007) also reported a decrease in earthworm biomass during laboratory experiments. Quality of food, animal stress during experimental preparation and size of the microcosms used can cause such a biomass decrease. In our experiment, the decrease of earthworm biomass in both kind of treatments, i.e. only earthworm and mixed earthworm-enchytraeid treatments, can be explained by the quality of the available food as well as by the earthworm stress which, probably, inhibited earthworms feeding for a certain period of time. We observed the highest biomass decrease for *L. terrestris,* a primary decomposer which feeds directly on plant litter, compared with *A. caliginosa,* a secondary decomposer which forages mainly in mineral soil on pre-decomposed material. It is known that cereal straw is a poor source of nitrogen compared with manure and therefore consumed after being partially decomposed. Cattle manure, on the other hand, is a source of high nitrogen content and can be consumed immediately. Consequently, we can assume that the decrease of earthworm biomass was caused by reduced feeding activity of *L. terrestris* during the first part of the experiment, especially when straw was used as organic material.

The total earthworm biomass loss was higher in mixed earthworm and enchytraeid treatments compared to single earthworm treatments. This fact may indicate an antagonistic relationship between enchytraeids and earthworms which conducted to a slower feeding activity of earthworms or a greater energy input for the interspecific competition. Since the amount of food was not limited in our experiment, other aspects like excretion and secretion processes of the enchytraeids may be responsible for the biomass loss of the earthworms. However, additional research is needed to prove this assumption.

Enchytraeids reached highest densities in mixed earthworm-enchytraeid treatments compared with single enchytraeid treatments. This result suggests a beneficial effect of the presence of earthworms toward enchytraeids. Dawod & Fitzpatrick (1993) showed that earthworm casts are used as a food source by enchytraeids, while Schrader & Seibel (2001) reported earthworm middens to be a favourable habitat for soil mesofauna like enchytraeids. Likewise van Vliet et al. (2004) explained the increase of the enchytraeid population in a microcosm experiment by the availability of a light organic matter pool. The high amount of casts in our treatments was a good food source for enchytraeids and conducted to the highest enchytraeid densities in the presence of earthworms. At the same time, the presence of earthworms enhanced microbial activity and thus the availability of organic compounds that can be further metabolized by enchytraeids. This can also explain the increased enchytraeid number in mixed treatments.

<u>Consumption rate</u>

The presence of animals influenced directly community consumption rate in all treatments. The efficient use of available sources and the enhancement of organic matter mineralization contributed to this result. A significant increase of consumed organic matter was observed in mixed earthworm-enchytraeid treatments compared to other treatments. This result differs to that observed by Cortet et al. (2003), who reported no effect of enchytraeids on litter decomposition in an experiment with different biotic community. Anyway, our results agreed with Helling et al. (1998) who reported a strong correlation between enchytraeids feeding activity and number of individuals present. In our mixed animal treatments enchytraeids reached highest densities and, consequently, the highest feeding activity. More than that, recent studies (Briones et al. 2004, Vetter et al. 2004) demonstrated a positive influence of enchytraeids on metabolic activity of the soil microflora. A more intense microbial metabolic activity lead to a more intense decomposition process of organic sources which was consumed more rapidly.

Community consumption rate depends also on substrate quality. Previous research (Shipitalo et al. 1988, Helling et al. 1998) showed that organic matter with a low C/N ratio is preferentially consumed by different soil organisms. This was also valid for our experiment where cattle manure was consumed more intensely than straw.

Regarding straw palatability, significant differences were observed in consumption rates of small sized straw, i.e. 2.5 cm, compared with those of larger sizes, i.e. 5 cm and 10 cm. Although some studies showed that both anecic and endogeic species can feed at the soil surface (Whalen et al. 2004), we considered *L. terrestris* as main responsible for surface straw consumption. *L. terrestris* buries surface litter into the soil even if it does not consume it until litter is partially decomposed. Small sized straw is easier to handle and to push into the burrows, so that earthworms prefer it. For agricultural practices this aspect should be considered when straw mulch is used in the field.

<u>Microbial biomass</u>

Microbial biomass showed different values in our treatments according to the structure of biotic community and the type of organic matter used. The highest value of the microbial biomass was found in single earthworm treatments while mixed earthworm-enchytraeid treatments and single enchytraeid treatments showed smallest microbial biomass compared to control. Beneficial effects of earthworms on microbial biomass are well documented (Scheu 1987, Bohlen et al. 1997, Cortez et al. 2000, Sandor & Schrader 2007). Recently, Postma-Blaauw et al. (2006) reported no effect of mixed species of *L. terrestris* and *A. caliginosa* on microbial biomass in an experiment where different earthworm species combinations were studied. Contrary to this result, in our mixed *L. terrestris* and *A. caliginosa* experiments, microbial biomass reached highest values indicating a stimulatory effect of earthworms. This effect can be caused by the intense casting and burrowing activity (data not shown) in the presence of both species.

In mixed earthworm-enchytraeid experiments as well as in single enchytraeid experiments the lowest values of microbial biomass were measured compared to control. The presence of enchytraeids caused a decrease of microbial biomass independently of the type of organic matter. A decrease of microbial biomass in the presence of enchytraeids was also reported in other studies (Cole et al. 2002, Cortet et al. 2003, van Vliet et al. 2004). Considering that enchytraeids feed partially on bacteria and fungi, enchytraeids grazing activity resulted in a decrease of microbial biomass. It might be expected that microbial biomass would have been higher in manure treatments than in straw treatments. Actually, for biotic communities, the straw treatments had a higher microbial biomass than manure treatments. Cattle manure contains easily mineralizable compounds, which increase microbial biomass shortly after application. Therefore, as soon as the available organic compounds were mineralized, the microbial biomass decreased. On the other hand, straw is a poor source of nutrients and contains hardly decomposable compounds which are used as substrate by a small group of fungi. During our experiment, faunal feeding activity contributed to the availability of simple organic compounds for microorganisms, resulting in the high values of the microbial biomass in straw treatments measured at the end of the experiment.

Soil chemical parameters

After incubation, soil pH was higher compared with the start value in all treatments. Biological activity and added organic material increased soil pH. This fact was more visible in straw treatments than in cattle manure treatments.

Studies of Williams & Griffiths (1989), Schrader (1994), Briones et al. (1998) reported that earthworms and enchytraeids are able to increase soil pH. Our results do not confirm these findings because pH values measured in single earthworm and in single enchytraeid treatments were quite similar with those of non-faunal control treatments. But in the interaction treatments with both faunal groups soil pH reached the highest value. This suggests a higher pH buffer capacity of the soil in the presence of both groups.

The highest soil N-NO_3^- was measured in single earthworm treatments for both kinds of organic matter. The increase of soil N-NO_3^- in the presence of earthworms was also reported by other authors (Blair et al. 1997, Cortez et al. 2000). A more intense mineralization rate in the presence of earthworms and the excretion products, resulted from their metabolism, can be responsible for that.

It is known that cattle manure contains larger quantities of nitrogen compared to straw, and the use of manure as organic source results in a higher soil N-NO_3^- amount. This was also observed in our experiments in which the amount of soil N-NO_3^- in manure treatments was significantly higher compared with straw treatments for any kind of biotic community.

The presence of enchytraeids in the soil microcosms conducted to the increase of inorganic nitrogen as reported also by van Vliet et al. (2004). In our experiments we obtained the same results only for cattle manure, while in straw treatments soil N-NO_3^- content was lowest. As we already pointed out, straw treatments supported a high microbial biomass so that nitrogen immobilization might have taken place. It was also suggested (Cole et al. 2002) that increased mineralization of nutrients in the presence of enchytraeids can be masked by rapid re-utilization of nutrients by the microbial community.

Contrary to the other treatments, in mixed earthworm-enchytraeid treatments, a significant decrease of the N-NO_3^- content was found. Surprisingly, this happened in treatments where the organic matter decomposition rate had the highest values and consequently we expected the highest amount of N-NO_3^-. A possible explanation for not meeting these expectations could be the immobilization of nitrogen in microbial biomass and enchytraeid tissue. To support this theory, we emphasize that the highest density of enchytraeids was observed in mixed earthworm-enchytraeid treatments. The enchytraeids grazed on microorganisms and lead to the nitrogen immobilization in their tissue. Nicolardot et al. (2007) showed that surface mulch associated fungi are able to transfer soil nitrogen to the surface. This could have happened also in our experiment, conducting to the decrease of soil N-NO_3^-.

Soil N-NH_4^+ did not change significantly between treatments. A slight increase was observed in single earthworm treatments at the end of the experiment. The increase was more evident for manure treatments compared with straw treatments. Other studies (van Vliet et al. 2004) showed an increase of soil N-NH_4^+ during first part of experiments followed by a concentration decrease later on. Therefore, we can assume that during the first part of the experiment ammonification took place, while during the last part of the experiment nitrification became more important.

The short period of the experiment resulted in no important changes of soil total nitrogen. Anyway, faunal diversity could have influenced soil total nitrogen through soil organic nitrogen mineralization processes.

The soil organic carbon dynamic was significantly related with biotic community structure. In contrast to single earthworm treatments, the final C_{org} values in mixed earthworm-enchytraeid treatments as well as in single enchytraeid treatments were significantly lower compared to control. Therefore, we assume that the presence of enchytraeids conducted to the decrease of soil organic carbon indicating high mineralization processes. Similar results were reported by Marinissen & Didden (1997) and van Vliet et al. (2004). The increase of soil microbial metabolic activity in the presence of enchytraeids conducted to carbon mineralization and a CO_2 release from soil. Our results are in line with other studies (Hedlund & Augustsson 1995, Cole et al. 2002, Vetter et al. 2004) which revealed a significant influence of enchytraeids on CO_2 emission. Hedlund & Augustsson (1995) considered higher CO_2 production in the presence of fungivorus species as a result of their higher metabolic activity. The same kind of mechanism could be responsible for the decrease of soil organic carbon in our experiments.

Conclusions

The results of this study indicate that the interaction of earthworms and enchytraeids caused a higher consumption and decomposition rate compared to single group treatments. The density of enchytraeids in mixed faunal treatments was higher than in single enchytraeid treatments indicating a beneficial effect of earthworms on enchytraeids. Regarding soil parameters in mixed faunal treatments, soil pH was higher compared with single group treatments while microbial biomass, N-NO_3^- and C_{org} contents were lower respectively. Consequently, interaction effects might have important impacts on soil fertility which was not found in the case of single group activity.

Both organic sources were used as energy supply by biotic communities, cattle manure being more palatable than barley straw. Straw treatments supported higher microbial biomass than manure treatments and small sized straw (i.e. 2.5 cm) was more intensely consumed than larger sized straw (i.e. 5 cm and 10 cm).

Further investigation is needed for a better understanding of how interaction between soil faunal groups influence soil processes in order to develop appropriate mineralization models in agricultural soils.

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